Studies on the Disease of Root Tumor of Melon(Cucumis melo L.)

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Studies on the Disease of Root Tumor of Melon (Cucumis melo L.)

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Summary

In March, 1982, a new disease which formed a number of tumors on roots was discovered for the first time in semi-forcing cultured melon plants (Cucumis melo L.), cultivars Amusu and Kosack (both rootstock: Kenkyaku), growing in Nishiki, Kuma, Kumamoto Prefecture in Japan. The first subject in this study was to determine the causal pathogen which was revealed to be a species of actinomycete. Its taxonomic characterization, identification and morphogenetical study were undertaken. A method for intensive isolation of the pathogen was established. In addition, spore germination and its activation, and pathogenicity and host range of the pathogen were investigated. Then, several experiments were performed to define the appearance of symptom, parasitic site of the pathogen in roots, histological changes of diseased roots and environmental soil conditions on the occurrence. The results are outlined as follows.

1. Circumstances relating to the onset of the disease

From 1982 when the disease was first detected to 1986, the disease of melon had spread to all prefectures in Kyushu district except Fukuoka and Saga. The occurrence of the

same disease was also confirmed in cucumber-growing fields at Kumamoto City (1984) and Kagoshima City (1986). The disease had spread not only over Kyushu district but also to Kochi and Kanagawa Prefectures until 1990. The disease was also observed at a total of 30 cities, towns and villages in 7 prefectures from 1982 to 1993.

2. Symptoms

The first symptom is growth retardation and infected plants wilt in the daytime. When the disease is very severe, infected plants withered finally. Under the ground, many pale-brownish or whitish nodules with coarse surface and tiny protuberances are formed on roots. The size of each nodule was approximately 1-15 mm in diameter. These nodules are often fused together to form a large nodal appearance with a typical symptom of "tumor". When the disease further advanced, these tumors are discolored to brown or dark brown and the tumor tissue degenerated easily to corky and rotted situation. The formation of these tumors progressed on the lateral root surfaces. Tumors formed on branched roots grew as if adhering to the surfaces of main roots with hypertrophy of tumor tissue.

3. Transmissibility

The disease was transmissible from infested field soil and tissue of root tumors. However, the pathogen in infested soil and tumor tissue was completely inactivated by autoclaving at 121℃ for 20 min. The transmissibility of

the pathogen in the tumor tissue was reduced by treatment with 5% antiformin solution.

4. Identification of causal organism and naming of the disease

A kind of bacterium was isolated from the tumor tissue. When the isolate was artificially inoculated to a young root of melon, the same symptom was reproduced. This isolate showed gram-positive reaction in gram-staining test. It formed bacterium-like colonies on the potato semi-synthetic agar medium, which were circular, capitate and entire. It formed a fungus-like colonies on the albumin agar medium, which were circular, flat and filamentous. From these characteristics, the causal pathogen was identified as a species belonging to Actinomycetales. This is the first record of this melon disease caused by actinomycete. Thus the English name of "Root tumor" and the Japanese name of "Ganshu-byo" were proposed.

5. Taxonomic characterization of the causal actinomycete

The causal actinomycete had true mycelia, and mycelial filaments tended to remain intact and were not fragmentary. The actinomycete did not produce sclerotia, pycnidia or sporangia, but it was characterized by the formation of pseudosporangia. The spores were produced in chains on aerial mycelia, and the morphology of the spore chains was classified as retinaculum-apertum (RA) type. The color of matured, sporulated aerial-mycelia was in the gray series,

and the spore surface was smooth. Whole cell hydrolysates of the actinomycete contained LL-diaminopimelic acid but no diagnostically important sugars. These results indicated that this causal actinomycete belonged to cell wall type I, and should be assigned to the genus Streptomyces. The pathogen grew very well in the medium at 27-35 ℃ and pH 6.5-7.7, and it utilized D-glucose and 13 other carbohydrates as carbon sources but not D-mannitol and 3 others. The actinomycete was negative in melanoid pigment and hydrogen sulfide productions and xantine dissolution, whereas it was positive in gelatin liquefaction, milk decomposition, starch hydrolysis, nitrate reduction and calcium malate dissolution. The highest concentration of NaCl to allow growth was 4%. In addition, there were no known Streptomyces species that were identical to the present actinomycete. This suggested that the pathogen will be a new species of genus Streptomyces. The name Streptomyces tuberis sp. nov. was proposed for this pathogenic actinomycete causing root tumor of melon.

6. Morphogenesis of the pathogen

Spores of the pathogenic Streptomyces sp. began to germinate 3 hr after incubation at 28 ℃. All spores had swollen by 3 hr after incubation, and most spores germinated until 24 hr. Germ tube (hypha) appeared from 1-3 points of the spore surface. Branched multiplying hyphae formed colonies of substratal mycelia 24-48 hr after incubation. Besides, growing hyphal tips of these mycelia began to swell,

and produced hook-shaped aerial hyphae on the colonies. The aerial hyphae swelled more and transformed into hook or open-loop shapes 72 hr after incubation. Ripened aerial-mycelia developed into not only spore chains by formation of septa but also pseudosporangia consisting of spore chains from 72 hr to 5 days after incubation. The morphology of the spore chains indicates that it belongsto a type of RA. Then, in these colonies the number of pseudosporangia increased and the number of spore chains decreased with incubation time. Some of the pseudosporangia were covered with mucoid-like substances after incubation for 10 days. During 14-21 days of incubation, these colonies maintained a stable state with pseudosporangia, spore masses and spore chains on the substratal mycelial mats overspread with mucoid-like substances.

7. Method for intensive isolation of the pathogen

Pre-treatment of the diseased tissue with phenol at 140-fold diluted solution for 10 min was extremely effective to decrease the population of the bacterial contaminants in dilution plate technique. Moreover, the addition of 50 ppm of kanamycin as an inhibitor to the culture medium showed the highest effect for reducing the contaminants. The best medium for the isolation was the basal medium for rhizosphere microorganisms introduced by Lochhead and Chase (medium B), and the optimum incubation period was for 5 days at 28 ℃. The isolation method on medium B containing kanamycin (50

ppm) by incubation at 28 ℃ for 5 days using the sample surface-disinfested with the phenol solution (x 140) was considered as the best one. This improved isolation procedure for the pathogenic Streptomyces sp. could increase the values of its isolation ratio (number of successful isolation tests / number of isolation tests x 100) from the root tumor, its percentage in the total number of actinomycetes and mean number of the isolates per isolating plate as compared to non-treated isolation procedure on medium B; from 42.9% to 100%, from 12.5% to 71.2% and from 0.3 to 80.6, respectively.

8. Spore germination and activation of the pathogen

In the pathogenic Streptomyces sp., spores which were formed on the culture medium by incubation at 28 ℃ for 14 days ("nascent spore") and those stored at 5 ℃ for 28 days after sporulation by incubation at 28 ℃ for 28 days ("matured spore") began to germinate within 3 hr incubation at 28 ℃. Most germinating nascent spores germinated within 24 hr incubation. While, germination of matured spores was retarded and the time of maximum germination rate was 1-2 days later than that of nascent spores. The rates of germination were 84.0-87.0% at nascent spores and 81.2-83.3% at matured spores, and about 10-20% of these spores remained ungerminate. A heat shock treatment at 40 ℃ for 20 min was the most effective for activation of the germinability of the spores, whereby the colony forming ratio to the non-treated control increased to 110.0-115.1%. In addition to the heat

shock (40℃, 20 min), treatment with 0.00625-0.05% sodium dodecyl sulfate (SDS) or 1.0-2.0% yeast extract as spore-activating agents further improved spore germination; especially a treatment with 0.025% SDS increased the colony forming ratio to 121.2% of the control (only heat shock treatment). These results suggest that this pathogen contains ungerminating spores which are considered to be in a dormant phase and this dormancy can easily be broken by heat shock treatment at 40℃ for 20 min with 0.025% SDS solution.

9. Appearance of the symptoms in inoculated melon

Appearance of the root tumor in both melon plants inoculated by pouring and mixing of the pathogen to the growing soil and grown in the infested soil indicated that the latent period of this disease was for ca. 11-14 days. In the melon sown in infested soil, root tumor was firstly observed on branching parts of its primary branched roots from the main root. Then these tumors began to form on many branching parts with time. Although the number of root tumors increased until about 42 days, the number of tumors newly formed decreased from about 49 days after sowing. During 49 days after sowing the growth of the infected melon was inhibited to less than 40% of healthy melon in plant length and 15% in the number of foliage leaves. In the inoculation test for melon plants of different growth stages, the severest symptom was observed at the inoculation to 14- to 21-days old plants, after that the severity of the disease

was mitigated with the aging of melon. The pathogen also caused morphological abnormality on the aerial parts of melon by injecting inoculation. The pathogenicity was far more severe on hypocotyl tissue than on stem and petiole. And these abnormalities were more conspicuous when inoculated with mycelia than with spores.

10. Existence of the pathogen and histological changes in the roots of diseased melon

Most of the root tumors were formed at branching parts of the roots. In optical microscopic observation of the cross sections of tissue of spontaneous tumors, a stain which could represent the existence of microorganisms was recognized on tumor surface and in an epidermal intercellular space of tumor tissue. It was also observed at the boundary between the root and tumor. Besides, tissue surrounding vascular bundle structure and outer-layer tissue of branched roots containing tumors were also stained in the same manner. In scanning electron microscopic observation of the diseased roots that were artificially inoculated, a great deal of the proliferated pathogen were found on the surface of root tumors, namely, on the surface of epidermis and at one of the endodermis which appeared in the ruptures of cortex due to ejection of branching roots. However, the pathogen was hardly observed on the surfaces of hypertrophied parenchymal cells which were exposed from the broken endodermis. The growing pathogen was recognized only in intercellular spaces of 1-2 outer layers of tumor tissue. Tissues of tumors

always contained branching roots or vascular bundle structure. Root tumors were composed of hyperplastic tissues made of parenchymal cells in endodermis, pericycle and inner cortex or around the vascular bundle structure. Moreover, the outer layer cells of tumor tissue were hypertrophied. Then, the surface of the tumor presented a coarse structure, because warty hypertrophied cells were exposed on the surface with its development.

11. Pathogenicity and host range of the pathogen

The pathogen was parasitic to a wide range of cucurbitaceous plants such as melon, cucumber, watermelon, pumpkin, bottlegourd, oriental pickling melon and balsam pear. It had especially strong pathogenicity to cucumber and oriental pickling melon, because the three different isolates of the pathogen caused the disease in all cultivars of these species tested. The pathogenicity in melon, watermelon and pumpkin differed by cultivars, isolates and methods of inoculation. The pathogen seemed to have weaker pathogenicity to watermelon and pumpkin, compared to other cucurbitaceous plants. In Solanaceae such as tomato, eggplant and sweet pepper, the disease appeared but its pathogenicity was weak. In addition to Cucurbitaceae and Solanaceae, the pathogen was also parasitic to cauliflower in Cruciferae, spinach and chard in Chenopodiaceae, and edible burdock, lettuce and sunflower in Compositae, although the pathogenicity was comparatively weak to these plants except

for chard and sunflower. Thus, the hosts of this pathogen involved a total of 16 members of 5 plant families.

12. Relation between the occurrence of the disease and environmental soil conditions

Young growing melons sown in kuroboku soil and inoculated with a high concentration (more than ca. 5×10^3 cfu/ml moist soil) of the pathogen showed a symptom of seedling damping-off. But the degree of the severity of damping-off varied with the types of soil used. Root tumor was observed in plants which were grown in soil infested with more than about 10 cfu of the pathogen per ml of moist soil. The severest disease appeared when melon was sown in soil infested with ca. 10^3-10^4 cfu/ml of the soil. The disease occurred at a soil temperature range of 15-35 ℃ and a soil pH range of 4.6-7.5, with the highest severity at 35 ℃ and pH 6.5-7.0. No disease occurred at less than pH 5.5. The disease was severer in soil moistened with pF 2.4 of irrigation point than in those with pF 1.8 and 2.7. In investigation of effect of soil texture on occurrence of the disease, the disease severity varied with the kind of soils tested within the same group of soil texture. This result suggests that the soil texture has no direct influence on the occurrence of the disease but the moisture content in cultivating soils is closely associated with the severity of the disease. Studies on the relation between occurrence of the disease and soil sterilization suggested that the occurrence of the disease was enhanced by simplified

microflora in cultured soil. The disease recurred in soil collected from a submerged paddy field where rice was cropped once after infestation of soil with the pathogen. The pathogen in the soil inoculated with the pathogenic Streptomyces sp. was not inactivated completely by continuous submerging treatment for 150 days. Existence of various microorganisms in nonsubmerged soils seemed important for the inactivation of this pathogen.

