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On the Strains of *Erwinia carotouora* subsp. *carotouora* Isolated from Radish Seedlings and Broccoli Plants

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Twenty bacterial strains isolated from edible radish seedlings (Kaiware-daikon : *Raphanus sativus* L.) and broccoli plants (*Brassica oleracea* var. *italica* Plen.) showing soft rot were compared on the basis of pathogenicity and physiological, biochemical and serological properties. Ten strains isolated from each host were homogeneous and they were identical in being positive for the following properties : rod shaped cells with peritrichous flagellation, anaerobic growth, fermentative metabolism of glucose, growth at 36°C, catalase, H_2S production, pectate degradation, gelatin liquefaction, nitrate reduction, utilization of lactose, rhamnose, trehalose and citrate. They were also identical in being negative in the following tests: Gram stain, oxidase, production of reducing substances from sucrose, amino acid decarboxylases, phosphatase, utilization of maltose, α-methyl glucoside, palatinose, malonate and tartrate. Casein hydrolysis was variable. From these results both strains were identified as *Erwinia carotovora* subsp. *carotovora*. These strains caused soft rot by inoculation to slices of potato, carrot and cucumber as well as to radish seedlings and detached broccoli tissues. The pathogenicity to onion and tomato, however, was different between the radish strains and broccoli strains, suggesting variation in pathogenicity. Bacterial strains from either radish seedling or broccoli were distinguishable each other in the serological reaction (agar gel diffusion test) to their reciprocal antisera.

INTRODUCTION

Bacterial soft rot disease occurred on edible seedlings of Japanese radish (*Raphanus sativus* L. cv. Osaka yonju-nichi) which is so-called Kaiware-daikon grown in culture bed in the greenhouse (Plate I-l) and broccoli (*Brassica* ***oleracea*** var. *italica* Plen.) in the farmer's fields, respectively, in Amagi, Fukuoka Prefecture, Japan, in June 1985.

On radish seedlings the symptoms appeared in both hypocotyls and cotyledons. Drooping of the cotyledons with a dark brown rot of glassy or watery glittering was remarkable in the initial stage, then seedlings were decayed finally (Plate I-2).

On broccoli plant, on the other hand, the leaf, stem, peduncle and flower organs showed dark brown decay with bacterial ooze (Plate I-3). In advanced stages of the disease, the vascular bundle systems of the stem showed dark brown decay and unpleasant odor was produced (Plate I-4).

The pathogenic bacteria isolated from each infected plant were identical with

Erwinia carotovora in many respects.

This study was designed to compare the strains of causal bacteria in pathogenicity and bacteriological and serological properties. The abstract of the study was reported elsewhere (Tsuchiya *et al.*, 1986).

MATERIALS AND METHODS

Bacterial strains used

To isolate the causal organisms, samples from the margins of rotten tissue of infected radish seedlings and broccoli plants were surface-disinfected with 70 % ethanol for a few seconds followed by 1 % sodium hypochlorite for 1 min, rinsed in sterile distilled water, and crushed with glass tissue grinder. The resulting suspensions were streaked onto potato semi-synthetic agar (PSA) medium (Wakimoto, 1955). Culture plates were incubated at 25°C for 3 days. The colonies of the suspect bacteria were isolated and single colony isolation was practised further on PSA medium three times. Ten bacterial strains isolated from radish seedlings and broccoli plants were designated as K1~K10 and B1~B10, respectively. The bacterial strains were stab cultured followed by sealing with sterile liquid paraffin and preserved at 4°C or lyophilized in PS broth (PSA minus agar) for long term preservation. Stock cultures of *E. carotovora* subsp. *carotovora* N7129 (radish strain) and *Enterobacter* species *(E. aerogenes, E. cloacea, E. agglomerans* and *E. sakazaki*) were also used as controls for testing bacteriological properties.

Physiological and biochemical properties

Unless otherwise mentioned, the following tests were employed as described in references (Dye, 1968 and 1969 ; Cowan, 1974 ; Lelliot and Dickey, 1984 ; Schaad, 1980) to characterize the suspect bacterial strains from infected plants ; anaerobic growth, utilization of carbohydrates as a sole source of carbon, production of reducing substances from sucrose, effect of NaCl concentration, growth factor requirement, amino acid decarboxylases, phenylalanine deaminase, catalase, oxidase, acetoin and urease production, H_2S production, hydrolysis of casein and starch, tests for mode of utilization of glucose, gelatin liquefaction, indole production and nitrate reduction. Pectolytic and cellulolytic (Cx) activities were tested by agar plate method as reported previously (Tsuchiya *et al.*, 1983).

