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(54) Title: ANTIFUNGAL BIOCONTROL AGENTS, A PROCESS FOR PREPARING AND TREATING THE SAME

(57) Abstract

The present invention relates to antifungal biocontrol agents which enhance plant growth and reduce plant diseases by suppressing fungal pathogens, and the manufacturing and application methods thereof. The antifungal biocontrol agents of the present invention consist of one of the newly isolated strains of *Streptomyces* sp. WYE 20 (KCTC 0341BP) and WYE 324 (KCTC 0342BP) and a delivery medium which carries and stabilizes the cells. In particular, WYE 20 and WYE 324 of the present invention exhibit strong antifungal activity against *Rhizoctonia solani* and *Phytophthora capsici* and can be used in various ways to reduce fungal diseases in plants such as cucumber (*Cucumis sativus* L.), pepper (*Capsicum annuum* L.), and golf course turfgrasses.

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ANTIFUNGAL BIOCONTROL AGENTS, A PROCESS FOR PREPARING AND TREATING THE SAME

FIELD OF THE INVENTION

The present invention relates to antifungal biocontrol agents, and to a process for preparing and treating the same. In particular, the present invention relates to two antifungal biocontrol strains of *Streptomyces* sp., WYE 20 and WYE 324, capable of enhancing plant growth and reducing plant diseases by suppressing fungal pathogens colonized in rhizosphere soils of host plants, and the preparation method and application method thereof.

BACKGROUND OF THE INVENTION

It is well known that soilborne fungal phytopathogens cause enormous economic losses in the agricultural and horticultural industries. In particular, *Rhizoctonia solani* is one of the major fungal phytopathogens exhibiting strong pathogenicity; it is associated with seedling diseases as well as foliar diseases such as seed rot, root rot, damping-off, leaf and stem rot of many plant species and varieties, resulting in enormous economic losses. For example, *Rhizoctonia solani* AG 1 (IB) causes plant diseases in crops such as cucumber (*Cucumis sativus* L.) and pepper (*Capsicum annuum* L.) as well as brown patches of golf green creeping bentgrass, *Rhizoctonia solani* AG 2-2 causes large patches in fairway turfgrass on large areas of golf courses resulting in enormous economic losses. In addition, *Phytophthora capsici* is a widespread and highly destructive soilborne fungal phytopathogen that causes root and crown rot as well as the aerial blight of leaves, fruit, and the stems of peppers (*Capsicum annuum* L.). It is very difficult to suppress Phytophthora blight in pepper plants if they are infected by a *Phytophthora capsici*. *Phytophthora capsici* is a particularly destructive phytopathogen responsible for blight of pepper plants during hot and humid rainy seasons resulting in the killing of the pepper plants and consequently enormous economic losses. As mentioned above, both *Rhizoctonia solani* and *Phytophthora*

capsici are major fungal phytopathogens which exhibit strong pathogenicity and produce spores which survive for a long time in harsh conditions. Thus, they repeatedly cause plant diseases in large areas when conditions for incidence of the disease are optimal.

Therefore, growers generally apply a mixture of fungicides to plants on a regular basis to control plant diseases caused by both *Rhizoctonia solani* and *Phytophthora capsici.* However, it is becoming increasingly difficult to control Phytophthora blight of pepper plants and Rhizoctonia brown patches of creeping bentgrass because of the emergence of strains of fungal phytopathogens resistant to fungicides. It is also becoming increasingly difficult to control these diseases due to the widespread incidence of *Rhizoctonia solani* resistant to fungicides in golf courses and the proliferation of *Phytophthora capsici* resulting from the successive cultivation of pepper plants.

Intensive use of agrochemicals has also provoked residual toxicity and environmental problems. Furthermore, agrochemicals are considered ineffective in controlling soilborne plant diseases due to wash out, and the lack of an efficient application which allows effective penetration into rhizosphere soils. It is also very difficult to expect long term protection of plants with an application of chemical fungicides. Therefore, using certain rhizosphere bacteria as a biocontrol agent can provide not only more effective and economical practices for the control of plant diseases such as Phytophthora blight caused by *Phytophthora capsici* and golf course turfgrass diseases caused by *Rhizoctonia solani,* but also increased environmental conservation.

It has been shown that use of antagonistic microorganisms is an attractive way to control fungal pathogens (Suh, Ph.D. Dissertation. University of Idaho, Idaho, USA, 1992; Crawford *et al*, Appl. Environ. Microbiol. 59:3899-3905, 1993; U.S. Pat. No. 5,403,584). The inventors disclosed in the prior art that certain *Streptomyces* species strains can be used to control plant pathogens by using a delivery medium containing peat moss, sand, and cornmeal, in the potting mixture/soil or by coating plant seeds with sodium alginate containing the

biocontrol agent, resulting in the colonizing of the roots (U.S. Pat. No. 5,403,584). It has also been disclosed in the prior art that peat containing a beneficial biocontrol agent, can be used to control plant pathogens (U.S. Pat. No. 4,595,589) The delivery medium methods in the prior arts are suitable for the application of seedlings/potting mixtures, but are not quite suitable for direct application to plant seeds or plant roots to achieve efficient protection against fungal phytopathogens.

The object of the present invention is to provide novel strains of *Streptomyces* species which are capable of controlling fungal phytopathogens by direct application to plant seeds or plant roots utilizing specially designed formulations comprising delivery media containing a biologically pure culture selected from the newly isolated strains of *Streptomyces* species.

Further, it is an object of the present invention to provide antifungal biocontrol agents and describe their use in protecting plants from infections caused by soilborne fungal phytopathogens.

SUMMARY OF THE INVENTION

The present invention has been achieved through the isolation of *Streptomyces* sp. WYE 20 and WYE 324 which are capable of protecting plants against *Rhizoctonia solani* and *Phytophthora capsici*. The strains, *Streptomyces* sp. WYE 20 and WYE 324, are effective in preventing the incidence of fungal diseases in plants and in enhancing plant growth in greenhouse and agricultural field trials. Therefore, one aspect of the present invention is microbially pure cultures of *Streptomyces* sp. WYE 20 and WYE 324.

The present invention also encompasses the various delivery media suitable for the treatment of plant seeds, seedling beds/pots, or potting mixtures/soil with *Streptomyces* sp. WYE 20 and WYE 324. The delivery medium is very useful for carrying *Streptomyces* sp. WYE 20 and WYE 324 to inhibit the plant diseases caused by fungal phytopathogens.

In a particular embodiment according to the present invention, the delivery medium consists of wood sawdust, wheat bran, chitin, chitosan, and

Pharmamedia (trade name; made by The Budkeye Oilseed Products Company, Texas, USA) together with *Streptomyces* sp. WYE 20 or WYE 324. In another particular embodiment, the delivery medium consists of pectin and colloidal chitin and water together with *Streptomyces* sp. WYE 20 or WYE 324. In the preferred embodiment, the delivery medium comprises at least 10^5 colony forming units of *Streptomyces* sp. WYE 20 or WYE 324 per gram of delivery medium.

The present invention also encompasses methods for reducing fungal infection in seeds, seedlings and/or growing plants prior to or during sowing and growing seasons through the treatment of plant seeds, seedling beds/pots, potting mixture/soils, spraying or in-furrow application.