Explanation of Plates

Plate I

Optical micrographs of the morphogenesis in several stages of growing process from spore germination to sporulation and pseudosporangium formation of the pathogenic Streptomyces sp. causing root tumor of melon on the yeast-starch agar medium at 28 ℃. Bars represent 50 ㎛.

1. Spores in 12 hr after incubation. Most spores germinated and a little of germ tubes were branching.
2. Twenty-four hr after incubation. Germinated and branched hyphae have been starting to formation of substratal mycelia.
3. Seventy-two hr after incubation. Aerial mycelia were formed on the colonies of substratal mycelia, and these tips formed itself into hook or open-loop shapes.
4. Five days after incubation. Spore chains were produced in some of the aerial mycelia by appearance of septa.
5. Seven days after incubation. Pseudosporangia were formed in spore chains.
6. Fourteen days after incubation. Structures of pseudosporangia, spore chains and masses of mucoid-like substances were overspread on the colonies of substratal mycelia.

Plate II, III

Scanning electron micrographs of the morphogenesis in several stages of growing process from spore germination to sporulation and pseudosporangium formation of the pathogenic Streptomyces sp. causing root tumor of melon on the yeast-starch agar medium at 28 ℃. Bars represent 1 ㎛ (7-9,16) and 5 ㎛ (10-15,17-20).

7. Inoculated spores on the culture medium.
8. Germinating spore incubated for 3 hr. Germ tube was emerging from one side of spore.
9. Germinated spore in 3 hr after incubation. Trace of spore sheath was observed between spore and germ tube.
10. Twelve hr after incubation. Germinated hyphae have been starting to branch.
11. Germination from three places points of the spore incubated for 12 hr.

12. Twenty-four hr after incubation. Colonies of substratal mycelia were consisted of multiplied hyphae, and some of the growing tips of these hyphae were become swelling.
13. Forty-eight hr after incubation. Hook shaped aerial hyphae were formed on the colonies of substratal mycelia.
14. Open-loop or hook shaped aerial mycelia in 72 hr after incubation.
15. Five days after incubation. Spore chains were begining to produce from the aerial mycelia by formation of septa.
16. Retinaculum-Apertum (RA) type spore chain in 5 days after incubation.
17. Pseudosporangia formed from spore chains in 7 days after incubation.
18. Matured pseudosporangia in 10 days after incubation. Some of the pseudosporangia were covered with mucoid-like substances.
19. Pseudosporangia, spore masses and spore chains in 14 days after incubation.
20. Fourteen days after incubation. Matured pseudosporangia and spore masses or spore chains were observed on the colonies of substratal mycelia overspread with mucoid-like substances.

Plate Ⅳ, Ⅴ

Cross sections of healthy root of melon plant stained with thionin-orange G and the diseased root tumor appeared on the plants cultured in natural infested soil. Bars represent 500 ㎛. Arrows indicate the places at where the pathogens exist. Abbreviations; R:root, T:tumor, BR:branched root, EP:epidermis, CO:cortex, EN:endodermis, PE:pericycle, VB:vascular bundle, SG:starch grain, HP:hyperplasia tissue, HT:hypertrophy tissue, VS:vascular bundle structure.

1. Healthy root. Tissues consisted of cells which were arranged systematically around the vascular bundle, and these cells contained starch grains.
2. Branching part of healthy root. Branched root derived from pericycle.

3,4. Diseased root tumor. Tumor tissues contained vascular bundle structure, and its surroundings tissues were hyperplasia.

5-8. Continuous cross sections of root tumor containing branching part of root. Tumor tissues were composed of inner hyperplasia parenchyma (hyperplasia one around vascular bundle structure) and outer hypertrophy one.

Plate Ⅵ,Ⅶ

Scanning electron micrographs of the root of melon plant infected artificially and cultured on agar medium in test tube. Bars represent 500 μm (9-11,13,15, 16,19), 50 μm (12,17,18,20) and 5 μm (14). Arrows indicate the pathogen. Abbreviations; R:root, T:tumor, BR:branched root, RU:rupture, RH:root hair, EP:epidermis, CO:cortex, EN:endodermis, VB:vascular bundle, HP:hyperplasia tissue, HT:hypertrophy tissue, VS:vascular bundle structure.

9,10. Diseased root tumor. Root tumors were formed at branching parts of root.

11. Rupture caused by rooting in the diseased root. Proliferated mycelia of the pathogen were recognized on the surface of epidermis and endodermis of root (epidermis of tumor).

12. Pathogen on the surface of endodermis appeared in the rupture.

13. Exposed hypertrophying parenchyma cells on the surface of tumor. There was little pathogen on these surfaces.

14. Surface of root aparted from tumor tissues in the diseased melon. Quantity of the pathogen was very little.

15,16. Cross cutting faces of tumor tissues in the diseased root. Tumor tissues were consisted of hyperplasia tissues which were derived from parenchyma cells in endodermis, pericycle and inner cortex or around vascular bundle structure and hypertrophied tissues originated from outer layer of cortex cells.

17. Cross cutting face of outer layer tissue of tumor. Tissue had loosely binding hypertrophied cells.

18. Epidermis of tumor. Proliferated pathogen could be found in intercellular space of epidermis.

19. Branching part of healthy root. The rupture was formed by rooting.

20. Cross cutting face of healthy root . Normal cells were arranged systematically.

Plate Ⅷ, Ⅸ

Scanning electron micrographs of the diseased root of melon plant cultured in artificially infested soil in test tube. Bars represent 500 μm (21,25-27, 31), 50 μm (23,28,30,32) and 5 μm (22,24,29). Arrows indicate the pathogen. Abbreviations; R:root, T:tumor, BR:branched root, RH:root hair, EP:epidermis, CO:cortex, VB:vascular bundle, HP:hyperplasia tissue, HT:hypertrophy tissue, VS:vascular bundle structure.

21,22. Surface of epidermis of tumor (from endodermis of root). Mycelia and spores of the pathogen were recognized but the quantity was less than that of the above experiment (11,12).

23. Surface of outer layer tissue of tumor. Epidermis of tumor was torn with enlarging tumor, then surface of the tumor became to be coarse structure with appearance of warty hypertrophied cells.

24. Surface of root aparted from tumor tissues in the diseased melon. The pathogen was very little.

25,26. Cross cutting faces of tumor tissues. Tumor contained part of branched root or vascular bundle structure. Besides, tumor tissue was composed of hyperplasia tissues made from parenchyma cells of endodermis, pericycle and inner cortex or around vascular bundle structure. Hypertrophied tissues of outer layer, and these warty hypertrophied cells were exposed on the surface of enlarging tumor.

27. Outer layer tissue of tumor. Tissues of epidermis and cortex of root were broken by enlarging tumor tissue.

28,29. Epidermis of tumor. Proliferated pathogen could be detected only in intercellular space of epidermis or 1-2 outer layer tissues of tumor.