Pathogenicity tests

Scales of onion, slices of potato tuber, roots of radish and carrot as well as detached broccoli tissues (stem and peduncle) placed in Petri dishes were inoculated to test pathogenicity of the bacterial strains. Tomato plants, cvs. 'Tōkō', 'Oogata fukuju', 'Sekai-ichi', 'Kyoryoku gokō' were grown in pots in greenhouse, and 21 day-old seedlings were also used for pathogenicity test.

Inoculations were performed by spraying or pricking method with bacterial suspensions (ca. 10^9 cells/ml).

Serological property

Two strains of *E. c.* subsp. *carotovora, N7129* (radish isolate) and Bl (broccoli isolate) were used for antisera preparation. Each bacterial suspension was injected 6 times into the ear vein of adult New Zealand white rabbits (2.5-3.0 kg) with dose increasing from 0.5 to 2 ml at 4-day intervals. Seven days after the final injection, the

rabbits were bled and antisera were prepared by ordinary procedures.

For preparing antigens, each bacterial culture grown on PSA slants was suspended in 0.85 % NaCl solution and washed twice by centrifugation at 10,000 x g for 15 min. Bacteria were resuspended in the same solution to adjust the concentration at ca. 10^9 cells/ml. The antigen and antisera thus prepared were stored at -40°C until needed. Serological properties of the bacterial strains were tested by agar gel diffusion test (Tsuchiya et al., 1982).

RESULTS

Bacteriological properties of the present strains

All bacterial strains isolated from both radish seedlings (radish strains) and broccoli plants (broccoli strains) were identical in being positive for the following properties : rod shaped cells with peritrichous flagellation (Plate II-1 and 2), anaerobic growth, fermentative metabolism of glucose, growth at 36°C, catalase reaction, H_2S production, acetoin production, pectate degradation, gelatin liquefaction, nitrate reduction, growth in 5 % NaCl, erythromycin resistance, utilization of melibiose, inositol, raffinose, cellobiose, lactose, rhamnose, xylose, trehalose, glycerol and citrate. They were also identical in being negative in the following tests : Gram stain, growth factor requirement, oxidase test, production of reducing substances from sucrose, methyl red test, phenylalanine deaminase, lecithinase, amino acid decarboxylases (arginine, ornithine, lysine and glutamic acid), indole production, phosphatase, utilization of maltose, sorbitol, melezitose, dulcitol, palatinose, a-methyl glucoside, malonate and tartrate. The radish strains hydrolyzed casein while the broccoli strains did not (Table 1).

Pathogenicity test

All the present radish strains and broccoli strains used in this experiment were highly virulent to the slices of potato, radish, carrot as well as to respective host plants and intermediately virulent to those of cucumber (Table 2, Plate I-5, 6 and 7). On the other hand, pathogenicity to onion bulb and tomato plant was variable depending upon the strains. Radish strains were pathogenic to both plants, though some variation in virulence to four varieties of tomato plants were observed. Pathogenicity of broccoli strains to these two plants were variable among strains showing weak virulence or avirulence alternatively to either plant (Table 2, Plate I-7 and 8).

Serological property

Twenty bacterial strains from both radish seedlings and broccoli plants were subjected to gel diffusion test with two antisera produced against *E. c.* subsp. *carotovora* N7129 (radish strain) and Bl (present broccoli strain) to compare serological properties with one another. All of these strains produced a common precipitin band with both antisera. All of radish strains were serologically homogeneous in the reaction against anti-N7129-serum. They produced specific precipitin band "a" which was identical with that produced by homologous strain of N7129, besides one common band (Plate II-3).

Against anti-Bl-serum, both radish and broccoli strains produced two precipitin bands, one of which was common to all strains, while the other one named "b" (Surang *et al.* unpublished) was specific to broccoli strains (Plate 11-3).

Table 1. Biochemical and physiological properties of the present strains.