DETAILED DESCRIPTION OF THE INVENTION

According to the present invention , *Streptomyces* sp. WYE 20 and WYE 324 are provided that are effective in inhibiting fungal diseases and in enhancing the growth of plants such as pepper, cucumber, and turfgrasses, although their scope is not limited to these examples.

The present invention encompasses the various delivery media that are useful for carrying *Streptomyces* sp. WYE 20 and WYE 324 to inhibit the plant diseases caused by fungal phytopathogens.

In a particular embodiment of the present invention, the delivery medium consists of 40 to 65 w/w% of wheat bran, 1 to 5 w/w% of chitosan, 30 to 55 w/w% of wood sawdust, 1 to 3 w/w% chitin, and 1 to 3 w/w% of Pharmamedia on the basis of the total weight of the delivery medium. In the preferred embodiment, the delivery medium further consists of 0.2 to 3.5 w/w% of sporulation medium as an additional component on the basis of the total weight of the delivery medium. The preferred sporulation medium is selected from ATCC #5 sporulation medium or yeast extract-glucose-mineral salts, but the presentation does not exclude other sporulation media.

In another particular embodiment, the delivery medium consists of 1.0 to 3.0 w/w% of pectin and 0.1 to 0.6 w/w% of colloidal chitin in water.

In the preferred embodiment, the delivery medium comprises 10^5 to 10^{10}, preferably 10^7 to 10^8, colony forming units of *Streptomyces* sp. WYE 20 or WYE 324 per gram of delivery medium.

The present invention includes a process for the production of mycelium and spore inocula of *Streptomyces* sp. WYE 20 and WYE 324. Viable cells of *Streptomyces* sp. WYE 20 and WYE 324 are produced by incubating vegetative cells in the yeast extract-glucose-mineral salts or modified bennett liquid medium under the proper conditions.

A process for preparing an antifungal biocontrol agent comprises as follows;

Streptomyces sp. WYE 20 or WYE 324 are prepared by incubating at 130 rpm to 300 rpm, at a temperature of 25 ℃ to 33 ℃ for 3 to 7 days.

After harvesting cells of *Streptomyces* sp. WYE 20 or WYE 324, the cells are lyophilized or mixed directly into a delivery medium.

Further, according to the present invention, a process for preparing an antifungal biocontrol agent comprises the following steps; preparing a delivery medium containing 40 to 65 w/w% of wheat bran, 1 to 5 w/w% of chitosan, 30 to 55 w/w% of wood sawdust, 1 to 3 w/w% of chitin and 1 to 3 w/w% of Pharmamedia on the basis of the total weight of the delivery medium;

autoclaving the resulting delivery medium;

incorporating *Streptomyces* sp. WYE 20 (KCTC 0341BP) or *Streptomyces* sp. WYE 324 (KCTC 0342BP) into the delivery medium;

incubating incorporated cells of *Streptomyces* sp. WYE 20 (KCTC 0341BP) or *Streptomyces* sp. WYE 324 (KCTC 0342BP) at 25 ℃ to 33 ℃ for 5-14 days; and

aseptically drying the resulting product to obtain an antifungal biocontrol agent.

Preferably, the process further comprises aseptically blending the resulting dried product.

In the above process, the delivery medium is optionally pelletized and then coated with 0.2 to 3.5 w/w% of sporulation medium on the basis of the total

weight of the delivery medium.

Streptomyces sp. WYE 20 or WYE 324 is incorporated to a concentration of 10^5-10^{10}, preferably 10^7-10^8 colony forming unit per gram of delivery medium.

Autoclaving of the delivery medium is carried out at 121 ℃ for 30 to 40 minutes.

In another embodiment of the present invention, a process is provided for preparing an antifungal biocontrol agent which comprises :

preparing a delivery medium consisting of 1.0 to 3.0 w/w% of pectin and 0.1 to 0.6 w/w% of colloidal chitin in water;

autoclaving the resulting delivery medium; and

incorporating *Streptomyces* sp. WYE 20 (KCTC 0341BP) or *Streptomyces* sp. WYE 324 (KCTC 0342BP) into the delivery medium.

In the above embodiment of the present invention, *Streptomyces* WYE 20 or WYE 324 is added to the delivery medium to a final concentration of 10^5-10^{10}, preferably 10^7-10^8 colony forming unit per gram of delivery medium.

Further, the present invention relates to a method for treating the antifungal biocontrol agent which comprises coating, mixing, spraying or in-furrow applicating the agent to plant seeds, potting mixture, plants, or soils thereof.

The present invention also relates to an antifungal biocontrol agent which comprises one of the newly isolated strains of *Streptomyces* sp. WYE 20 and WYE 324. Therefore, it is appreciated that an antifungal biocontrol agent containing microorganisms having an antifungal property equal to that of the strains together with the delivery medium according to the present invention is within the true spirit and scope of the present invention.

The present invention will be further described in the following examples. However, the present invention is not limited to the following examples.

Materials and Methods

1. Culture Medium of Microorganism

All bacterial culture media used distilled water and were sterilized by autoclaving prior to use. All bacterial samples were treated by standard aseptic

laboratory techniques to maintain purity.

1) CYD (casamino acid/yeast extract/dextrose/agar) medium contains casamino acids (Difco: 0.5g/l), yeast extract (Difco: 0.8g/l), D-glucose (0.4 g/l), K_2HPO_4 (2.0g/l; pH 7.2-7.4), and agar (18g/l) in distilled water.

2) WYE (water/yeast extract/agar) medium, modified from Reddi and Rao (1971) contains yeast extract (0.25g/l), K_2HPO_4 (2.0g/l; pH 7.2-7.4) and agar (18g/l) in distilled water.

3) YGM (yeast extract/glucose/mineral salts) medium comprises 0.6% (w/v) yeast extract (Difco Laboratories, Detroit, MI, U.S.A.), 1.0%(w/v) glucose, and phosphate mineral salt solution (5.3g of Na_2HPO_4, 1.98g of KH_2PO_4, 0.2g of $MgSO_4$ $7H_2O$, 0.2g of NaCl, 0.05g of $CaCl_2$ $2H_2O$, plus 1.0 ml of trace elements (Pridham and Gottlieb, J. Bacteriology 56:107-114, 1948) per liter of deionized H2O; pH 7.1 to 7.2). The solution of trace elements consists of 0.64g of $CuSO_4$ $5H_2O$, 0.11g of $FeSO_4$ $7H_2O$, 0.79g of $MnCl_2$ $4H_2O$, 0.15g of $ZnSO_4$ $7H_2O$ in 100 ml of distilled water.

4) CM (chitin/mineral salts/agar) medium comprises 0.4 % to 0.6% (w/v) colloidal chitin prepared by the previously known method (Hsu and Lockwood, Applied Microbiology, p422-426, 1975), 0.6% (w/v) $(NH_4)_2SO_4$, and 2.0% (w/v) agar per liter of phosphate mineral salt solution described above; pH 7.0 to 7.2.