30. Cross cutting face of healthy root.

31. Cross cutting face of branching part of healthy root.

32. Epidermis and cortex of healthy root.

Plate I

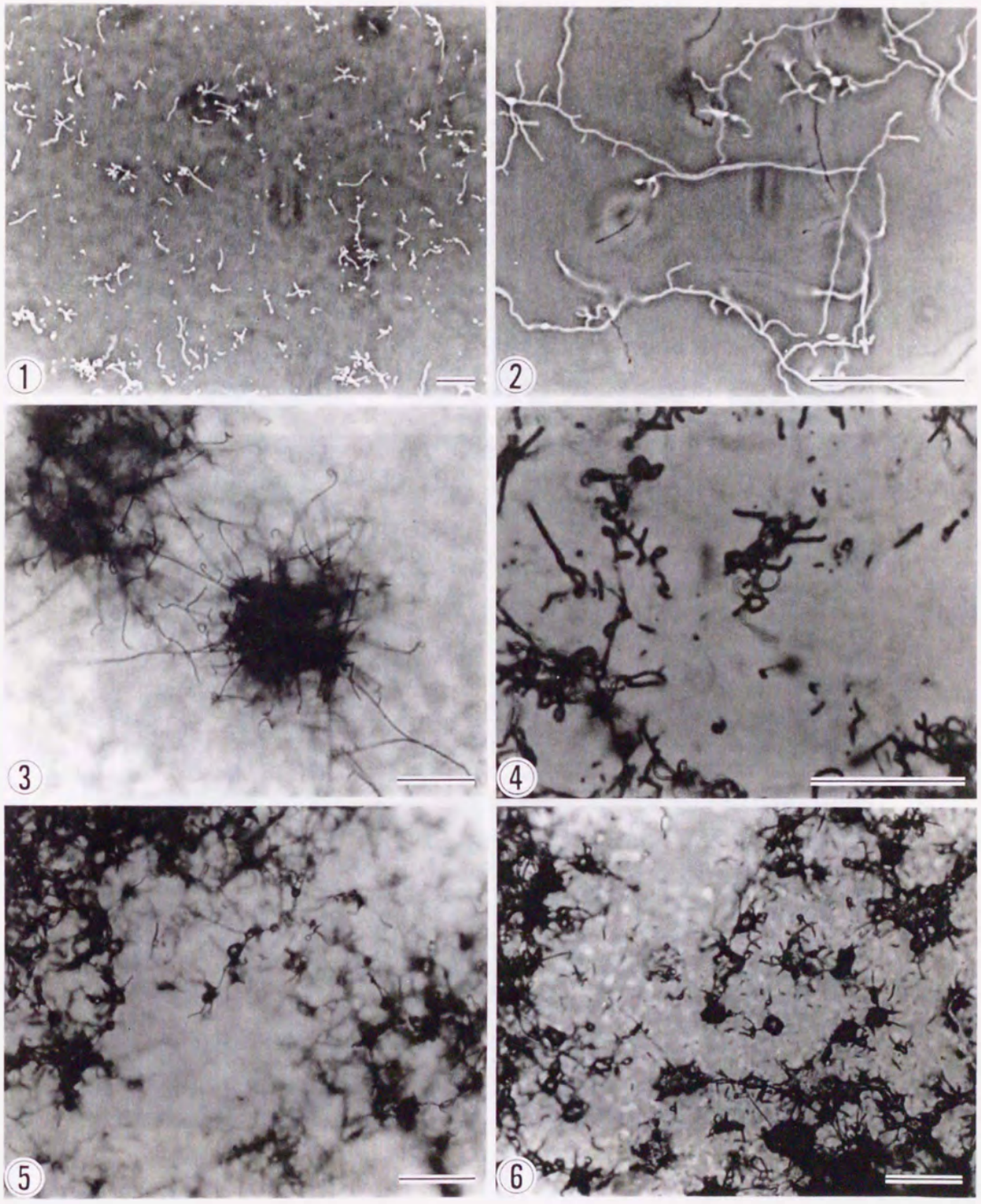


Plate II

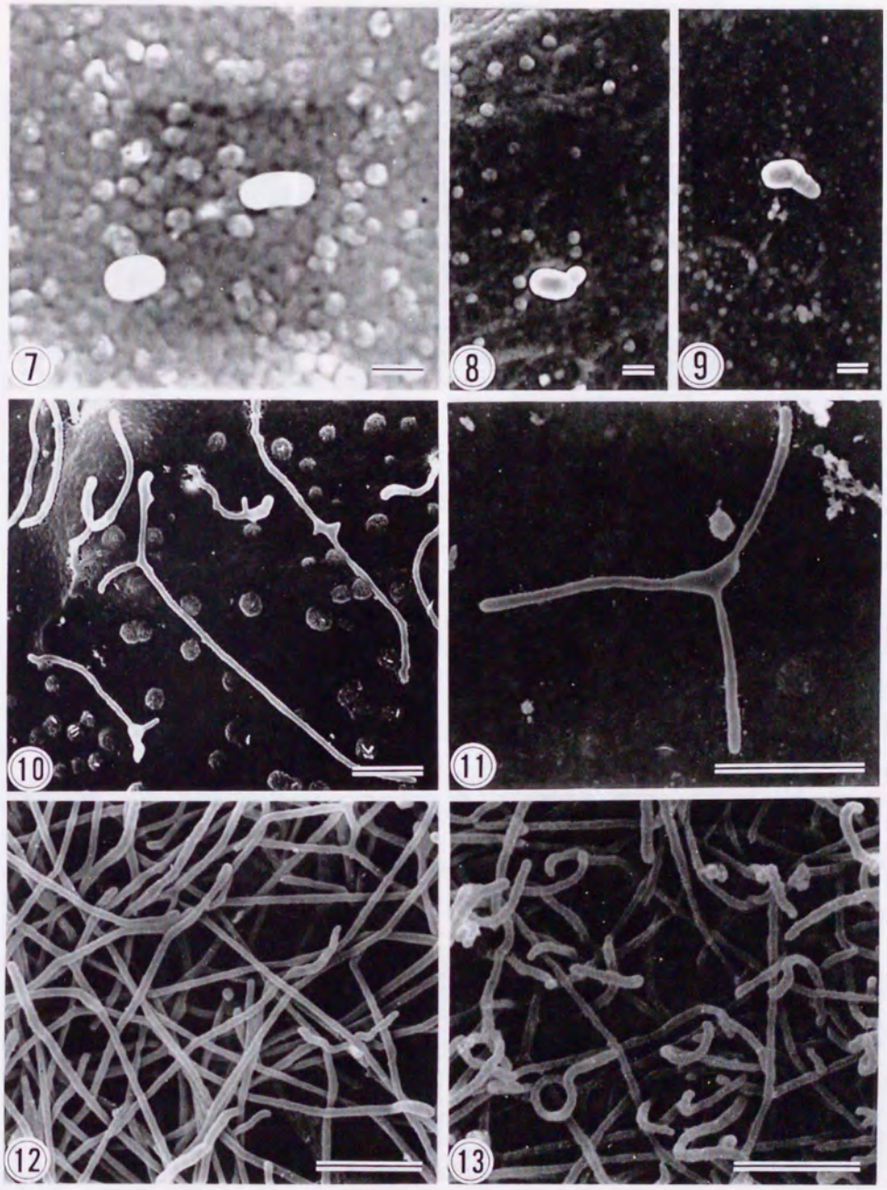


Plate Ⅲ

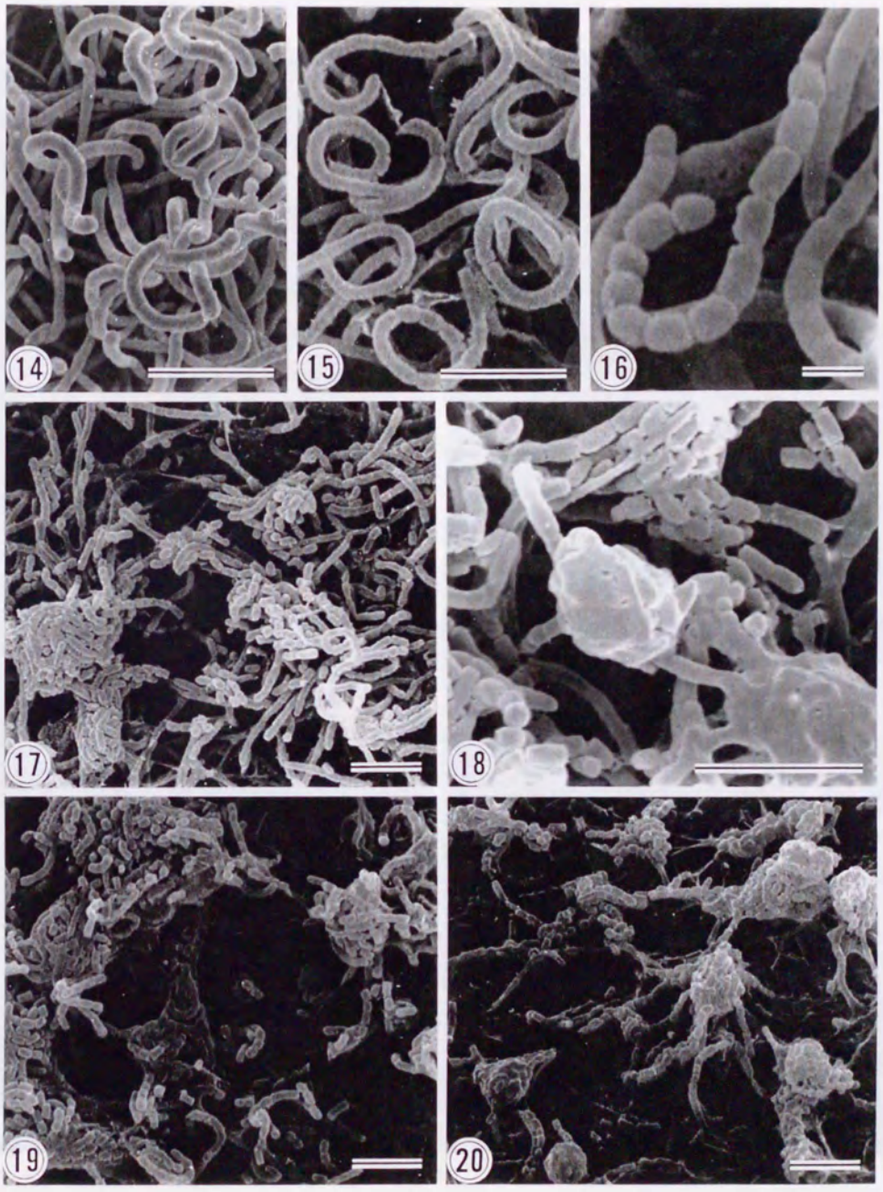


Plate Ⅳ

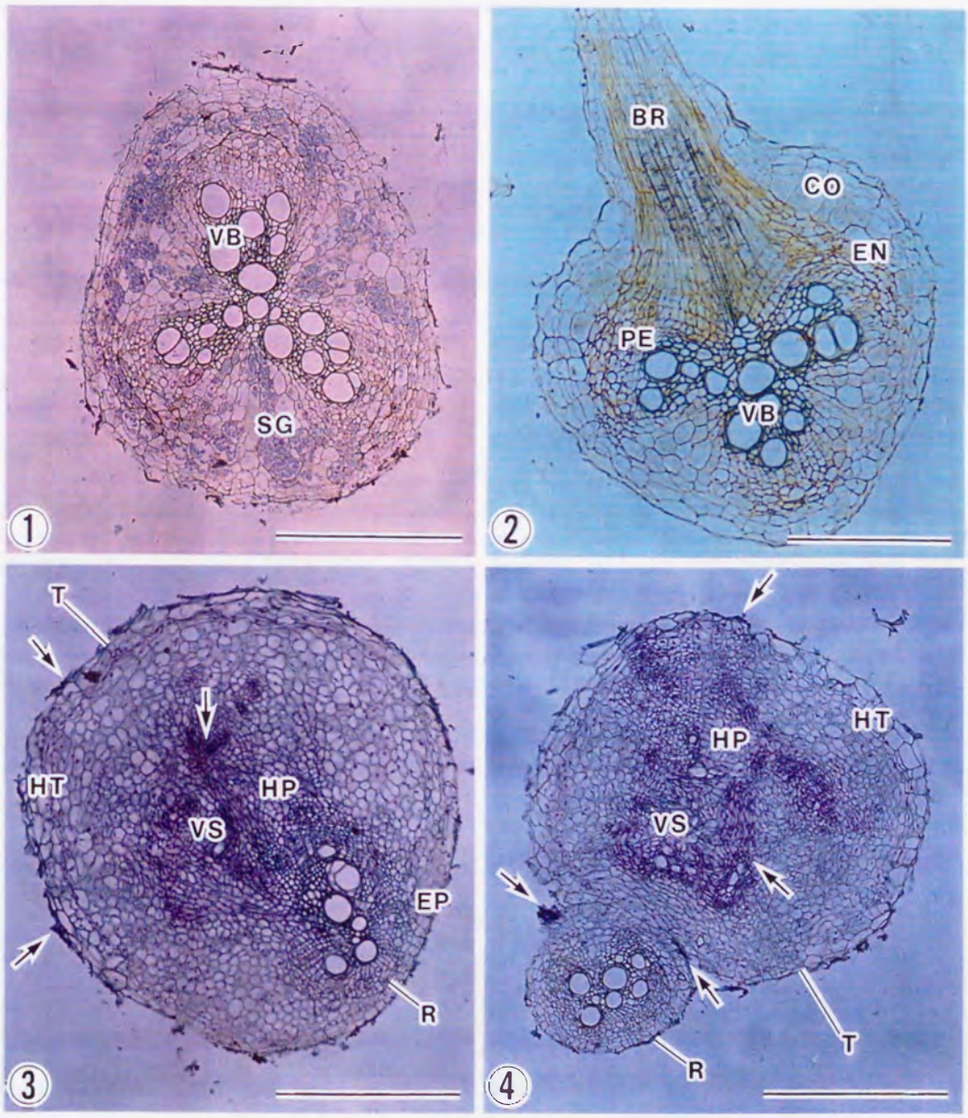


Plate V

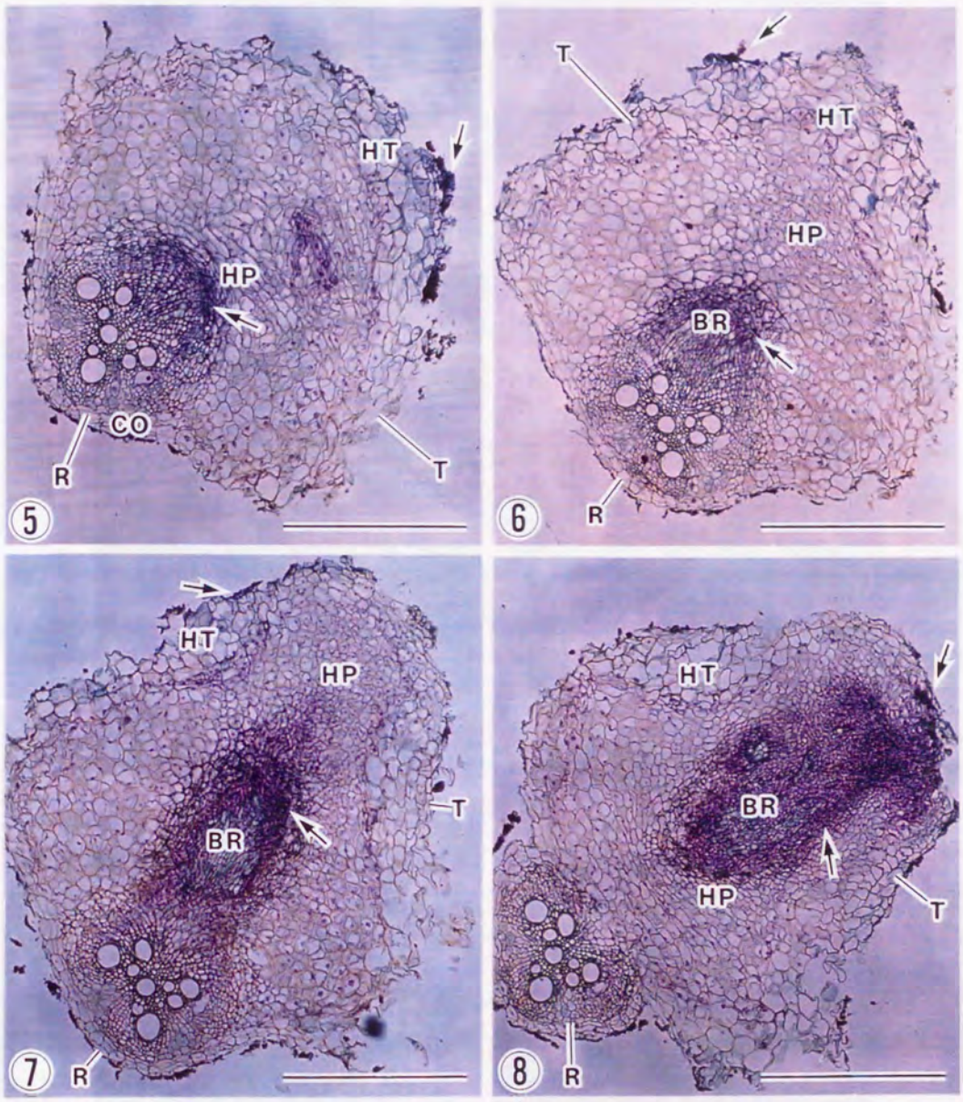


Plate VI