Property	Radish strain	Broccoli strain	*Ecc*[2]	*Eca*[2]	*Ecb*[2]
Motility	+[1]		+	+	+
Flagellation	peritrichous	peritrichous			
Anaerobic growth	+	+	+	+	+
Growth factor required		−			−
Mucoid growth	(+)	(+)	d	−	d
Growth at 36°C	+	+	+		+
Oxidase	−		−		−
Catalase	+	+	+	+	+
H_2S production	+		+	+	+
Reducing substances from sucrose			−	+	+
Acetoin production	+	+	+	+	+
Pectate degradation	+		+	+	+
Casein hydrolysis	(+)		+	+	−
Gelatin liquefaction	+	(+)	+	+	+
Phenylalanine deaminase			−		−
Decarboxylases for					
Arginine			−		−
Lysine					−
Ornithine				−	−
Glutamic acid				−	−
Indole production				−	−
Nitrate reduction	+		+	+	+
Growth in 5% NaCl	+	+	+	+	+
Phosphatase	−		−	−	
Lecithinase	−		−	−	
Sensitivity to erythromycin (15 #g/disk)	−	−	−	−	−
Utilization of :					
Melibiose	+		+	+	−
Inositol	+	+	+	−	+
Raffinose		+	+	+	+
Maltose		−		+	+
Sorbitol			−		−
Cellobiose	+		+	+	
Lactose	+	+	+	+	+
Rhamnose	+	+	+	+	+
Xylose	+	+	+	+	+
Trehalose	+		+	+	+
Glycerol	+	+	+	d	+
Melezitose					−
Dulcitol					−
Palatinose			−	+	+
a-methyl glucoside				+	+
Galacturonate (Na)	+		+	+	
Citrate (Na)	+		+	+	
Malonate (Na)					−
Tartrate (Na)	−		−		−

1) + = positive, (+) = weakly positive, − = negative.

2) *Ecc*, *Eca* and *Ecb* *E. carotovora* subsp. *carotovora*, subsp. *atroseptica* and subsp. *betavasculorum*, respectively : data on these species are mostly from Dickey (1979) and Lelliot and Dickey (1984) with supplemental data from Dye (1969) and Thomson *et al.* (1981).

Table 2. Pathogenicity of the present strains to various plant tissues.

Test plant (variety)	Pathogenicity	
	Radish strain	Broccoli strain
Potato ('May Queen')	++[1)]	++
Radish ('Minowase')	++	++
Carrot spp.	++	++
Broccoli sp.	++	++
Cucumber ('Nashio')	++	++
Onion ('Satsuki', 'Momiji')	tt	(+)/−[2)]
Tomato ('Oogata fukuju')	++	(+)/−[3)]

1) ++ = highly virulent, + = intermediately virulent, (+) = weakly virulent, − = avirulent.
2) Strains B7 and B8 were avirulent, while others were weakly virulent.
3) Strains B2, B3, B9 and B10 were avirulent, while others were weakly virulent.

DISCUSSION

The strains of rot-causing erwinias were differentiated into several groups such as *E. c.* subsp. *carotovora*, *E. c.* subsp. *atroseptica*, *E. c.* subsp. *betavasculorum*, *E. rhapontici*, *E. chrysanthemi* and *E. cypripedii* on the basis of pathological, physiological, biochemical and nutritional properties (Dye, 1968 ; Lelliot and Dickey, 1984 ; Thomson *et al.*, 1977 and 1981).

The present strains isolated from radish seedlings and broccoli plants were identical in their physiological and biochemical properties with only exception of casein hydrolysis (Table 1). They were distinct from *E. chrysanthemi* in being negative in phosphatase, indole production and sensitivity to erythromycin and they also differed from *E. rhapontici* and *E. cypripedii* in being positive in pectate degradation and gelatin liquefaction as well as in some other properties such as pink diffusible pigment production, acetoin production and phenylalanine deaminase (Dickey, 1979 ; Lelliot and Dickey, 1984 ; Schaad, 1980). Furthermore, the present strains were distinguishable from *E. carotovora* subspecies *atroseptica* and *betavasculorum* in the following properties which are presumptive characteristics to differentiate subspecies (Dickey, 1979): growth at 36°C, reducing substances from sucrose, and utilization of inositol, maltose and α-methyl glucoside (Table 1).

From the results shown in Table 1, the radish strains were evidently identical to *E. carotovora* subsp. *carotovora* on the basis of overall similarity (97.7 %) in the phenotypic characteristics. The strains isolated from broccoli plants also identified as *E. c.* subsp. *carotovora* because of high similarity (95.3 %) in the properties tested, although they differed from the typical strains of the species in the negative reaction in the casein hydrolysis (Table 1). Variation in casein hydrolysis ability in the strains of *E. c.* subsp. *carotovora* had already reported as well as some other variable properties (Dickey, 1979 ; Lelliot and Dickey, 1984).