5) Laminarin agar medium comprises 0.25% (w/v) laminarin, 0.6% (w/v) $(NH_4)_2SO_4$, and 2.0% (w/v) agar per liter of phosphate mineral salt solution described above; pH 7.2 to 7.4.

6) Modified Bennett liquid medium contains yeast extract (2g/l), beef extract (2g/l), peptone (2g/l), glucose (10g/l), and nystatin (5 μg/ml; pH 6.5-7.5) in distilled water.

7) Modified Bennett Agar medium contained yeast extract (2g/l), beef extract (2g/l), peptone (2g/l), glucose (10g/l), nystatin (5 μg/ml; pH 6.5-7.5), and agar (20g/l) in distilled water.

8) ISP medium #2 (Difco).

9) ISP medium #3 (Difco).

10) ISP medium #4 (Difco).

11) Sporulation medium (ATCC medium #5) contains yeast extract (1.0g/l), beef extract (1.0g/l), tryptose (2.0g/l), $FeSO_4$(0.01g/l), glucose (10g/l), and agar (15g/l) (17th Edition ATCC Catalogue of Bacteria and Bacteriophages).

2. Identification of *Streptomyces* sp. WYE 20 and WYE 324

Strains WYE 20 and WYE 324 were identified as *Streptomyces* species on the basis of the morphological, physiological, and chemical characteristics of the genus *Streptomyces,* as defined in Bergey's Manual of Systematic Bacteriology (1989); the International Streptomyces Project (ISP) (1974)); and Williams, et al. (1983). The summarized results of these strains in the respects of morphological, physiological, and chemical characteristics are reported in Tables I to V.

3. Preparation of seed culture and storage of *Streptomyces* sp. WYE 20 and WYE 324

Spores from a CYD agar slant were inoculated with an inoculation loop into 500ml flasks containing 50ml of modified bennett liquid medium (pH 6.5-7.5). The medium was sterilized by autoclaving for 15 minutes at 121 ℃ prior to inoculation. The inoculated flasks were then incubated with shaking at 130 to 300 rpm at 25 ℃ to 33 ℃ for 1-4 days to provide a standard inoculum.

For short-term use, *Streptomyces* sp. WYE 20 and WYE 324 were incubated on CYD or WYE agar slant at 25 ℃ until sporulated and then stored at 4 ℃ until used. The cultures were transferred every 4 weeks. Spores suspended in autoclaved glycerol (15%-30%) (121 ℃, 15 minutes) were used for long-term storage at -70 ℃.

4. Harvesting and production of mycelia and spores in liquid culture

One liter flasks containing 200ml of modified bennett liquid medium or 200 ml of YGM (pH 6.5-7.5) medium were inoculated with 8ml of each seed culture for the production of viable cells of mycelia and spores of *Streptomyces* sp. WYE 20 and WYE 324. The inoculated flasks were incubated with shaking at 130 to 300 rpm at 25 ℃ to 33 ℃ for 3 to 7 days. Viable cells of mycelia and

spores produced through the above process were aseptically harvested by centrifugation at 4,000 rpm for 10 minutes.

5. Production of viable cells of mycelia and spores with delivery medium and preparation of antifungal biocontrol agent.

(1) Preparation of antifungal biocontrol agent in the form of powder comprising *Streptomyces* sp. WYE 20 or WYE 324 and a delivery medium

The delivery medium of the present invention which consisted of wheat bran of 40 to 65 w/w%, chitosan of 1 to 5 w/w%, wood sawdust of 30 to 55 w/w%, chitin of 1 to 3 w/w%, Pharmamedia (The Budkeye Oilseed Products Company, Fortworth, Texas, U.S.A.) of 1 to 3 w/w% on the basis of the total weight of the medium was thoroughly mixed. The resulting delivery medium was pelletized and spray-coated with sporulation medium (ATCC #5 or YGM medium) of 0.2 to 3.5 w/w%, and then autoclaved at 121 ℃ for 30 to 40 minutes. There was no effect on cellular growth when the sporulation medium was added below 0.2 w/w%, whereas the initial cellular growth was retarded when the sporulation medium was added over 3.5 w/w%.

One hundred to two hundred ml of *Streptomyces* sp. WYE 20 and WYE 324 (10^5-10^7 cfu/ml) were inoculated into the resulting autoclaved delivery medium and then incubated at 30 ℃ for 5 to 14 days. *Streptomyces* sp. WYE 20 and WYE 324 in the delivery medium were aseptically harvested and dried in U.V.- sterilized laminar flow bench. The dried product was aseptically blended to obtain an antifungal biocontrol agent comprising *Streptomyces* sp. WYE 20 or WYE 324 and the delivery medium.

(2) Preparation of antifungal biocontrol agent comprising *Streptomyces* sp. WYE 20 or WYE 324 and a liquid delivery medium.

Preparation of the antifungal biocontrol agent was carried out by incorporating viable cells of mycelia and spores of *Streptomyces* sp. WYE 20 or WYE 324 obtained in the above into autoclaved delivery medium of 1.0 to 3.0 w/w% of pectin and 0.1 to 0.6 w/w% of colloidal chitin in water.

6. Fungal Pathogens

The following fungal pathogens were used for an antifungal test: *Pythium ultimum, Pythium graminicola, Rhizoctonia solani, Rhizoctonia solani* AG 1 (IB), *Rhizoctonia solani* AG 2-2 (IV), *Fusarium oxysporum, Fusarium sambucinctum, Fusarium solani, Phytophthora capsici, Phytophthora parasitica, Sclerotinia sclerotiorum, Sclerotium cepivorum,* and *Verticillium dahliae.* All strains were grown on potato dextrose agar (Difco) or corn meal agar (Difco) at 25 ℃ and stored at 4 ℃.

7. Determination of Cell Number in Delivery Medium

One gram of delivery medium containing *Streptomyces* sp. WYE 20 or WYE 324 was added to 9ml of sterile distilled water and thoroughly mixed with vortexTM. The resulting suspension was serially diluted and spread on CMA plates to determine colony forming unit (cfu) per gram. The plates were incubated at 30 ℃ and colony formation was observed. The same method was used to determine cfu/ml of delivery medium in the form of liquid.

8. *In Vivo* Bioassay to Determine Antifungal Activity of *Streptomyces* sp. WYE 20 and WYE 324

The activity of *Streptomyces* sp. WYE 20 or WYE 324 to enhance plant growth and to reduce plant diseases caused by fungal pathogens was determined by treating cucumber, pepper, or golf course turfgrasses with *Streptomyces* sp. WYE 20 or WYE 324 in a delivery medium, and then planting treated and untreated seeds in a suitable growing medium or agricultural fields. The activity of *Streptomyces* sp. WYE 20 or WYE 324 in a delivery medium as a biocontrol agent was measured in terms of emergence, outgrowth of emerged plants, plant height, and capability of disease control.