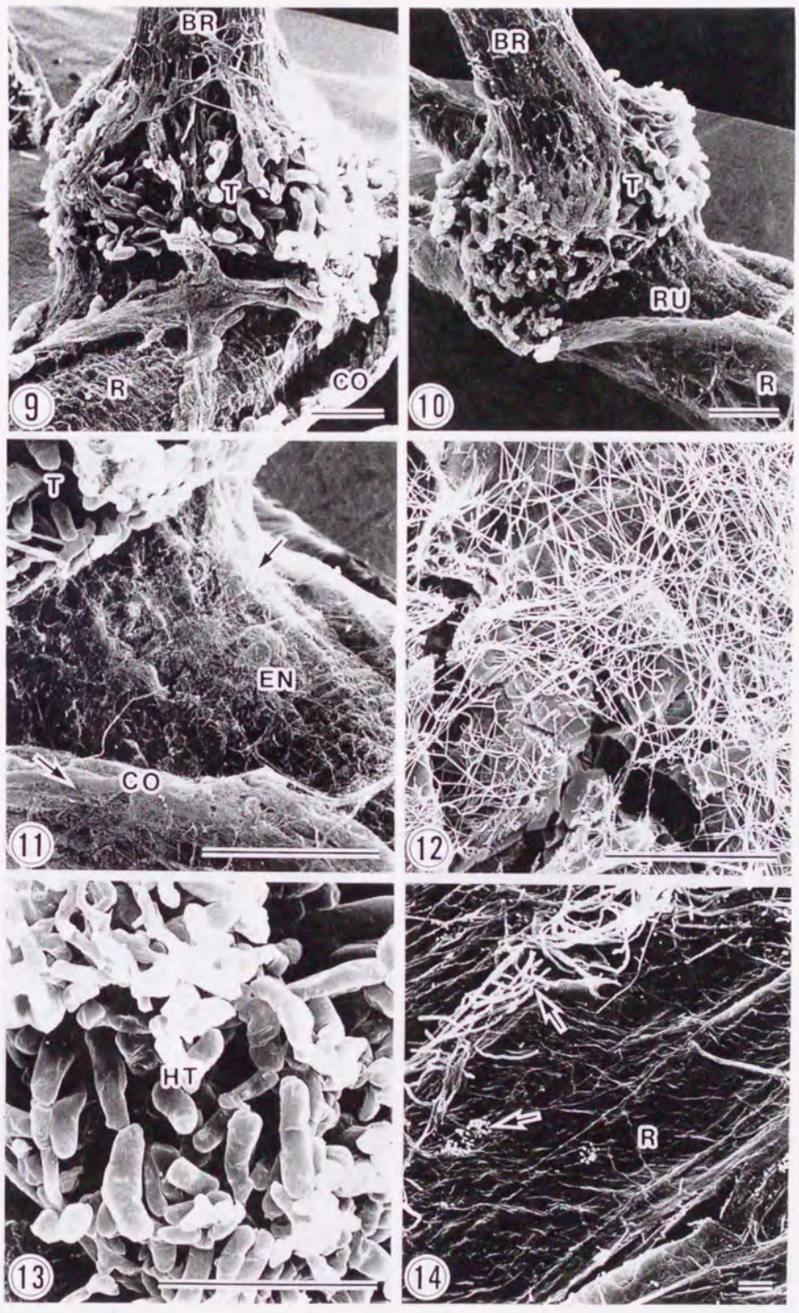
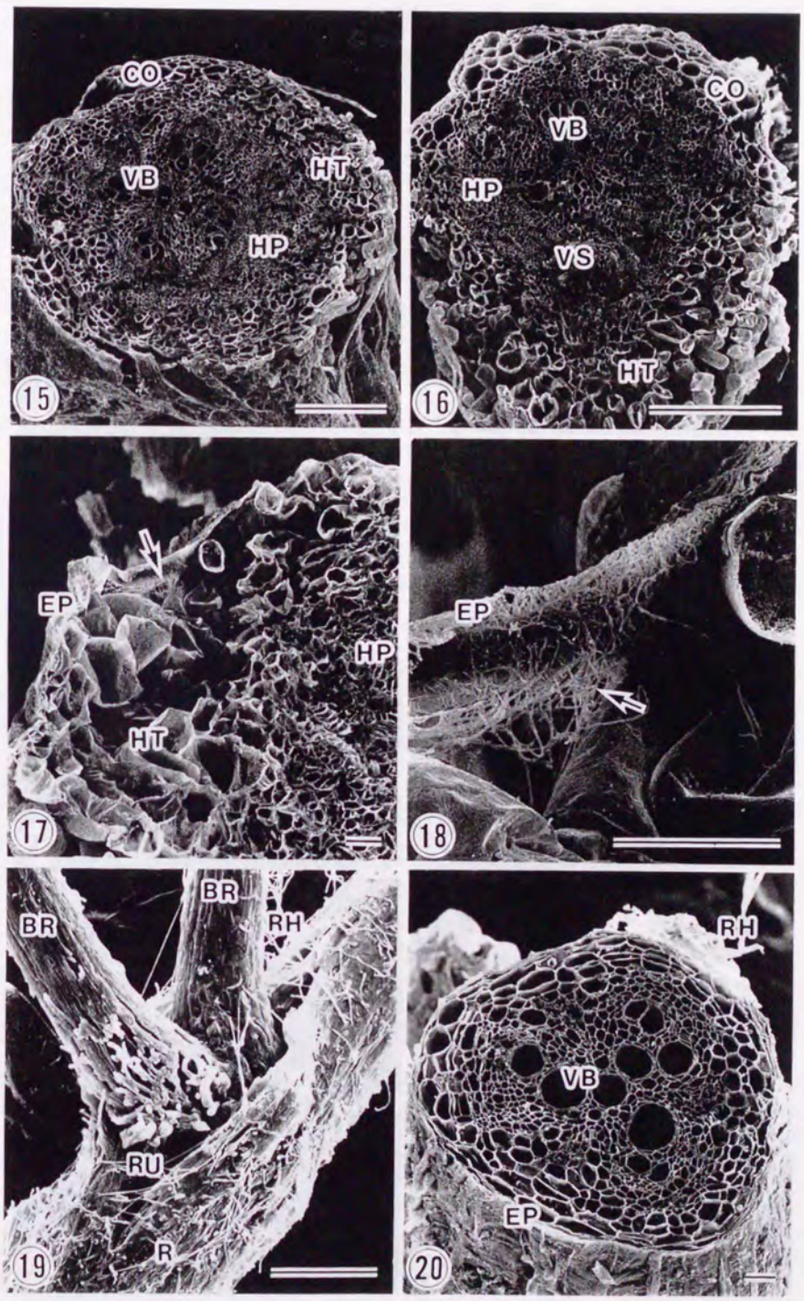


Plate Ⅶ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VB
HT
HP
15
CO
VB
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VS
HT
16
EP
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BR
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RH
RU
R
19
RH
VB
EP
20

Plate Ⅷ

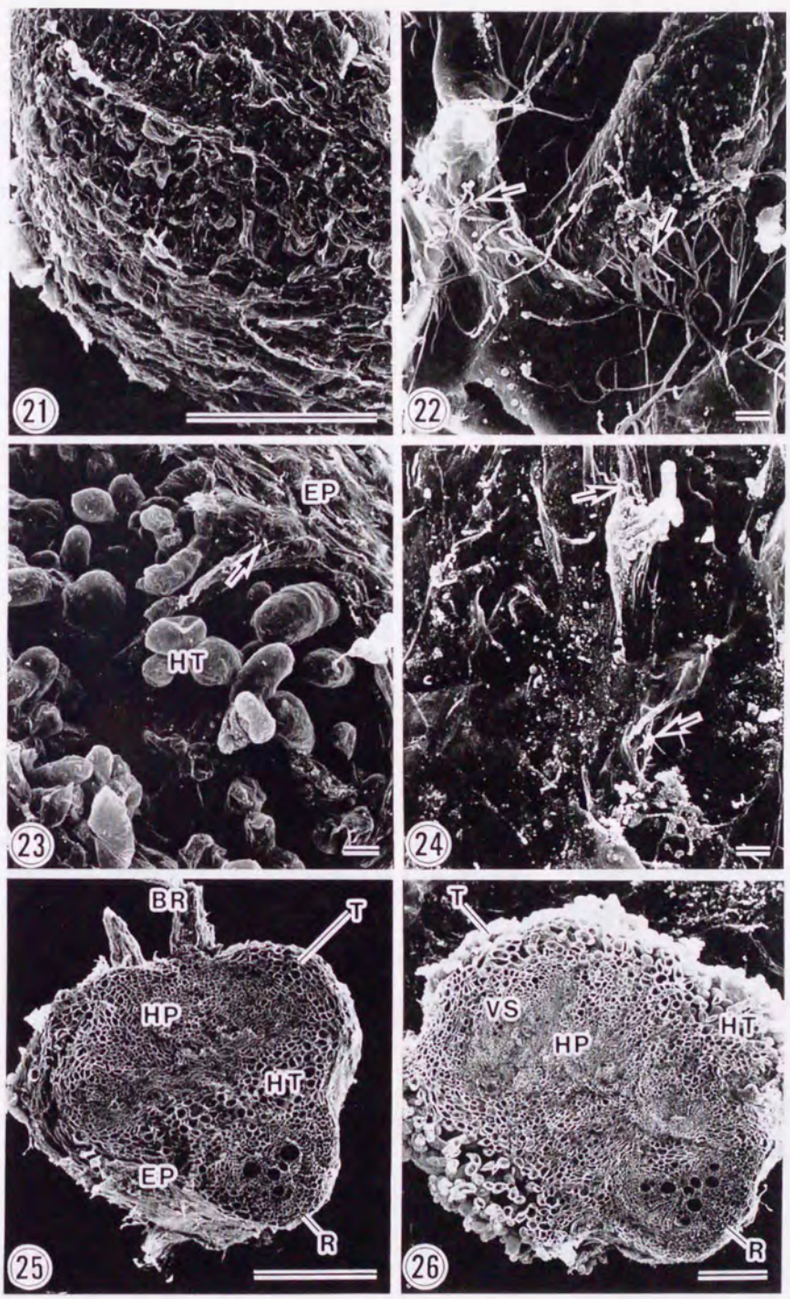
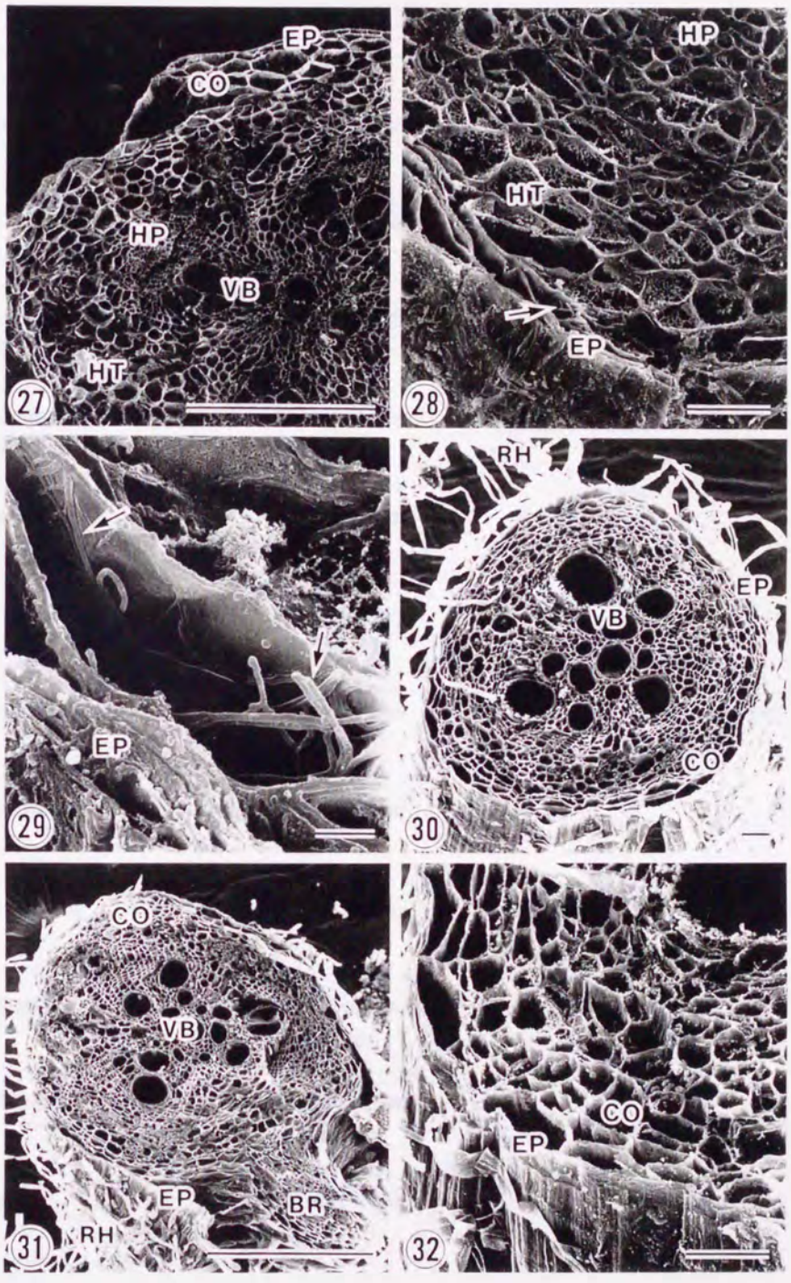