All the present strains from radish seedlings and broccoli tissues were pathogenic and caused soft rot to the slices of potato, radish, carrot and cucumber as well as to detached broccoli tissues, although some differences exist in virulence among strains.

Pathogenicity to 2 varieties of onion and 4 varieties of tomato plants was different among strains, that is, the radish strains were highly or intermediately virulent to the plants tested, whereas the broccoli strains were weakly virulent or avirulent, suggesting that pathogenic variability exists in *E. c.* subsp. *carotovora* as in the case of pathovars in *E. chrysanthemi* (Dickey, 1979 and 1981 ; Dye *et al.,* 1980), although no distinct information about it has been reported so far. It has been recently reported that 29 and 51 strains of *Erwinia carotovora* isolated from various kinds of vegetables collected in Thailand and Japan, respectively, could be divided into 4 pathovars and 7 biovars, although no relationship between pathovar and biovar was found (Karnjanarat *et al.,* 1986).

The bacterial strains isolated from either radish seedlings or broccoli plants were distinguishable each other in serological reaction to reciprocal antisera. One precipitin band which was identical to band "a" that produced between *E. c.* subsp. *carotovora* strain N7129 and anti-N7129-serum was formed by radish strains but not by broccoli strains, and the reaction to anti-Bl-serum was *vice versa* (Plate 11-3). Each distinguishable main band was also formed by heat treated respective bacterial cells, suggesting that thermostable somatic antigen might concerned. A precipitin band commonly formed between two antisera and all bacterial strains was thermolabile. The results obtained here indicated that serological variation existed among present strains on the basis of either flagella or LPS O-antigens as reported with *E. carotovora* subspecies (De Boer *et al.,* 1979 ; De Boer, 1980 ; De Boer *et al.,* 1985 ; Karnjanarat *et al.,* 1986).

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REFERENCES

Cowan, S. T. 1974 Cowan *and Steel's Manual for the Identification of Medical Bacteria,* 2nd ed. Camb. Univ. Press, England

De Boer, S. H. 1980 Serological relationship among flagella of *Erwinia carotovora* var. *atroseptica* and some *E. carotovora* var. *carotovora* serogroups. Can. *J. Microbiol., 26* : *567-571*

De Boer, S. H., J. J. Bradshaw-Rouse, L. Sequeira and M. E. McNaughton 1985 Sugar composition and serological specificity of *Erwinia carotovora* lipopolysaccharides. *Can. J. Microbiol., 31* : *583-586*

De Boer, S. H., R. J. Copeman and H. Vruggink 1979 Serogroups of *Erwinia carotovora* potato strains determined with diffusible somatic antigens. *Phytopathology, 69* : 316-319

Dickey, R. S. 1979 *Eminia chrysanthemi* : A comparative study of phenotypic properties of strains from several hosts and other *Erwinia* species. *Phytopathology, 69* : 324-329

Dickey, R. S. 1981 *Eminia chrysanthemi* : Reaction of eight plant species to strains from several hosts and to strains of other *Eminia* species. *Phytopathology,* 71: 23-29

Dye, D. W. 1968 A taxonomic study of the genus *Eminia* I. The "amylovora" group. N. *Z. Jl. Sci.,* 11: 590-607

Dye, D. W. 1969 A taxonomic study of the genus *Erwinia II.* The "carotovora" group. N. Z. *Jl. Sci.,* 12: 81-97

Dye, D. W., J. F. Bradbury, M. Goto, A. C. Hayward, R. A. Lelliot and M. N. Schroth 1980 International standards for naming pathovars of phytopathogenic bacteria and a list of pathovar names and pathotype strains. *Rev. Plant Pathol., 59* :153-158

Karnjanarat, S., K. Tsuchiya, N. Matsuyama and S. Wakimoto 1986 Comparative studies on pathogenicity, biochemical, physiological and serological properties among strains of *Erwinia carotovora* isolated from Thailand and Japan. *Ann. Phytopath. Soc. Japan, 52: 506*

Lelliot, R. A. and R. S. Dickey 1984 Genus VII. Erwinia Winslow, Broadhurst Buchanan, Krumwiede, Rogers and Smith 1920. *In* "Bergey's Manual of Systematic Bacteriology", Vol. 1 : 469-476. Williams and Wilkins, Baltimore

Schaad, N. W. 1980 Laboratory guide for identification of plant pathogenic bacteria. *Bact. Comm. Amer. Phytopath. Soc. Minn.*