EXAMPLE I

Isolation of *Streptomyces* sp. WYE 20 and WYE 324

Soil samples were obtained from four different rhizosphere-associated soils in Goesan, Chungbuk Province, Korea. Isolation of strains was carried out by serial-dilution/spread-plate technique on WYE agar plates. The obtained

strains were tested with respect to antagonistic activity against *Rhizoctonia solani* and *Phytophthora capsici;* enzyme activities exhibiting fungal cell wall degradation; root colonization, growth at a low temperature (4 ℃, 8 ℃); and the possibility of large cell mass production to isolate two novel strains. The two novel strains were named *Streptomyces* sp. WYE 20 and WYE 324, respectively. Purification of *Streptomyces* sp. WYE 20 and WYE 324 was carried out by incubating the strains on WYE plates at 25 ℃ for 4 to 10 days to allow the strains to sporulate, and then their colonies were isolated and streaked onto new WYE agar plates to obtain pure cultures. Pure cultures of *Streptomyces* sp. WYE 20 and WYE 324 were transferred to CYD agar slants, incubated at 25 ℃ - 30 ℃ until sporulated, and stored at 4 ℃. The cultures were transferred every 4 weeks.

EXAMPLE II

Identification and Characterization of *Streptomyces* sp. WYE 20 and WYE 324

The morphological, physiological, and chemical characteristics of microorganisms obtained in Example I were investigated on the basis of Bergey's Manual of Systematic Bacteriology (1989). The results are shown in Tables I to V.

Table I

Medium	Cellular Growth		Aerial Mycelium		Substrate Mycelium		Color of Spore Mass	
	WYE 20	WYE 324	WYE 20	WYE 324	WYE 20	WYE 324	WYE 20	WYE 324
ISP #2	Good	Good	Abun-dant	Abun-dant	Light Yellow	Light Yellow	Grayish Pink	Grayish Pink
ISP #3	Good	Good	Abun-dant	Abun-dant	Pale Yellow	Pale Yellow	Grayish Pink	Grayish Pink
ISP #4	Good	Good	Abun-dant	Abun-dant	Pale Yellow	Pale Yellow	Grayish Pink	Grayish Pink

Table IIa

Characteristics	Strain WYE 20	Strain WYE 324
Spore chain morphology	Spirales	Spirales
Spore chain ornamentation	Smooth	Smooth
Spore shape	Cylinderical	Cylinderical
Diffusible pigments	-	-
Melanin production	+ (brown-dark brown)	+ (brown-dark brown)
Antimicrobial activity:		
Aspergillus niger	+	+
Bacillus subtilis NCIB 3610	+	+
Streptomyces murinus	+	+
Candida albicans	-	-
Saccharomyce cerevisiae CBS 1172	-	-
Micrococcus luteus NCIB 196	+	+
Lecithinase activity	+	+
Pectin hydrolysis	-	-
Skim milk hydrolysis	+	+
Starch hydrolysis	+	+
Nitrate reduction	+	+
H_2S production	$+^{4week}$	-

Table IIa(continue)

Characteristics	Strain WYE 20	Strain WYE 324
Antibiotic resistance (μg/ml):		
Oleandomycin (100)	-1mm giz	-1mm giz
Neomycin (50)	+	+
Rifampicin (50)	-2mm giz	-
Lincomycin (100)	+	+
Novobiocin (100)	-	-
Ganamycin (100)	-2mm giz	-3mm giz
Ampicilin (100)	+	+
Streptomycin (100)	-3mm giz	-2mm giz
Kasugomycin (100)	+	-1mm giz
Tetracycline (100)	+	+
Chloramphenicol (100)	-	-3mm giz
	-: growth inhibition zone (giz) > 4 mm	+: no growth inhibition zone

Table IIb

Characteristics	Strain WYE 20	Strain WYE 324
Degradation activity:		
Xanthine	-	-
Elastin	+	+
Arbutin	-	-
Xylan	+	+
L-tyrosine	+	+
Allantoin	+	+
Growth at 45 ℃	-	-
Growth at pH 4.3	-	-
Growth in the presence of chemical inhibitors (%, w/v):		
NaCl (7.0)	-	-
Sodium azide (0.01)	-	-
Phenol (0.1)	+	+
Potassium tellurite (0.001)	+	+
Crystal violet (0.0001)	+	-
Neolin (0.5)	+	+
Neolin (1.0)	+	+
Tween 20 (0.5)	+	+
Tween 20 (1.0)	+	+
Rizorax™ (0.1)	+	+
Rizorax™ (1.0)	+	+
Gochutan™ (0.1)	+	+
Smirax™ (0.1)	-	-
Lidomil MG™ (0.125)	+	±
Lidomil MG™ (0.25)	-	-
Angcol™ (0.1)	-	-

Table IIb(continue)

Characteristics	Strain WYE 20	Strain WYE 324
Nitrogen utilization (0.1% w/v):		
L-asparagine	+	+
DL- α -amino-n-butyric acid	-	-
L-cysteine	±	+
L-valine	±	+
L-phenylalanine	-	±
L-histidine	+	+
L-hydroxyproline	+	+
L-arginine	+	+
L-methionine	±	+
Potassium nitrate	-	-
L-serine	+	+
L-threonine	+	+

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Table IIc

Characteristics	Strain WYE 20	Strain WYE 324
Carbon utilization(1.0% w/v):		
Sucrose	+	+
Meso-inositol	-	-
Mannitol	-	-
L-Rhamnose	+	-
Raffinose	-	-
D-Melezitose	+	+
Adonitol	-	-
D-Melibiose	±	±
Dextran	±	-
Xylitol	±	-
L-Arabinose	±	±
D-Fructose	+	+
D-Galactose	+	+
D-Glucose	+	+
D-Salicin	±	+
D-Xylose	+	+
Sorbose	+	+
D-Lactose	+	+
D-Mannose	+	+
Trehalose	+	+
Maltose	+	+
Cellobiose	+	+
Inulin	-	-
Sodium acetate (0.1)	+	+
Sodium citrate (0.1)	+	+
Sodium malonate (0.1)	±	±
Sodium propionate (0.1)	+	+
Sodium pyruvate (0.1)	+	+
Diaminopimelic acid	LL	LL

Table IV

Fatty acid	*Streptomyces* sp. WYE 324						
	Content (%)						
	1	2	3	4	Minimum	Maximum	Average
Saturated fatty acid							
14:0	0.28	0.26	0.42	0.45	0.26	0.45	0.35
15:0	2.22	2.19	1.23	1.25	1.23	2.22	1.72
16:0	4.34	4.40	5.89	6.19	4.34	6.19	5.21
17:0	0.40	0.43	-	-	nd	nd	nd
Unsaturated fatty acid							
15:1 B	0.20	0.15	-	-	nd	nd	nd
16:1 cis 9	6.40	6.39	5.66	5.54	5.54	6.40	6.00
17:1 cis 9	1.40	1.43	0.78	0.71	0.71	1.43	1.08
Methyl group branch							
13:0 iso	0.08	0.10	-	-	nd	nd	nd
13:0 anteiso	0.13	0.14	-	-	nd	nd	nd

Table IV(continue)