Plate Ⅸ


EP
CO
HP
VB
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27
HP
HT
EP
28
EP
29
RH
VB
EP
CO
30
CO
VB
EP
BR
RH
31
CO
EP
32

Attached list 1-1. Cultural characteristics of the actinomycete isolate B-7-2, pathogen of root tumor of melon, on various media

Medium[a]	Substratal mycelium		Aerial mycelium		Soluble pigment[d] (Color)
	Growth[b]	Color[c]	Growth[b]	Color[c]	
Yeast extract-malt extract agar(ISP No.2)	+++	Pale yellowish brown (2.5Y7/6)	+++	Gray (10YR7/1)	-
Oatmeal agar (ISP No.3)	+++	Pale yellowish brown (5Y8/4)	+++	Brownish gray (10YR5/2)	+(Yellowish brown)
Inorganic salts-starch agar(ISP No.4)	+++	Milky brown (2.5Y6/3)	+++	Brownish gray (10YR6/1)	-
Glycerol-asparagine agar(ISP No.5)	++	Milky white (5Y9/3)	-		-
Tyrosine agar (ISP No.7)	++	Milky brown (10YR8/4)	-		+(Yellowish brown)
Sucrose-nitrate agar	++	Milky brown (2.5Y8/4)	-		+(Yellowish brown)
Glucose-asparagine agar	+	Milky brown (10YR8/2)	+	Brownish gray (10YR6/2)	-
Glycerol-nitrate agar	++	Milky white (5Y9/2)	-		±(Faint yellowish brown)
Calcium malate agar	++	Milky white (5Y9/2)	-		-
Starch agar	++	Milky brown (2.5Y6/3)	+	Gray (5Y7/2)	-
Nutrient agar	+	Milky brown (2.5Y8/4)	-		-
Water agar	+	Colorlessness	+	Whitish gray (10YR8/1)	-

a) ISP:International Streptomyces Project medium[123].
b) -:no growth, ±:very poor, +:poor, ++:moderate, +++:good.
c) Symbol in parenthesis is color code in standard color chart conformed to JIS Z 8721[44].
d) -:none, ±:scant, +:pigment.

Attached list 1-2. Cultural characteristics of the actinomycete isolate OTP-3-1, pathogen of root tumor of melon, on various media

Medium[a]	Substratal mycelium		Aerial mycelium		Soluble pigment[d] (Color)
	Growth[b]	Color[c]	Growth[b]	Color[c]	
Yeast extract-malt extract agar(ISP No.2)	+++	Milky brown (10YR6/4)	++	Gray (10YR7/1)	-
Oatmeal agar (ISP No.3)	+++	Milky brown (2.5Y8/3)	++	Brownish gray (10YR5/2)	+(Yellowish brown)
Inorganic salts-starch agar(ISP No.4)	+++	Milky brown (2.5Y6/3)	++	Gray (10YR7/1)	-
Glycerol-asparagine agar(ISP No.5)	++	Milky white (5Y9/3)	-		-
Tyrosine agar (ISP No.7)	+++	Milky brown (10YR8/4)	±	White (9/N)	+(Yellowish brown)
Sucrose-nitrate agar	+++	Milky brown (10YR8/4)	±	White (9/N)	+(Yellowsih brown)
Glucose-asparagine agar	+	Milky white (2.5Y9/2)	-		-
Glycerol-nitrate agar	+++	Milky brown (10YR7/4)	-		+(Yellowish brown)
Calcium malate agar	++	Milky white (5Y9/2)	-		-
Starch agar	++	Milky brown (2.5Y8/3)	-		-
Nutrient agar	+	Milky brown (2.5Y8/4)	-		-
Water agar	+	Colorlessness	+	Whitish gray (10YR8/1)	-

a),b),c),d) See Attached list 1-1.

Attached list 1-3. Cultural characteristics of the actinomycete isolate OTP-4-2, pathogen of root tumor of melon, on various media

Medium[a]	Substratal mycelium		Aerial mycelium		Soluble pigment[d] (Color)
	Growth[b]	Color[c]	Growth[b]	Color[c]	
Yeast extract-malt extract agar(ISP No.2)	+++	Milky brown (10YR6/4)	+++	Gray (10YR7/1)	-
Oatmeal agar (ISP No.3)	+++	Milky brown (2.5Y8/3)	+++	Brownish gray (10YR5/2)	+(Yellowish brown)
Inorganic salts-starch agar(ISP No.4)	+++	Milky brown (2.5Y6/3)	+++	Brownish gray (10YR4/2)	-
Glycerol-asparagine agar(ISP No.5)	++	Milky brown (2.5Y7/4)	-		-
Tyrosine agar (ISP No.7)	+++	Milky brown (10YR8/4)	±	Whitish gray (10YR8/1)	+(Yellowish brown)
Sucrose-nitrate agar	+++	Milky brown (10YR8/4)	±	White (9/N)	+(Yellowish brown)
Glucose-asparagine agar	+	Milky brown (10YR6/3)	+	Gray (10YR7/1)	-
Glycerol-nitrate agar	+++	Milky brown (10YR7/4)	±	White (9/N)	+(Yellowish brown)
Calcium malate agar	++	Milky white (5Y9/2)	-		-
Starch agar	++	Milky brown (2.5Y8/3)	-		-
Nutrient agar	+	Milky brown (2.5Y8/4)	-		-
Water agar	+	Colorlessness	+	Whitish gray (10YR8/1)	-

a),b),c),d) See Attached list 1-1.

Attached list 1-4. Cultural characteristics of the actinomycete isolate KM-1-1, pathogen of root tumor of melon, on various media

Medium[a]	Substratal mycelium		Aerial mycelium		Soluble pigment[d] (Color)
	Growth[b]	Color[c]	Growth[b]	Color[c]	
Yeast extract-malt extract agar(ISP No.2)	+++	Pale yellowish brown (2.5Y6/6)	+++	Brownish gray (5YR3/1)	-
Oatmeal agar (ISP No.3)	+++	Milky brown (2.5Y8/3)	+++	Dark brown (10YR3/1)	+(Yellowish brown)
Inorganic salts-starch agar(ISP No.4)	+++	Milky brown (2.5Y6/3)	+++	Brownish gray (10YR6/1)	-
Glycerol-asparagine agar(ISP No.5)	++	Milky white (5Y9/3)	-		-
Tyrosine agar (ISP No.7)	+++	Milky brown (10YR8/4)	++	Whitish gray (10YR8/1)	+(Yellowish brown)
Sucrose-nitrate agar	+++	Milky brown (2.5Y8/4)	±	White (9/N)	+(Yellowish brown)
Glucose-asparagine agar	+	Milky brown (10YR6/3)	+	Brownish gray (10YR6/2)	-
Glycerol-nitrate agar	++	Milky brown (10YR7/4)	+	Whitish gray (10YR8/1)	+(Yellowish brown)
Calcium malate agar	++	Milky white (5Y9/2)	-		-
Starch agar	++	Milky brown (2.5Y7/3)	±	Gray (5Y7/2)	-
Nutrient agar	+	Milky brown (2.5Y8/4)	-		-
Water agar	+	Colorlessness	+	Whitish gray (10YR8/1)	-

a),b),c),d) See Attached list 1-1.

Attached list 1-5. Cultural characteristics of the actinomycete isolate KM-2-1, pathogen of root tumor of melon, on various media

Medium[a]	Substratal mycelium		Aerial mycelium		Soluble pigment[d] (Color)
	Growth[b]	Color[c]	Growth[b]	Color[c]	
Yeast extract-malt extract agar(ISP No.2)	+++	Pale yellowish brown (2.5Y6/6)	+++	Brownish gray (5YR3/1)	-
Oatmeal agar (ISP No.3)	+++	Milky brown (2.5Y8/3)	+++	Gray (10YR8/2)	+(Yellowish brown)
Inorganic salts-starch agar(ISP No.4)	+++	Milky brown (2.5Y6/3)	+++	Gray (10YR5/1)	-
Glycerol-asparagin agar(ISP No.5)	++	Milky white (5Y9/3)	-		-
Tyrosine agar (ISP No.7)	+++	Milky brown (10YR8/4)	+	Whitish gray (10YR8/1)	+(Yellowish brown)
Sucrose-nitrate agar	++	Milky brown (2.5Y8/4)	±	White (9/N)	±(Faint yellowish brown
Glucose-asparagine agar	+	Milky white (2.5Y9/2)	-		-
Glycerol-nitrate agar	++	Milky brown (10YR7/4)	-		±(Faint yellowish brown)
Calcium malate agar	++	Milky white (5Y9/2)	-		-
Starch agar	++	Milky brown (2.5Y7/3)	±	Gray (5Y7/2)	-
Nutrient agar	+	Milky brown (2.5Y8/4)	-		-
Water agar	+	Colorlessness	+	Whitish gray (10YR8/1)	-

a),b),c),d) See Attached list 1-1.

Attached list 1-6. Cultural characteristics of the actinomycete isolate Cu-2-1, pathogen of root tumor of melon, on various media

Medium[a]	Substratal mycelium		Aerial mycelium		Soluble pigment[d] (Color)
	Growth[b]	Color[c]	Growth[b]	Color[c]	
Yeast extract-malt extract agar(ISP No.2)	+++	Pale yellowish brown (2.5Y7/6)	+	Whitish gray (5YR8/1)	-
Oatmeal agar (ISP No.3)	+++	Milky brown (2.5Y8/3)	+++	Gray (10YR5/1)	+(Yellowish brown)
Inorganic salts-starch agar(ISP No.4)	+++	Milky brown (2.5Y6/3)	+++	Brownish gray (10YR6/1)	-
Glycerol-asparagine agar(ISP No.5)	++	Milky white (5Y9/3)	-		-
Tyrosine agar (ISP No.7)	++	Milky brown (10YR8/4)	±	Whitish gray (10YR8/1)	+(Yellowish brown)
Sucrose-nitrate agar	++	Milky brown (2.5Y8/4)	±	White (9/N)	+(Yellowish brown)
Glucose-asparagine agar	+	Milky white (2.5Y9/2)	-		-
Glycerol-nitrate agar	++	Milky white (2.5Y9/2)	-		±(Faint yellowish brown)
Calcium malate agar	++	Milky white (5Y9/2)	-		-
Starch agar	++	Milky brown (2.5Y7/3)	+	White (5Y9/1)	-
Nutrient agar	+	Milky brown (2.5Y8/4)	-		-
Water agar	+	Colorlessness	+	Whitish gray (10YR8/1)	-

a),b),c),d) See Attached list 1-1.

Attached list 1-7. Cultural characteristics of the actinomycete isolate Cu-3-1, pathogen of root tumor of melon, on various media

Medium[a]	Substratal mycelium		Aerial mycelium		Soluble pigment[d] (Color)
	Growth[b]	Color[c]	Growth[b]	Color[c]	
Yeast extract-malt extract agar(ISP No.2)	+++	Milky brown (10YR6/4)	+	Whitish gray (5YR8/1)	-
Oatmeal agar (ISP No.3)	+++	Milky brown (2.5Y8/3)	++	Gray (10YR8/1)	+(Yellowish brown)
Inorganic salts-starch agar(ISP No.4)	+++	Milky brown (2.5Y6/3)	++	Gray (10YR7/1)	-
Glycerol-asparagine agar(ISP No.5)	++	Milky white (5Y9/3)	-		-
Tyrosine agar (ISP No.7)	++	Milky brown (10YR8/4)	-		+(Yellowish brown)
Sucrose-nitrate agar	++	Milky brown (2.5Y8/4)	±	White (9/N)	+(Yellowish brown)
Glucose-asparagine agar	+	Milky brown (10YR8/2)	+	Brownish gray (10YR6/2)	-
Glycerol-nitrate agar	++	Milky brown (10YR7/4)	-		±(Faint yellowish brown)
Calcium malate agar	++	Milky white (5Y9/2)	-		-
Starch agar	++	Milky brown (2.5Y7/3)	±	Gray (10YR7/2)	-
Nutrient agar	+	Milky brown (2.5Y8/4)	-		-
Water agar	+	Colorlessness	+	Whitish gray (10YR8/1)	-

a),b),c),d) See Attached list 1-1.