Thomson, S. V., D. C. Hildebrand and M. N. Schroth 1981 Identification and nutritional differentiation of the Erwinia sugar beet pathogen from members of *Erwinia carotovora* and *Erwinia* chrysanthemi. *Phytopathology,* 71: 1037-1042

Thomson, S. V., M. N. Schroth, F. J. Hills, E. D. Whitney and D. C. Hildebrand 1977 Bacterial vascular necrosis and soft rot of sugarbeet : general description and etiology. *Phytopathology, 67* : 1183-1189

Tsuchiya, K., J. E. Choi, T. W. Mew and S. Wakimoto 1982 Serological properties of mutant strains of *Xanthomonas campestris* pv. *oryzae* induced by N-methyl-N'-nitro-N-nitroso-guanidine. *Ann. Phytopath. Soc.* Japan, *48* : 9-18

Tsuchiya, K., Karnjanarat and S. Wakimoto 1986 On the *Erwinia carotovora* subsp. *carotovora* strains isolated *from* radish seedlings (Kaiware-daikon) and broccoli plants showing soft rot symptoms. *Ann. Phytopath. Soc. Japan, 52: 107*

Tsuchiya, K., N. Matsuyama and S. Wakimoto 1983 Studies on the relationship between virulence and bacteriological properties in *Erwinia carotovora* subsp. *carotovora*. *J. Fac. Agr., Kyushu Univ., 27* : 197-207

Wakimoto, S. 1955 Studies on the multiplication of OP, phage *(Xanthomonas oryzae* bacteriophage) 1. One-step growth experiment under various conditions. *Sci. Bull. Fac. Agr., Kyushu Univ.,* 15: 151-160

Explanation of Plate I

Fig. 1. Edible radish seedlings (Kaiware-daikon: *Raphanus* ***sativus*** L. cv. Osaka yonju-nichi) growing in culture bed,

Fig. 2. Radish seedlings showing soft rot symptom on hypocotyl and cotyledon.

Fig. 3. Soft rot symptom developed on flower buds and peduncles of broccoli.

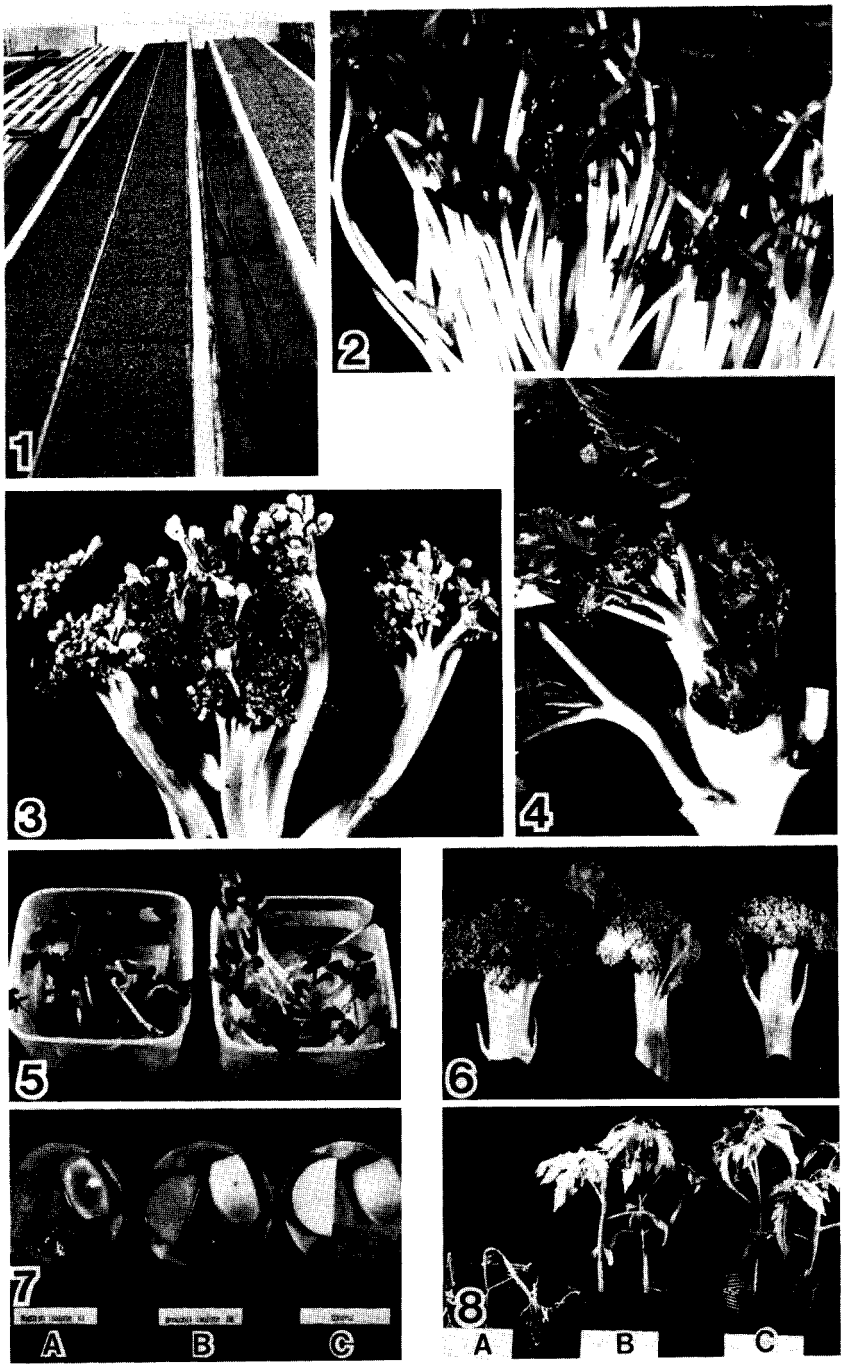
Fig. 4. Soft rotting with decay on vascular bundle systems of broccoli stem.