Fatty acid	*Streptomyces* sp. WYE 324						
	Content (%)						
	1	2	3	4	Minimum	Maximum	Average
14:0 iso	1.64	1.62	2.18	2.30	1.62	2.30	1.94
15:0 iso	7.48	7.48	8.15	8.10	7.48	8.15	7.80
15:0 anteiso	30.25	30.10	31.65	31.76	30.10	31.76	30.94
16:1 iso H	2.59	2.57	3.34	3.23	2.57	3.34	2.93
16:0 iso	14.05	14.06	16.67	16.69	14.05	16.69	15.37
16:0 9? CH_3	3.56	3.56	3.29	3.12	3.12	3.56	3.38
17:1 anteiso C	6.68	6.65	5.97	5.90	5.90	6.68	6.30
17:0 iso	1.77	1.82	2.14	2.16	1.77	2.16	1.97
17:0 anteiso	10.78	10.95	10.68	10.72	10.68	10.95	10.78
17:0 10 CH_3	0.18	0.18	-	-	nd	nd	nd
18:1 iso H	0.49	0.50	-	-	nd	nd	nd
Hydroxyl group branch							
17:0 iso 2OH	0.50	0.51	-	-	nd	nd	nd

Table III(continue)

Fatty acid	*Streptomyces* sp. WYE 20						
	Content (%)						
	1	2	3	4	Minimum	Maximum	Average
Unknown 17.595 SM	0.78	0.82	0.50	0.55	0.50	0.82	0.66

Note: The first and second time: Cells grown in Trypticase Soy Broth (TSB).

The third and fourth time: Cells grown on Trypticase Soy Agar (TSA).

- : no detection. nd: not determined.

Table IV

Fatty acid	*Streptomyces* sp. WYE 324						
	Content (%)						
	1	2	3	4	Minimum	Maximum	Average
Saturated fatty acid							
14:0	0.28	0.26	0.42	0.45	0.26	0.45	0.35
15:0	2.22	2.19	1.23	1.25	1.23	2.22	1.72
16:0	4.34	4.40	5.89	6.19	4.34	6.19	5.21
17:0	0.40	0.43	-	-	nd	nd	nd
Unsaturated fatty acid							
15:1 B	0.20	0.15	-	-	nd	nd	nd
16:1 cis 9	6.40	6.39	5.66	5.54	5.54	6.40	6.00
17:1 cis 9	1.40	1.43	0.78	0.71	0.71	1.43	1.08
Methyl group branch							
13:0 iso	0.08	0.10	-	-	nd	nd	nd
13:0 anteiso	0.13	0.14	-	-	nd	nd	nd

Table IV(continue)

Fatty acid	*Streptomyces* sp. WYE 324						
	Content (%)						
	1	2	3	4	Minimum	Maximum	Average
14:0 iso	1.64	1.62	2.18	2.30	1.62	2.30	1.94
15:0 iso	7.48	7.48	8.15	8.10	7.48	8.15	7.80
15:0 anteiso	30.25	30.10	31.65	31.76	30.10	31.76	30.94
16:1 iso H	2.59	2.57	3.34	3.23	2.57	3.34	2.93
16:0 iso	14.05	14.06	16.67	16.69	14.05	16.69	15.37
16:0 9? CH_3	3.56	3.56	3.29	3.12	3.12	3.56	3.38
17:1 anteiso C	6.68	6.65	5.97	5.90	5.90	6.68	6.30
17:0 iso	1.77	1.82	2.14	2.16	1.77	2.16	1.97
17:0 anteiso	10.78	10.95	10.68	10.72	10.68	10.95	10.78
17:0 10 CH_3	0.18	0.18	-	-	nd	nd	nd
18:1 iso H	0.49	0.50	-	-	nd	nd	nd
Hydroxyl group branch							
17:0 iso 2OH	0.50	0.51	-	-	nd	nd	nd

Table IV(continue)

Fatty acid	*Streptomyces* sp. WYE 324						
	Content (%)						
	1	2	3	4	Minimum	Maximum	Average
17:0 3OH	0.24	0.19	-	-	nd	nd	nd
Cyclopropane							
17:0 Cyclopropane	3.15	3.14	1.95	1.89	1.89	3.15	2.53
Unknown 17.595 SM	0.68	0.69	-	-	nd	nd	nd

Note: The first and second time: Cells grown in Trypticase Soy Broth (TSB).

The third and fourth time: Cells grown on Trypticase Soy Agar (TSA).

- : no detection. nd: not determined.

Table V

Characteristics	*Streptomyces* sp. WYE 20	*Streptomyces colombiensis* ATCC 27425	*Streptomyces* sp. WYE 324	*Streptomyces goshikiensis* ATCC 23914
Spore chain: Rectiflexibles	-	+	-	-
Spore chain: Retinaculiaperti	-	-	-	-
Spore chain: Spirales	+	+	+	+
Spore chain: Verticillati	-	-	-	-
Spore surface ornamentation: Smooth	+	+	+	+
Spore surface ornamentation: Rugose	-	-	-	-
Color of spore mass: Red	-	+	-	+
Color of spore mass: Gray	-	-	-	-
Color of spore mass: Green	-	-	-	-
Diffusible pigment: Red/Orange	-	-	-	-
Diffusible pigment: Yellow/Brown	-	-	-	-
Melanin production	+	+	+	+
Fragmentation	-	-	-	-
Use of DL- α -amino-n-butyric acid(0.1%, w/v)	-	-	-	-

Table V(continue)

Characteristics	*Streptomyces* sp. WYE 20	*Streptomyces colombiensis* ATCC 27425	*Streptomyces* sp. WYE 324	*Streptomyces goshikiensis* ATCC 23914
Use of L-histidine (0.1%, w/v)	+	-	+	-
Use of L-hydroxyproline (0.1%, w/v)	+	+	+	-
Lecithinase activity	+	+	+	+
Pectin hydrolysis	-	-	-	-
Nitrate reduction	+	+	+	+
H2S Production	+	+	-	-
Bacillus subtilis NCIB	+	+	+	+
Streptomyces murinus	+	+	+	+
Aspergillus niger	+	+	+	+
Xanthin degradation activity	-	+	-	+
Allantoin degradation activity	+	+	+	+
Arbutin degradation activity	-	+	-	-
Neomycin (50 μ g/ml) resistance	+	+	+	+
Rifampicin(50 μ g/ml) resistance	-	-	-	-

Table V(continue)

Characteristics	*Streptomyces* sp. WYE 20	*Streptomyces colombiensis* ATCC 27425	*Streptomyces* sp. WYE 324	*Streptomyces goshikiensis* ATCC 23914
Growth at 45 ℃	-	-	-	-
Growth at NaCl (7%, w/v)	-	-	-	-
Growth at sodium azide (0.01%, w/v)	-	-	-	-
Growth at phenol (0.1%, w/v)	+	+	+	+
Utilization of D-xylose (1.0%, w/v)	+	-	+	-
Utilization of meso-inositol (1.0%, w/v)	-	-	-	-
Utilization of mannitol (1.0%, w/v)	-	-	-	-
Utilization of D-fructose (1.0%, w/v)	+	+	+	+
Utilization of L-rhamnose (1.0%, w/v)	+	-	-	-
Utilization of raffinose (1.0%, w/v)	-	-	-	-
Utilization of inulin (1.0%, w/v)	-	-	-	-
Utilization of adonitol (1.0%, w/v)	-	-	-	-
Utilization of cellobiose (1.0%, w/v)	+	+	+	+

The two strains obtained in Example I were identified as the genus *Streptomyces*, as a result of analyzing the data of Tables I to V on the basis of Bergey's Manual of Systematic Bacteriology (1989); International Streptomyces Project (ISP) (1974)); and William et al (1983).