Attached list 2-1. Pathogenicity of isolate B-9-1 of the pathogenic Streptomyces sp. against gourd family plants by pouring and mixing inoculation (test-I)

Species	Cultivar	Percentage of diseased plants	Disease severity	Number of tumors per diseased plant
Melon	Kenkyaku(Stock)	100	75.0	70.0
	Kosack	100	82.1	79.2
	Prince	100	58.3	47.4
	Kyoei(Stock)	100	58.3	40.9
	Amusu	100	89.2	100.0
	Homerunstar	100	46.4	25.2
	Nanshoarusu kashukei	100	42.9	34.0
	Andesu	100	42.9	25.1
	Michizure(Stock)	100	42.9	25.4
	Arususeinunatu I	100	53.6	36.0
Cucumber	Asomidori	100	67.9	54.5
	Hijiri	100	57.1	41.4
	Suyo	100	28.6	14.5
	Honmyo	100	57.1	48.5
	Kagaaonagafushinari	100	46.4	31.4
Pumpkin	Benkei(Stock)	71.4	17.1	6.2
	Hayato	57.1	14.3	5.1
	Ebisu	100	35.7	17.6
	Tetsukabuto	100	25.0	6.9
	Kurodane(Stock)	100	25.0	3.4
	Shintosa No.1(Stock)	100	32.1	14.4
Watermelon	Tenryu No.2	100	57.1	47.2
	Fujihikari	100	35.8	18.8
	Shimaomacks KE	100	42.9	29.5
Bottle gourd	Sakigake(Stock)	100	35.7	20.6
	Kachidoki(Stock)	100	67.9	64.1
Oriental pickling melon	Yokauri	100	50.0	34.7
	Nagasakitsukeuri	100	39.3	21.0
Balsam pear	Satsumaonagareishi	100	35.0	22.0

Attached list 2-2. Pathogenicity of isolate B-9-1 of the pathogenic *Streptomyces* sp. against nightshade family plants by pouring and mixing inoculation (test-I)

Species	Cultivar	Percentage of diseased plants	Disease severity	Number of tumors per diseased plant
Tomato	Yuyake A	100	35.7	28.4
	BF Okitsu No.101	100	25.0	11.1
	Zuiken	100	28.6	15.7
	Oomiya	100	25.0	12.3
	Petittomato	100	39.3	22.1
Eggplant	Kokuyo	28.6	7.1	3.5
	Kuronishiki No.2	28.6	7.1	2.5
Sweet pepper (Bell type)	Ace	25.0	6.3	3.0
	Mansaku	42.9	10.7	3.0
Sweet pepper (Shishito type)	Wakato	0	0	0
		0	0	0
Potato	Dejima	0	0	0
	Mayqueen	0	0	0

Attached list 2-3. Pathogenicity of isolate B-9-1 of the pathogenic *Streptomyces* sp. against mustard family plants by pouring and mixing inoculation (test-I)

Species	Cultivar	Percentage of diseased plants	Disease severity	Number of tumors per diseased plant
Cabbage	Fujiwase	0	0	0
Cauliflower	Nozakiwase	100	32.1	18.5
Chinese cabbage	Muso	0	0	0
	Shin No.6	0	0	0
Japanese radish	Natuminowase No.3	0	0	0
Turnip	Taibyohikari	0	0	0

Attached list 2-4. Pathogenicity of isolate B-9-1 of the pathogenic Streptomyces sp. against other family plants by pouring and mixing inoculation (test- I)

Family	Species	Cultivar	Percentage of diseased plants	Disease severity	Number of tumors per diseased plant
Goosefoot	Spinach	Atorasu	57.1	14.2	2.8
	Chard	Shiroguki	100	35.7	17.5
Composite	Lettuce	Greatrekusu No.54	100	25.0	5.8
		Sakuramento	85.7	21.4	6.0
	Garland chrysanthemum	Oobashingiku	0	0	0
	Edible burdock	Watanabewase	28.6	7.1	3.0
	Sunflower	Dairinhimawari	100	58.3	43.2
Parsley	Carrot	Shinkurodagosun	0	0	0
		Oonaga	0	0	0
	Parse	Maruta	0	0	0
	Japanese hornwort	Shiroguki	0	0	0
Lily	Welsh onion	Kintyonegi	0	0	0
	Onion	Unzengokuwasekitamanegi No.1	0	0	0
	Chinese chive	Hirohaba	0	0	0
Mallow	Okra	Betterfive	0	0	0
Grass	Rice	Nihonbare	0	0	0
	Corn	Honeybantam	0	0	0

Attached list 3-1. Pathogenicity of isolate OTP-3-1 of the pathogenic Streptomyces sp. against gourd family plants by pouring and mixing inoculation (test- I)

Species	Cultivar	Percentage of diseased plants	Disease severity	Number of tumors per diseased plant
Melon	Kenkyaku(Stock)	100	39.3	22.3
	Kosack	100	28.6	11.8
	Prince	100	25.0	9.5
	Kyoei(Stock)	57.1	17.9	12.8
	Amusu	83.3	29.2	7.4
	Homerunstar	57.1	14.3	2.8
	Nanshoarusu kashukei	42.9	10.7	3.6
	Andesu	100	39.3	19.2
	Michizure(Stock)	71.4	17.9	2.2
	Arususeinunatu I	85.7	17.9	2.2
Cucumber	Asomidori	100	39.3	21.7
	Hijiri	100	30.0	10.6
	Suyo	100	35.7	20.9
	Honmyo	100	39.3	25.0
	Kagaaonagafushinari	100	28.6	12.1
Pumpkin	Benkei(Stock)	0	0	0
	Hayato	0	0	0
	Ebisu	14.3	3.6	1.0
	Tetsukabuto	57.1	14.3	1.8
	Kurodane(Stock)	71.4	17.9	2.4
	Shintosa No.1(Stock)	57.1	14.2	4.3
Watermelon	Tenryu No.2	0	0	0
	Fujihikari	0	0	0
	Shimaomacks KE	14.3	3.6	2.0
Bottle gourd	Sakigake(Stock)	100	25.0	9.7
	Kachidoki(Stock)	100	33.3	20.0
Oriental pickling melon	Yokauri	100	25.0	8.5
	Nagasakitsukeuri	100	25.0	5.7
Balsam pear	Satsumaonagareishi	28.6	7.1	2.5

Attached list 3-2. Pathogenicity of isolate OTP-3-1 of the pathogenic *Streptomyces* sp. against nightshade family plants by pouring and mixing inoculation (test-I)

Species	Cultivar	Percentage of diseased plants	Disease severity	Number of tumors per diseased plant
Tomato	Yuyake A	85.7	21.4	1.8
	BF Okitsu No.101	85.7	21.4	4.0
	Zuiken	100	32.1	13.5
	Oomiya	85.7	21.4	3.0
	Petittomato	85.7	21.4	5.2
Eggplant	Kokuyo	14.3	3.5	1.0
	Kuronishiki No.2	0	0	0
Sweet pepper (Bell type)	Ace	14.3	3.6	2.0
	Mansaku	28.6	7.1	1.0
Sweet pepper (Shishito type)	Wakato	0	0	0
Potato	Dejima	0	0	0

Attached list 3-3. Pathogenicity of isolate OTP-3-1 of the pathogenic *Streptomyces* sp. against mustard family plants by pouring and mixing inoculation (test-I)

Species	Cultivar	Percentage of diseased plants	Disease severity	Number of tumors per diseased plant
Cabbage	Fujiwase	0	0	0
Cauliflower	Nozakiwase	14.3	3.5	1.0
Chinese cabbage	Muso	0	0	0
Japanese radish	Natuminowase No.3	0	0	0
Turnip	Taibyohikari	0	0	0

Attached list 3-4. Pathogenicity of isolate OTP-3-1 of the pathogenic *Streptomyces* sp. against other family plants by pouring and mixing inoculation (test-Ⅰ)

Family	Species	Cultivar	Percentage of diseased plants	Disease severity	Number of tumors per diseased plant
Goosefoot	Spinach	Atorasu	28.6	7.1	2.0
	Chard	Shiroguki	100	25.0	5.3
Composite	Lettuce	Greatrekusu No.54	57.1	—[a)]	—
	Garland chrysanthemum	Oobashingiku	0	0	0
	Edible burdock	Watanabewase	14.3	3.5	1.0
	Sunflower		85.7	21.4	2.7
Parsley	Carrot	Shinkurodagosun	0	0	0
	Parse	Maruta	0	0	0
	Japanese hornwort	Shiroguki	0	0	0
Lily	Welsh onion	Kintyonegi	0	0	0
	Onion	Unzengokuwasekitamanegi No.1	0	0	0
Mallow	Okra	Betterfive	0	0	0
Grass	Corn	Honeybantam	0	0	0

a) Recognized root scab but no root tumor formed.

Attached list 4-1. Pathogenicity of isolate Cu-2-1 of the pathogenic Streptomyces sp. against gourd family plants by pouring and mixing inoculation (test-I)

Species	Cultivar	Percentage of diseased plants	Disease severity	Number of tumors per diseased plant
Melon	Kenkyaku(Stock)	85.7	21.4	1.2
	Kosack	85.7	21.4	1.9
	Prince	100	25.0	6.0
	Kyoei(Stock)	0	0	0
	Amusu	100	25.0	2.2
	Homerunstar	0	0	0
	Nanshoarusu kashukei	28.6	7.1	1.5
	Andesu	71.4	17.9	3.0
	Michizure(Stock)	42.9	10.7	1.0
	Arususeinunatu I	28.6	7.1	1.5
Cucumber	Asomidori	66.7	16.6	5.3
	Hijiri	14.3	3.6	2.0
	Suyo	33.3	8.3	5.5
	Honmyo	42.6	14.3	12.0
	Kagaaonagafushinari	16.7	4.2	9.0
Pumpkin	Benkei(Stock)	0	0	0
	Hayato	0	0	0
	Ebisu	0	0	0
	Tetsukabuto	0	0	0
	Kurodane(Stock)	0	0	0
	Shintosa No.1(Stock)	0	0	0
Watermelon	Tenryu No.2	0	0	0
	Fujihikari	0	0	0
	Shimaomacks KE	0	0	0
Bottle gourd	Sakigake(Stock)	50.0	12.5	2.3
	Kachidoki(Stock)	28.6	7.1	2.0
Oriental pickling melon	Yokauri	80.0	20.0	6.8
	Nagasakitsukeuri	100	25.0	2.7
Balsam pear	Satsumaonagareishi	14.3	3.6	1.0

Attached list 4-2. Pathogenicity of isolate Cu-2-1 of the pathogenic Streptomyces sp. against nightshade family plants by pouring and mixing inoculation (test-I)

Species	Cultivar	Percentage of diseased plants	Disease severity	Number of tumors per diseased plant
Tomato	Yuyake A	100	25.0	2.4
	BF Okitsu No.101	85.7	21.4	2.3
	Zuiken	71.4	17.9	3.0
	Oomiya	28.6	7.1	1.0
	Petittomato	28.6	7.1	1.5
Eggplant	Kokuyo	14.2	3.6	2.0
	Kuronishiki No.2	42.9	10.7	2.0
Sweet pepper (Bell type)	Ace	14.3	3.5	2.0
	Mansaku	0	0	0
Sweet pepper (Shishito type)	Wakato	0	0	0
Potato	Dejima	0	0	0

Attached list 4-3. Pathogenicity of isolate Cu-2-1 of the pathogenic Streptomyces sp. against mustard family plants by pouring and mixing inoculation (test-I)

Species	Cultivar	Percentage of diseased plants	Disease severity	Number of tumors per diseased plant
Cabbage	Fujiwase	0	0	0
Cauliflower	Nozakiwase	0	0	0
Chinese cabbage	Muso	0	0	0
Japanese radish	Natuminowase No.3	0	0	0
Turnip	Taibyohikari	0	0	0

Attached list 4-4. Pathogenicity of isolate Cu-2-1 of the pathogenic Streptomyces sp. against other family plants by pouring and mixing inoculation (test-Ⅰ)

Family	Species	Cultivar	Percentage of diseased plants	Disease severity	Number of tumors per diseased plant
Goosefoot	Spinach	Atorasu	0	0	0
	Chard	Shiroguki	71.4	17.9	4.8
Composite	Lettuce	Greatrekusu No.54	71.4	—a)	—
	Garland chrysanthemum	Oobashingiku	0	0	0
	Edible burdock	Watanabewase	0	0	0
	Sunflower	Dairinhimawari	0	0	0
Parsley	Carrot	Shinkurodagosun	0	0	0
	Parse	Maruta	0	0	0
	Japanese hornwort	Shiroguki	0	0	0
Lily	Welsh onion	Kintyonegi	0	0	0
	Onion	Unzengokuwasekitamanegi No.1	0	0	0
Mallow	Okra	Betterfive	0	0	0
Grass	Corn	Honeybantam	0	0	0

a) See Attached list 3-4.