Fig. 5. Radish seedlings (cv. Osaka yonju-nichi) inoculated with radish strain K1 by spraying (left) and control (right), respectively.

Fig. 6. Broccoli tissues inoculated with broccoli strain B1 by pin-pricking method.

Fig. 7. Variability in pathogenicity of the present strains to onion scale and slices of radish and carrot. Radish strains caused soft rot to these plant tissues (A) but a broccoli strain, B8, was not pathogenic to onion scale (B). Non-inoculated controls were in (C).

Fig. 8. Tomato plants (cv. Oogata fukuju) inoculated with radish strain (A), broccoli strain (B) and sterile distilled water (C), respectively.


1
2
3
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A
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8
A
B
C

Explanation of Plate II

Fig. 1. Electron micrograph of bacterial cells of strain K1. Bar indicates 1 μm.

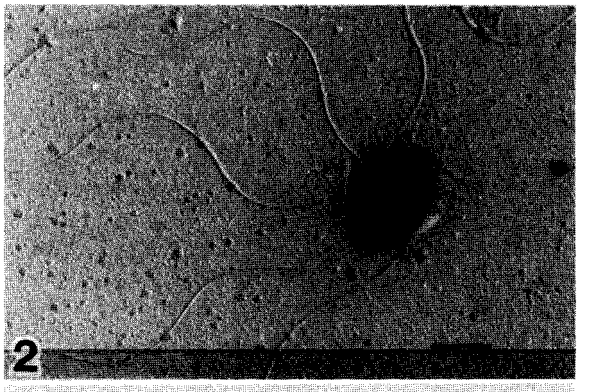
Fig. 2. Electron micrograph of bacterial cell of strain B1. Bar indicates 1 μm.

Fig. 3. Serological differentiation of the present bacterial strains from radish seedlings and broccoli plants by gel diffusion test.

Center wells A and B contain anti-N7129 (radish strain)-serum and anti-B1 (present broccoli strain)-serum, respectively.

Outer wells l-5 were filled with intact cell suspensions of K1, K2, B1, B2 and N7129, respectively.

1



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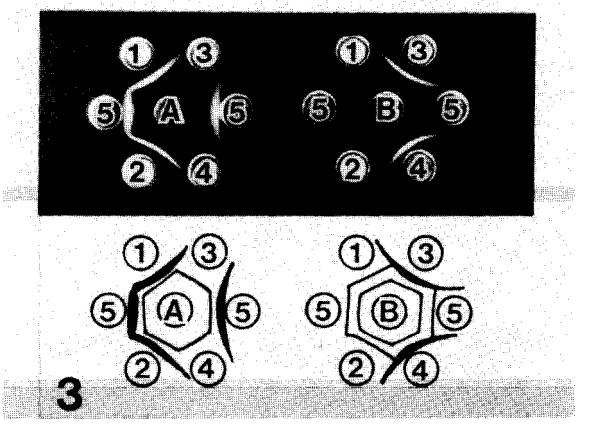

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