The two strains were named as WYE 20 and WYE 324, respectively. The strain WYE 20 might belong to the species *Streptomyces colombiensis* or a closely related species in cluster 61 on the basis of the morphological, physiological, and chemical characteristics of the genus *Streptomyces*, but the strain was identified as a noble strain of the genus *Streptomyces*. Strain WYE 324 might belong to the species *Streptomyces goshikiensis* or a closely related species in cluster 61 on the basis of the morphological, physiological, and chemical characteristics of the genus *Streptomyces*, but the strain was identified as a noble strain of the genus *Streptomyces*.

The strains WYE 20 and WYE 324 were deposited in the Korean Culture Type Collection (KCTC), Korea Research Institute of Bioscience and Biotechnology, Taejon, Korea on June 18, 1997 under the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure, assigned with the Accession No. KCTC 0341BP and KCTC 0342BP, respectively.

EXAMPLE III

Optimum Temperature for The Cellular Growth of *Streptomyces* sp. WYE 20 and WYE 324

To investigate the effect of temperature on the growth of the *Streptomyces* sp. WYE 20 and WYE 324, spores of strains WYE 20 and WYE 324 were streak-inoculated on modified bennett agar plates. The streaked plates were incubated at predetermined temperatures of 4 ℃, 8 ℃, 27 ℃, 37 ℃, and 45 ℃ for 3 weeks. The growth was recorded at 7, 14, and 21 days. The results are shown as good growth (+); slow growth (±); no growth (-) in Table VI.

Table VI

Strain	4 ℃		8 ℃		27 ℃		37 ℃		45 ℃	
	7 days	21 days	7 days	14 days	7 days	14 days	7 days	14 days	7 days	14 days
WYE 20	±	+	+	+	+	+	±	+	-	-
WYE 324	-	±	±	+	+	+	±	+	-	-

As shown in Table VI, strain WYE 20 represented slow growth after a week of incubation at 4 ℃ and good growth after 3 weeks' incubation, whereas strain WYE 324 represented slow growth after 3 weeks' incubation at 4 ℃. Strain WYE 20 represented good growth at both 8 ℃ and 27 ℃. However, strain WYE 324 represented slow growth after a week of incubation at 8 ℃ and represented good growth after 2 weeks' incubation at 8 ℃. In addition, strain WYE 324 represented good growth at 27 ℃. Strains WYE 20 and WYE 324 represented slow growth after a week of incubation at 37 ℃ and represented good growth after 2 weeks incubation at 37 ℃, but they did not grow at 45 ℃.

EXAMPLE IV

Enzyme Activity and Antagonism Assay

(1) Enzyme Activity Assay

To assay the enzyme activity of the strains WYE 20 and WYE 324, both chitinase and β-1,3-glucanase were used which were known as enzymes capable of parasitizing/inhibiting fungal pathogens.

In order to determine the chitinase activity of strains WYE 20 and WYE 324, each strain was streaked on CM agar plates which contained colloidal chitin as a sole carbon source, and then incubated at 30 ℃ for 14 days. Colony formation and growth were observed. The strains WYE 20 and WYE 324 were proved to have a chitinase activity from the above.

To determine β-1,3-glucanase activity of the strains WYE 20 and WYE 324, each strain was streaked on laminarin agar plates which contained laminarin as a sole carbon source, and then incubated at 27 ℃ for 7 days. Colony formation and growth were observed. The strains WYE 20 and WYE 324 were proved to have a β-1,3-glucanase activity from the above.

(2) Antagonism Assay

An *in vitro* plate assay was performed to test the antagonistic activity of strains WYE 20 and WYE 324 in terms of growth inhibition of fungal phytopathogens. Each strain was streak-inoculated on CMA plates and incubated at 30 ℃ for 6 to 10 days. A PDA (Potato Dextrose Agar) block containing actively growing fungal mycelia was then aseptically placed in the center of the plate and incubated at 25 ℃ for 24 to 192 hours. The above PDA block was inoculated into CMA plates not containing strain WYE 20 or WYE 324 and used as a control. The bioassay was replicated on three plates. The antagonism was recorded as a degree of inhibition of mycelial growth of fungal pathogens as shown in Table VII.

Table VII.

Fungal Pathogens	Antagonistic activity	
	WYE 20	WYE 324
Pythium ultimum[1]	+	+
Pythium graminicola[2]	++	++
Fusarium oxysporum	++	++
Fusarium sambucinctum	++	nt
Fusarium solani	++	++
Rhizoctonia solani	++	++
Rhizoctonia solani AG 1 (IB)	++	++
Rhizoctonia solani AG 2-2 (IV)	++	++
Phytophthora capsici	++	++
Phytophthora parasitica	++	++
Sclerotinia sclerotiorum	++	++
Sclerotium cepivorum[3]	++	++
Verticillium dahliae[3]	++	nt

[1] 24 hour incubation, [2]36 hour incubation, [3]192 hour incubation.

Remnant: 96 hour incubation

++: Strong inhibition with zone of inhibition $\geq$ 1.0 cm

+: Growth definitely retarded, with obvious zone of inhibition near colony

-: Not antagonistic, or very weakly antagonistic.

n.t.: not tested.

As shown in Table VII, strains WYE 20 and WYE 324 showed an obvious zone of inhibition against *Pythium ultimum*. Strain WYE 20 showed strong antagonism against *Pythium graminicola, Fusarium oxysporum, Fusarium*

sambucinctum, Fusarium solani, Rhizoctonia solani, Rhizoctonia solani AG 1 (IB), *Rhizoctonia solani* AG 2-2 (IV), *Phytophthora capsici, Phytophthora parasitica, Sclerotinia sclerotiorum, Sclerotium cepivorum* and *Verticillium dahliae.* In addition, strain WYE 324 also showed strong antagonism against *Pythium graminicola, Fusarium oxysporum, Fusarium solani, Rhizoctonia solani, Rhizoctonia solani* AG 1 (IB), *Rhizoctonia solani* AG 2-2 (IV), *Phytophthora capsici, Phytophthora parasitica, Sclerotinia sclerotiorum,* and *Sclerotium cepivorum.*

EXAMPLE V

Preparation of Antifungal Biocontrol Agent in the Form of Powder

A delivery medium was prepared by mixing wheat bran, chitosan, wood sawdust, chitin, and Pharmamedia according to Table VIII. The delivery medium was pelletized using an extruder. In the process of pelletizing, the degree of pellet formation was determined and recorded as good in the case of good pellet formation without debris and disruption; whereas recorded as poor in the case of poor pellet formation. The results are shown in Table VIII. It was also determined as poor, moderate, and good according to the air penetration and cellular growth of the delivery medium. The results are shown in Table VIII.