Attached list 5-1. Pathogenicity of isolate B-9-1 of the pathogenic Streptomyces sp. against gourd family plants by pouring and mixing inoculation (test-II)

Species	Cultivar	Percentage of diseased plants	Disease severity	Number of tumors per diseased plant
Melon	Kenkyaku(Stock)	100	31.3	13.1
	Kosack	100	25.0	2.4
	Prince	100	25.0	5.2
	Kyoei(Stock)	0	0	0
	Amusu	75.0	18.8	4.0
	Homerunstar	40.0	10.0	2.5
	Nanshoarusu kashukei	0	0	0
	Andesu	0	0	0
	Michizure(Stock)	40.0	10.0	1.0
	Arususeinunatu I	0	0	0
Cucumber	Asomidori	60.0	15.0	7.3
	Hijiri	100	25.0	8.2
	Suyo	20.0	5.0	2.0
	Honmyo	50.0	12.5	4.5
	Kagaaonagafushinari	40.0	10.0	2.5
Pumpkin	Benkei(Stock)	20.0	5.0	0.6
	Hayato	60.0	15.0	2.7
	Ebisu	0	0	0
	Tetsukabuto	20.0	5.0	1.0
	Kurodane(Stock)	0	0	0
	Shintosa No.1(Stock)	0	0	0
Watermelon	Tenryu No.2	0	0	0
	Fujihikari	0	0	0
	Shimaomacks KE	75.0	18.8	3.7
Bottle gourd	Sakigake(Stock)	40.0	10.0	1.5
	Kachidoki(Stock)	40.0	10.0	6.0
Oriental pickling melon	Yokauri	80.0	25.0	9.0
	Nagasakitsukeuri	60.0	15.0	7.3

Attached list 5-2. Pathogenicity of isolate B-9-1 of the pathogenic *Streptomyces* sp. against nightshade family plants by pouring and mixing inoculation (test-II)

Species	Cultivar	Percentage of diseased plants	Disease severity	Number of tumors per diseased plant
Tomato	Yuyake A	40.0	10.0	4.0
	BF Okitsu No.101	40.0	10.0	3.0
	Zuiken	20.0	5.0	10.0
	Oomiya	0	0	0
	Petittomato	80.0	20.0	2.8
Eggplant	Kokuyo	0	0	0
	Kuronishiki No.2	40.0	10.0	1.0
Sweet pepper (Bell type)	Ace	0	0	0
	Mansaku	40.0	10.0	1.5
Sweet pepper (Shishito type)	Wakato	0	0	0
Potato	Dejima	0	0	0
	Mayqueen	0	0	0

Attached list 5-3. Pathogenicity of isolate B-9-1 of the pathogenic *Streptomyces* sp. against mustard family plants by pouring and mixing inoculation (test-II)

Species	Cultivar	Percentage of diseased plants	Disease severity	Number of tumors per diseased plant
Cabbage	Fujiwase	0	0	0
Cauliflower	Nozakiwase	0	0	0
Chinese cabbage	Muso	0	0	0
	Shin No.6	0	0	0
Japanese radish	Natuminowase No.3	0	0	0
Turnip	Taibyohikari	0	0	0

Attached list 5-4. Pathogenicity of isolate B-9-1 of the pathogenic *Streptomyces* sp. against other family plants by pouring and mixing inoculation (test-II)

Family	Species	Cultivar	Percentage of diseased plants	Disease severity	Number of tumors per diseased plant
Goosefoot	Spinach	Atorasu	0	0	0
	Chard	Shiroguki	40.0	10.0	4.0
Composite	Garland chrysanthemum	Oobashingiku	0	0	0
	Edible burdock	Watanabewase	0	0	0

Attached list 6-1. Pathogenicity of isolate OTP-3-1 of the pathogenic *Streptomyces* sp. against gourd family plants by pouring and mixing inoculation (test-II)

Species	Cultivar	Percentage of diseased plants	Disease severity	Number of tumors per diseased plant
Melon	Kenkyaku(Stock)	100	43.8	22.9
	Kosack	100	25.0	12.5
	Prince	100	25.0	2.8
	Kyoei(Stock)	40.0	10.0	1.5
	Amusu	60.0	15.0	2.0
	Homerunstar	60.0	15.0	2.4
	Nanshoarusu kashukei	0	0	0
	Arususeinunatu I	0	0	0
Cucumber	Asomidori	100	35.0	17.4
	Hijiri	80.0	20.0	12.6
	Suyo	100	30.0	14.2
	Honmyo	75.0	18.8	6.3
	Kagaaonagafushinari	100	30.0	9.6
Pumpkin	Benkei(Stock)	60.0	10.0	1.7
	Hayato	60.0	15.0	3.3
	Ebisu	20.0	5.0	1.0
	Tetsukabuto	0	0	0
	Kurodane(Stock)	0	0	0
	Shintosa No.1(Stock)	0	0	0
Watermelon	Tenryu No.2	0	0	0
	Fujihikari	0	0	0
	Shimaomacks KE	40.0	10.0	1.0
Bottle gourd	Sakigake(Stock)	60.0	15.0	1.0
	Kachidoki(Stock)	50.0	12.5	5.5
Oriental pickling melon	Yokauri	60.0	15.0	5.0
	Nagasakitsukeuri	20.0	5.0	4.0

Attached list 6-2. Pathogenicity of isolate OTP-3-1 of the pathogenic *Streptomyces* sp. against nightshade family plants by pouring and mixing inoculation (test-II)

Species	Cultivar	Percentage of diseased plants	Disease severity	Number of tumors per diseased plant
Tomato	Yuyake A	20.0	5.0	4.0
	BF Okitsu No.101	40.0	10.0	2.0
	Zuiken	80.0	20.0	7.3
	Oomiya	0	0	0
	Petittomato	100	25.0	3.0
Eggplant	Kokuyo	0	0	0
	Kuronishiki No.2	20.0	5.0	1.0
Sweet pepper (Bell type)	Ace	0	0	0
	Mansaku	20.0	5.0	1.0
Sweet pepper (Shishito type)	Wakato	0	0	0
Potato	Dejima	0	0	0
	Mayqueen	0	0	0

Attached list 6-3. Pathogenicity of isolate OTP-3-1 of the pathogenic *Streptomyces* sp. against mustard family plants by pouring and mixing inoculation (test-II)

Species	Cultivar	Percentage of diseased plants	Disease severity	Number of tumors per diseased plant
Cabbage	Fujiwase	0	0	0
Cauliflower	Nozakiwase	0	0	0
Chinese cabbage	Muso	0	0	0
	Shin No.6	0	0	0
Japanese radish	Natuminowase No.3	0	0	0
Turnip	Taibyohikari	0	0	0

Attached list 6-4. Pathogenicity of isolate OTP-3-1 of the pathogenic Streptomyces sp. against other family plants by pouring and mixing inoculation (test-II)

Family	Species	Cultivar	Percentage of diseased plants	Disease severity	Number of tumors per diseased plant
Goosefoot	Spinach	Atorasu	0	0	0
	Chard	Shiroguki	0	0	0
Composite	Garland chrysanthemum	Oobashingiku	0	0	0
	Edible burdock	Watanabewase	0	0	0

Attached list 7-1. Pathogenicity of isolate Cu-2-1 of the pathogenic *Streptomyces* sp. against gourd family plants by pouring and mixing inoculation (test-II)

Species	Cultivar	Percentage of diseased plants	Disease severity	Number of tumors per diseased plant
Melon	Kenkyaku(Stock)	100	25.0	3.5
	Kosack	0	0	0
	Kyoei(Stock)	0	0	0
	Amusu	0	0	0
	Homerunstar	0	0	0
	Nanshoarusu kashukei	0	0	0
	Andesu	0	0	0
	Michizure(Stock)	0	0	0
	Arususeinunatu I	0	0	0
Cucumber	Asomidori	20.0	5.0	2.0
	Hijiri	20.0	10.0	21.0
	Suyo	20.0	5.0	2.0
	Honmyo	20.0	5.0	2.0
	Kagaaonagafushinari	20.0	5.0	1.0
Pumpkin	Hayato	60.0	15.0	2.3
	Ebisu	40.0	10.0	1.5
	Tetsukabuto	0	0	0
	Kurodane(Stock)	0	0	0
	Shintosa No.1(Stock)	0	0	0
Watermelon	Tenryu No.2	0	0	0
	Fujihikari	0	0	0
	Shimaomacks KE	0	0	0
Bottle gourd	Sakigake(Stock)	0	0	0
	Kachidoki(Stock)	20.0	5.0	2.0
Oriental pickling melon	Yokauri	60.0	20.0	9.3
	Nagasakitsukeuri	20.0	5.0	1.0

Attached list 7-2. Pathogenicity of isolate Cu-2-1 of the pathogenic Streptomyces sp. against nightshade family plants by pouring and mixing inoculation (test-II)

Species	Cultivar	Percentage of diseased plants	Disease severity	Number of tumors per diseased plant
Tomato	Yuyake A	20.0	5.0	1.0
	BF Okitsu No.101	0	0	0
	Zuiken	0	0	0
	Oomiya	0	0	0
	Petittomato	80.0	20.0	2.5
Eggplant	Kokuyo	0	0	0
	Kuronishiki No.2	40.0	10.0	1.0
Sweet pepper (Bell type)	Ace	20.0	5.0	1.0
	Mansaku	60.0	15.0	1.3
Sweet pepper (Shishito type)	Wakato	0	0	0
Potato	Dejima	0	0	0
	Mayqueen	0	0	0

Attached list 7-3. Pathogenicity of isolate Cu-2-1 of the pathogenic Streptomyces sp. against mustard family plants by pouring and mixing inoculation (test-II)

Species	Cultivar	Percentage of diseased plants	Disease severity	Number of tumors per diseased plant
Cabbage	Fujiwase	0	0	0
Cauliflower	Nozakiwase	0	0	0
Chinese cabbage	Muso	0	0	0
	Shin No.6	0	0	0
Japanese radish	Natuminowase No.3	0	0	0
Turnip	Taibyohikari	0	0	0

Attached list 7-4. Pathogenicity of isolate Cu-2-1 of the pathogenic *Streptomyces* sp. against other family plants by pouring and mixing inoculation (test-II)

Family	Species	Cultivar	Percentage of diseased plants	Disease severity	Number of tumors per diseased plant
Goosefoot	Spinach	Atorasu	20.0	5.0	1.0
	Chard	Shiroguki	0	0	0
Composite	Garland chrysanthemum	Oobashingiku	0	0	0
	Edible burdock	Watanabewase	0	0	0

Attached list 8-1. Pathogenicity of isolate B-9-1 of the pathogenic *Streptomyces* sp. by soil incorporating inoculation against gourd family plants

Species	Cultivar	Percentage of diseased plants	Disease severity	Number of tumors per diseased plant
Melon	Kenkyaku(Stock)	100	60.0	41.6
	Kosack	100	45.0	24.4
	Prince	100	25.0	10.0
	Kyoei(Stock)	100	35.0	11.4
	Amusu	100	30.0	19.0
	Homerunstar	100	35.0	18.8
	Nanshoarusu kashukei	100	35.0	11.4
	Andesu	100	25.0	12.2
	Michizure(Stock)	100	25.0	6.6
	Arususeinunatu I	0	0	0
Cucumber	Asomidori	100	25.0	10.0
	Hijiri	100	30.0	17.2
	Suyo	100	25.0	11.8
	Honmyo	100	25.0	13.2
	Kagaaonagafushinari	100	25.0	6.6
Pumpkin	Benkei(Stock)	0	0	0
	Hayato	0	0	0
	Ebisu	40.0	10.0	0.6
	Tetsukabuto	60.0	15.0	7.7
	Kurodane(Stock)	100	25.0	5.0
	Shintosa No.1(Stock)	60.0	15.0	5.0
Watermelon	Tenryu No.2	80.0	20.0	4.8
	Fujihikari	60.0	15.0	2.7
	Shimaomacks KE	100	25.0	3.6
Bottle gourd	Sakigake(Stock)	100	25.0	12.1
	Kachidoki(Stock)	75.0	18.8	5.7
Oriental pickling melon	Yokauri	100	30.0	13.2
	Nagasakitsukeuri	100	31.3	13.8
Balsam pear	Satsumaonagareishi	0	0	0

Attached list 8-2. Pathogenicity of isolate B-9-1 of the pathogenic Streptomyces sp. by soil incorporating inoculation against nightshade family plants

Species	Cultivar	Percentage of diseased plants	Disease severity	Number of tumors per diseased plant
Tomato	Yuyake A	80.0	20.0	2.8
	BF Okitsu No.101	60.0	15.0	7.3
	Zuiken	40.0	10.0	4.0
	Oomiya	25.0	6.3	9.0
	Petittomato	60.0	15.0	1.6
Eggplant	Kokuyo	0	0	0
	Kuronishiki No.2	0	0	0
Sweet pepper (Bell type)	Ace	20.0	5.0	1.0
	Mansaku	0	0	0
Sweet pepper (Shishito type)	Wakato	0	0	0
Potato	Dejima	0	0	0
	Mayqueen	0	0	0