One hundred fifty ml of the culture of *Streptomyces* sp. WYE 20 or WYE 324 (10^5-10^7 cfu/ml) obtained in "Materials and Methods" were inoculated into the resulting autoclaved delivery medium and then incubated at 30 ℃ for 5 to 14 days. The cultures of *Streptomyces* sp. WYE 20 or WYE 324 in each delivery medium were aseptically harvested and dried in a U.V.- sterilized laminar flow bench. The dried product was aseptically blended to obtain an antifungal biocontrol agent consisting of *Streptomyces* sp. WYE 20 or WYE 324 and the delivery medium, in the form of powder.

The number of cells of WYE 20 or WYE 324 in the delivery medium was adjusted to 10^5-10^{10} cfu/g delivery medium. The resulting antifungal biocontrol agent which consisted of WYE 20 or WYE 324 and a delivery medium was kept

at 4 ℃ and 25 ℃ for 3 months. The viability and growth of WYE 20 or WYE 324 in the delivery medium was determined at intervals of one month and recorded at 3 months.

One gram of delivery medium containing WYE 20 or WYE 324 was added to 9ml of sterile distilled water and thoroughly mixed by vortax. The cfu per gram of the resulting suspension was determined by serial-dilution and spread-plate technique and shown in Tables VIII and IX.

Table VIII

(unit: w/w%)

Delivery Medium (D.M.)	Wheat bran	Chitosan	Wood sawdust	Chitin	Pharma media	Pellet formation	Air penetration	Cellular growth
D.M. 1	65	1	30	2	2	Good	Moderate	Moderate
D.M. 2	65	2	30	1	2	Good	Moderate	Moderate
D.M. 3	65	2	30	2	1	Good	Moderate	Moderate
D.M. 4	65	1	30	1	3	Good	Moderate	Moderate
D.M. 5	65	1	30	3	1	Good	Moderate	Moderate
D.M. 6	63	5	30	1	1	Good	Moderate	Moderate
D.M. 7	60	5	30	2	3	Good	Moderate	Moderate
D.M. 8	50	1	45	2	2	Good	Good	Good
D.M. 9	45	5	45	2	3	Good	Good	Good
D.M. 10	45	3	50	1	1	Good	Good	Good
D.M. 11	40	3	55	1	1	Good	Good	Good
D.M. 12	40	1	55	3	1	Good	Good	Good

Table VIII(continue)

(unit: w/w%)

Delivery Medium (D.M.)	Wheat bran	Chitosan	Wood sawdust	Chitin	Pharma media	Pellet formation	Air penetration	Cellular growth
D.M. 13	40	1	55	1	3	Good	Good	Good
Control 1	90	2	5	2	1	Good	Poor	Poor
Control 2	80	5	10	3	2	Good	Poor	Poor
Control 3	70	3	25	1	1	Good	Poor	Poor
Control 4	30	5	60	2	3	Poor	Good	nt
Control 5	20	5	70	2	3	Poor	Good	nt
Control 6	10	5	80	2	3	Poor	Good	nt

nt: not tested

Table IX

(unit: w/w%)

Delivery Medium (D.M.)	Wheat bran	Chitosan	Wood sawdust	Chitin	Pharma media	Initial cell no. of WYE 20 or WYE 324(cfu/g)	Cell NO. of WYE 20 or WYE 324 after 3 months (cfu/g)		Cellular growth
							at 4 ℃	at 25 ℃	
D.M. 1	65	1	30	2	2	$1.2x10^7$	$1.0x10^6$	$2.3x10^5$	Good
D.M. 2	65	2	30	1	2	$1.9x10^7$	$1.5x10^6$	$2.9x10^5$	Good
D.M. 3	65	2	30	2	1	$1.3x10^7$	$1.4x10^6$	$2.5x10^5$	Good
D.M. 4	65	1	30	1	3	$2.1x10^7$	$1.7x10^6$	$3.6x10^5$	Good
D.M. 5	65	1	30	3	1	$2.0x10^7$	$1.2x10^6$	$3.1x10^5$	Good
D.M. 6	63	5	30	1	1	$2.7x10^7$	$1.1x10^6$	$2.7x10^5$	Good
D.M. 7	60	5	30	2	3	$1.5x10^7$	$1.3x10^6$	$3.3x10^5$	Good
D.M. 8	50	1	45	2	2	$1.6x10^8$	$2.1x10^7$	$2.1x10^6$	Good
D.M. 9	45	5	45	2	3	$2.6x10^8$	$1.1x10^7$	$2.5x10^6$	Good

Table IX(continue)

(unit: w/w%)

Delivery Medium (D.M.)	Wheat bran	Chitosan	Wood sawdust	Chitin	Pharma media	Initial cell no. of WYE 20 or WYE 324(cfu/g)	Cell NO. of WYE 20 or WYE 324 after 3 months (cfu/g)		Cellular growth
							at 4 ℃	at 25 ℃	
D.M. 10	45	3	50	1	1	$2.1x10^8$	$1.0x10^7$	$3.1x10^6$	Good
D.M. 11	40	3	55	1	1	$1.9x10^8$	$1.6x10^7$	$3.5x10^6$	Good
D.M. 12	40	1	55	3	1	$1.8x10^8$	$1.9x10^7$	$2.4x10^6$	Good
D.M. 13	40	1	55	1	3	$2.2x10^8$	$1.3x10^7$	$1.9x10^6$	Good

As shown in Tables VIII and IX, D.M. 1 to 13 were good and moderate in pellet formation, air penetration, and cellular growth, whereas Controls 1 to 6 with a different range of components were poor in pellet formation, air penetration, or cellular growth.

In addition, D.M. 1 to 13 pellets were coated with sporulation medium of YGM and determined the effects on cellular growth and viability. The results are shown in Table X.

Table X

(unit: w/w%)

Delivery Medium (D.M.)	Wheat bran	Chitosan	Wood sawdust	Chitin	Pharma media	YGM*	Initial cell no. of WYE 20 or WYE 324 (cfu/g)	Cell No. of WYE 20 or WYE 324 after 3 months (cfu/g)		Cellular growth
								at 4 ℃	at 25 ℃	
D.M. 14	65	1	30	2	2	0.2	$1.1x10^7$	$1.2x10^6$	$2.4x10^5$	Good
D.M. 15	65	2	30	1	2	0.5	$1.4x10^7$	$1.4x10^6$	$2.9x10^5$	Good
D.M. 16	65	2	30	2	1	1.0	$1.6x10^7$	$1.6x10^6$	$2.6x10^5$	Good
D.M. 17	65	1	30	1	3	1.5	$2.0x10^7$	$1.9x10^6$	$4.2x10^5$	Good
D.M. 18	65	1	30	3	1	1.8	$2.2x10^7$	$1.3x10^6$	$3.2x10^5$	Good
D.M. 19	63	5	30	1	1	2.0	$1.7x10^7$	$2.1x10^6$	$3.7x10^5$	Good
D.M. 20	60	5	30	2	3	3.5	$1.1x10^7$	$2.3x10^6$	$3.4x10^5$	Good
D.M. 21	50	1	45	2	2	3.5	$1.2x10^8$	$2.3x10^7$	$2.3x10^6$	Good
D.M. 22	45	5	45	2	3	2.5	$1.6x10^8$	$2.1x10^7$	$2.5x10^6$	Good
D.M. 23	45	3	50	1	1	2.0	$2.2x10^8$	$1.3x10^7$	$3.2x10^6$	Good

Table X

(unit: w/w%)

Delivery Medium (D.M.)	Wheat bran	Chitosan	Wood sawdust	Chitin	Pharma media	YGM*	Initial cell no. of WYE 20 or WYE 324 (cfu/g)	Cell No. of WYE 20 or WYE 324 after 3 months (cfu/g)		Cellular growth
								at 4 ℃	at 25 ℃	
D.M. 24	40	3	55	1	1	1.5	$2.9x10^8$	$2.1x10^7$	$3.6x10^6$	Good
D.M. 25	40	1	55	3	1	0.5	$1.4x10^8$	$1.9x10^7$	$2.6x10^6$	Good
D.M. 26	40	1	55	1	3	0.2	$1.2x10^8$	$1.3x10^7$	$2.9x10^6$	Good

YGM*: w/w% of total weight.