Attached list 8-3. Pathogenicity of isolate B-9-1 of the pathogenic Streptomyces sp. by soil incorporating inoculation against mustard family plants

Species	Cultivar	Percentage of diseased plants	Disease severity	Number of tumors per diseased plant
Cabbage	Fujiwase	0	0	0
Cauliflower	Nozakiwase	0	0	0
Chinese cabbage	Muso	0	0	0
	Shin No.6	0	0	0
Japanese radish	Natuminowase No.3	0	0	0
Turnip	Taibyohikari	0	0	0

Attached list 8-4. Pathogenicity of isolate B-9-1 of the pathogenic Streptomyces sp. by soil incorporating inoculation against other family plants

Family	Species	Cultivar	Percentage of diseased plants	Disease severity	Number of tumors per diseased plant
Goosefoot	Spinach	Atorasu	0	0	0
	Chard	Shiroguki	0	0	0
Composite	Lettuce	Greatrekusu No.54	0	0	0
	Garland chrysanthemum	Oobashingiku	0	0	0
	Edible burdock	Watanabewase	0	0	0
	Sunflower	Dairinhimawari	100	40.0	25.8
Grass	Rice	Nihonbare	0	0	0
	Corn	Honeybantam	0	0	0

Attached list 9-1. Pathogenicity of isolate OTP-3-1 of the pathogenic Streptomyces sp. by soil incorporating inoculation against gourd family plants

Species	Cultivar	Percentage of diseased plants	Disease severity	Number of tumors per diseased plant
Melon	Kenkyaku(Stock)	100	60.0	75.4
	Kosack	100	30.0	12.2
	Prince	80.0	20.0	3.9
	Kyoei(Stock)	100	35.0	20.4
	Amusu	100	45.0	18.6
	Homerunstar	100	80.0	111.0
	Nanshoarusu kashukei	40.0	10.0	1.2
	Andesu	80.0	15.0	5.2
	Michizure(Stock)	100	55.0	46.8
	Arususeinunatu I	0	0	0
Cucumber	Asomidori	100	55.0	37.4
	Hijiri	100	30.0	19.2
	Suyo	100	60.0	45.8
	Honmyo	100	60.0	26.3
	Kagaaonagafushinari	50.0	12.5	8.1
Pumpkin	Benkei(Stock)	40.0	10.0	0.6
	Hayato	0	0	0
	Ebisu	40.0	10.0	1.0
	Tetsukabuto	40.0	10.0	0.6
	Kurodane(Stock)	100	45.0	36.0
	Shintosa No.1(Stock)	0	0	0
Watermelon	Tenryu No.2	0	0	0
	Fujihikari	0	0	0
	Shimaomacks KE	80.0	20.0	6.8
Bottle gourd	Sakigake(Stock)	100	55.0	49.0
	Kachidoki(Stock)	75.0	25.0	10.3
Oriental pickling melon	Yokauri	100	45.0	37.0
	Nagasakitsukeuri	100	33.3	17.0
Balsam pear	Satsumaonagareishi	25.0	6.3	3.0

Attached list 9-2. Pathogenicity of isolate OTP-3-1 of the pathogenic *Streptomyces* sp. by soil incorporating inoculation against nightshade family plants

Species	Cultivar	Percentage of diseased plants	Disease severity	Number of tumors per diseased plant
Tomato	Yuyake A	60.0	15.0	12.3
	BF Okitsu No.101	40.0	15.0	18.5
	Zuiken	50.0	18.8	27.5
	Oomiya	25.0	6.3	1.0
	Petittomato	60.0	15.0	6.6
Eggplant	Kokuyo	0	0	0
	Kuronishiki No.2	0	0	0
Sweet pepper (Bell type)	Ace	0	0	0
	Mansaku	0	0	0
Sweet pepper (Shishito type)	Wakato	0	0	0
				0
Potato	Dejima	0	0	0
	Mayqueen	0	0	0

Attached list 9-3. Pathogenicity of isolate OTP-3-1 of the pathogenic *Streptomyces* sp. by soil incorporating inoculation against mustard family plants

Species	Cultivar	Percentage of diseased plants	Disease severity	Number of tumors per diseased plant
Cabbage	Fujiwase	0	0	0
Cauliflower	Nozakiwase	0	0	0
Chinese cabbage	Muso	0	0	0
	Shin No.6	0	0	0
Japanese radish	Natuminowase No.3	0	0	0
Turnip	Taibyohikari	0	0	0

Attached list 9-4. Pathogenicity of isolate OTP-3-1 of the pathogenic *Streptomyces* sp. by soil incorporating inoculation against other family plants

Family	Species	Cultivar	Percentage of diseased plants	Disease severity	Number of tumors per diseased plant
Goosefoot	Spinach	Atorasu	0	0	0
	Chard	Shiroguki	0	0	0
Composite	Lettuce	Greatrekusu No.54	0	0	0
	Garland chrysanthemum	Oobashingiku	0	0	0
	Edible burdock	Watanabewase	0	0	0
	Sunflower	Dairinhimawari	100	35.0	18.2
Grass	Rice	Nihonbare	0	0	0
	Corn	Honeybantam	0	0	0

Attached list 10-1. Pathogenicity of isolate Cu-2-1 of the pathogenic *Streptomyces* sp. by soil incorporating inoculation against gourd family plants

Species	Cultivar	Percentage of diseased plants	Disease severity	Number of tumors per diseased plant
Melon	Kenkyaku(Stock)	100	100	190.4
	Kosack	100	93.8	131.8
	Prince	100	90.0	113.4
	Kyoei(Stock)	100	60.0	56.6
	Amusu	100	95.0	128.2
	Homerunstar	100	60.0	39.2
	Nanshoarusu kashukei	100	25.0	9.6
	Andesu	100	50.0	39.6
	Michizure(Stock)	100	25.0	11.4
	Arususeinunatu I	100	25.0	8.0
Cucumber	Asomidori	100	25.0	12.2
	Hijiri	100	40.0	20.4
	Suyo	100	55.0	42.0
	Honmyo	100	50.0	35.4
	Kagaaonagafushinari	100	25.0	6.0
Pumpkin	Benkei(Stock)	60.0	15.0	3.0
	Hayato	100	35.0	17.8
	Ebisu	100	31.3	14.3
	Tetsukabuto	100	40.0	22.8
	Kurodane(Stock)	100	40.0	25.4
	Shintosa No.1(Stock)	100	70.0	54.0
Watermelon	Tenryu No.2	25.0	5.0	4.0
	Fujihikari	60.0	15.0	2.3
	Shimaomacks KE	100	30.0	14.4
Bottle gourd	Sakigake(Stock)	100	85.0	101.9
	Kachidoki(Stock)	100	50.0	31.1
Oriental pickling melon	Yokauri	100	65.0	56.9
	Nagasakitsukeuri	100	70.0	74.2
Balsam pear	Satsumaonagareishi	80.0	20.0	2.8

Attached list 10-2. Pathogenicity of isolate Cu-2-1 of the pathogenic *Streptomyces* sp. by soil incorporating inoculation against nightshade family plants

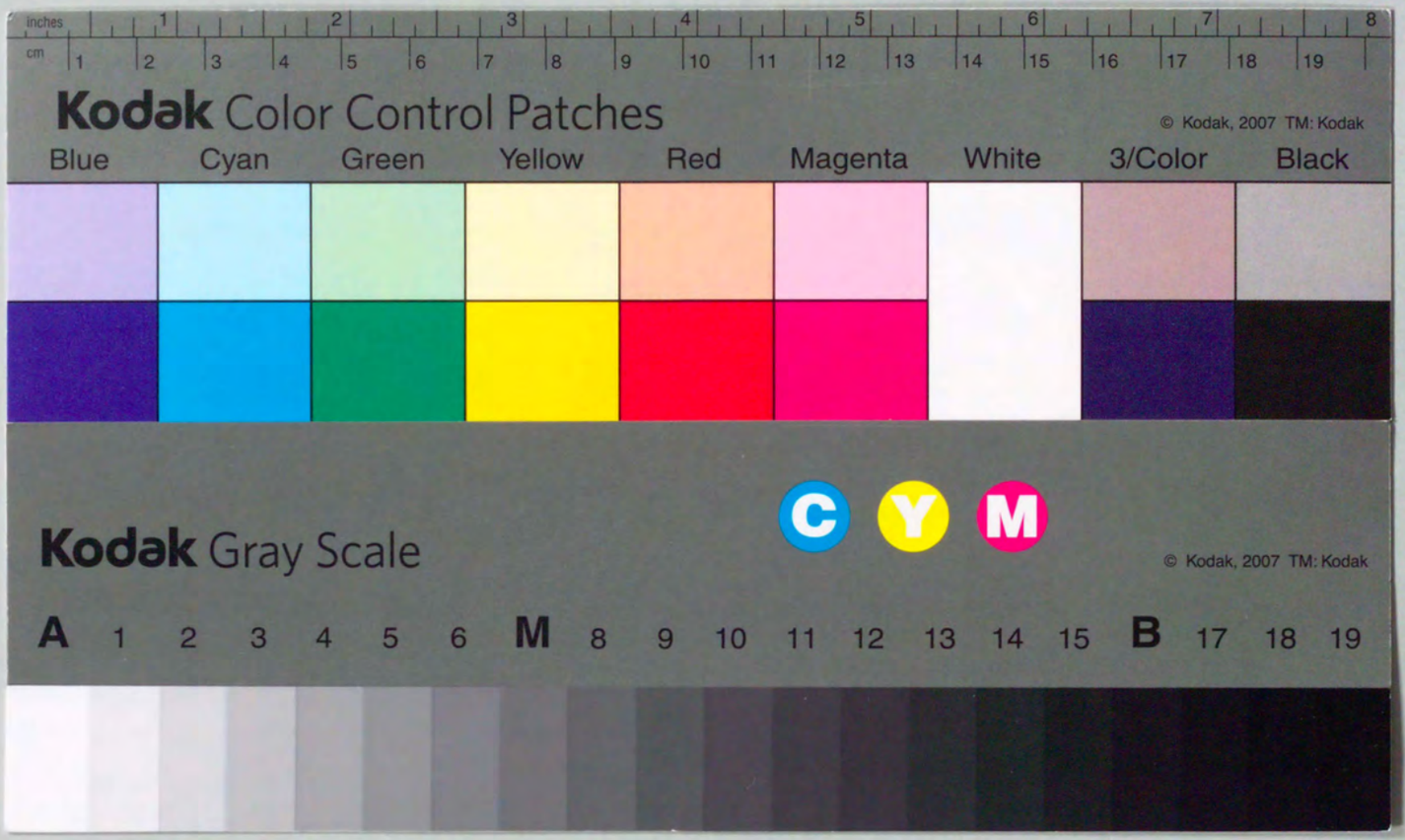
Species	Cultivar	Percentage of diseased plants	Disease severity	Number of tumors per diseased plant
Tomato	Yuyake A	100	25.0	9.0
	BF Okitsu No.101	40.0	15.0	18.5
	Zuiken	60.0	15.0	8.0
	Oomiya	40.0	10.0	1.5
	Petittomato	100	30.0	13.2
Eggplant	Kokuyo	0	0	0
	Kuronishiki No.2	0	0	0
Sweet pepper (Bell type)	Ace	0	0	0
	Mansaku	0	0	0
Sweet pepper (Shishito type)	Wakato	0	0	0
Potato	Dejima	0	0	0
	Mayqueen	0	0	0

Attached list 10-3. Pathogenicity of isolate Cu-2-1 of the pathogenic *Streptomyces* sp. by soil incorporating inoculation against mustard family plants

Species	Cultivar	Percentage of diseased plants	Disease severity	Number of tumors per diseased plant
Cabbage	Fujiwase	0	0	0
Cauliflower	Nozakiwase	0	0	0
Chinese cabbage	Muso	0	0	0
	Shin No.6	0	0	0
Japanese radish	Natuminowase No.3	0	0	0
Turnip	Taibyohikari	0	0	0

Attached list 10-4. Pathogenicity of isolate Cu-2-1 of the pathogenic *Streptomyces* sp. by soil incorporating inoculation against other family plants

Family	Species	Cultivar	Percentage of diseased plants	Disease severity	Number of tumors per diseased plant
Goosefoot	Spinach	Atorasu	0	0	0
	Chard	Shiroguki	0	0	0
Composite	Lettuce	Greatrekusu No.54	0	0	0
	Garland chrysanthemum	Oobashingiku	0	0	0
	Edible burdock	Watanabewase	0	0	0
	Sunflower	Dairinhimawari	100	80.0	91.8
Grass	Rice	Nihonbare	0	0	0
	Corn	Honeybantam	0	0	0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