As shown in Table IX and X, the cells of WYE 20 and WYE 324 were stabilized in D.M. 1 to 26 for a long period of time (> 3 months). The cells of WYE 20 and WYE 324 in D.M. 1 to 26 were shown $> 10^5$ cfu/g which was required for the biocontrol activity after 3 months storage. In particular, when D.M. 14 to 26 coated with the medium of YGM were used, the stability of the cells of WYE 20 and WYE 324 was improved.

EXAMPLE VI

Preparation of Biocontrol Agents in the Form of Suspension

A delivery medium comprising 20g of pectin and 2g of colloidal chitin and the remaining water on the basis of the total volume of 1.0 liter distilled water was autoclaved at 121 ℃ for 15 minutes. Preparation of a biocontrol agent in the form of suspension was achieved by incorporating *Streptomyces* sp. WYE 20 or WYE 324 as described in "Materials and Methods", into the resulting autoclaved delivery medium (1.2×10^7 cfu/ml).

The resulting product was kept at 4 ℃. The form of biocontrol agent was suitable for the treatment of plant seeds and seedling beds by in-furrow application, or spraying after dilution with water.

EXAMPLE VII

Antifungal Activity Assay

In vivo biocontrol assays were carried out to determine the efficacy of biocontrol agents prepared in Example V and Example VI. The biocontrol agents were tested for their ability to reduce fungal diseases and enhance plant growth by treating plants, plant seeds, plant roots, seedling beds, pots, potting mixtures, or soil.

(1) Biocontrol Activity to Rhizoctonia Damping-off of Cucumber (*Cucumis sativus* L.).

A biocontrol assay was carried out to test the efficacy of WYE 20 as a biocontrol agent in inhibiting Rhizoctonia damping-off of cucumber.

Cucumber seeds were treated with 20 ml of WYE 20 (1.2×10^7 cfu/ml) prepared in Example VI by immersing seeds therein for three hours and then being seeded in pots. Seeds were immersed for three hours in sterilized distilled water and a delivery medium, respectively; each resulting seed was used as a control.

Hortus (England) was used as a potting mixture. The potting mixture was autoclaved at 121 ℃ for 60 minutes, and placed for 12 hours at 25 ℃, and then autoclaved at 121 ℃ for 60 minutes again. This autoclaving cycle was repeated three times.

Rhizoctonia solani was cultured on PDB (Potato dextrose broth: Difco) at 25 ℃ for 14 days, harvested and mixed with autoclaved potting mixture so as to obtain an approximately 60% disease incidence. The cucumber seeds were treated with biocontrol agent and each control seed was seeded in pots containing the potting mixture into which *Rhizoctonia solani* had been artificially inoculated.

Disposable paper cups (diameter of 9cm) were used as seedling pots. Six cups containing three seeds per cup were prepared in each test. The cups were placed in a random block design in a glasshouse. Moisture was kept between 40% and 60% and additional water was supplied as needed. The temperature was maintained between 25 ℃ and 30 ℃ in daylight.

The results of emergence and occurrence of damping-off were periodically recorded. The final results are shown in Table XI.

Table XI

Treatments	No. Of Cucumber Seeds	Emerged Cucumber Seeds (%)	Healthy Plants (%)	Fresh Weight (g)/above ground
		7 days	14 days	18 days
No pathogen + control	18	18b[x] (100)	18b[x] (100)	2.61b[x]
Rhizoctonia solani + control	18	7a (39)	7a (39)	1.73a
Rhizoctonia solani + Liquid D.M. treatment	18	8a (44)	8a (44)	1.75a
Rhizoctonia solani + Liquid D.M. of WYE 20	18	18b (100)	18b (100)	2.51b

[x]Means in a column followed by the same letter are not significantly different at the P = 0.05 level. D.M.: Delivery Medium.

As shown in Table XI, cucumber seeds treated with WYE 20 in delivery medium as a biocontrol agent did not show Rhizoctonia damping-off of cucumber. In addition, cucumber plants from control seeds showed severe growth retardation resulting from *Rhizoctonia solani,* whereas plants from treated seeds did not. The results showed that WYE 20 was effective in controlling *Rhizoctonia solani* and in enhancing plant growth.

(2) Biocontrol Activity Against Powdery Mildew of Cucumber (*Cucumis sativus* L.).

A biocontrol assay was carried out to test the efficacy of WYE 324 (1.2 x 10^7 cfu/ml) in the biocontrol agent prepared in Example VI in inhibiting powdery

mildew of cucumber plants in pots.

Disposable paper cups (diameter of 9cm) were used as seedling pots. One cucumber seed was seeded in each cup containing potting mixture consisting of agricultural field soil and Hortus (England) (4:1 v/v ratio). The cups were placed in a glasshouse. Additional water was supplied as needed and the temperature was maintained between 25 ℃ and 30 ℃ in daylight.

Twenty ml of the biocontrol agent containing WYE 324 were sprayed on one cucumber plant (14-day-old) at the beginning of the assay and sprayed one more time after a week. Plants sprayed with the same amounts of water were used as a control. Three cucumber plants (one cucumber plant per cup) of each treated group and the control group were prepared in the assay.

To naturally induce powdery mildew of cucumber caused by *Sphaerotheca fuliginea*, three cups per group and three diseased cucumber plants (one plant per cup) were used and set in a random block arrangement in a glasshouse. Moisture was kept between 70% and 90% and additional water was supplied as needed. The temperature was maintained between 25 ℃ and 30 ℃ in a glasshouse. The incidence of cucumber powdery mildew was investigated. The experiment was continued for 2 weeks and the assay was repeated. The results are shown in Table XII.

Table XII

Treatments	Incidence of Powdery Mildew of Cucumber	
	Experiment 1	Experiment 2
Control	+	+
WYE 324 Treated	-	-

As shown in Table XII, powdery mildew was not detected in cucumber plants sprayed with the biocontrol agent containing WYE 324; whereas the disease was observed in control plants. The results showed that WYE 324 was

effective in controlling powdery mildew of cucumber (*Cucumis sativus* L.).

(3) Biocontrol Activity of *Streptomyces* sp. WYE 20 or WYE 324 to Rhizoctonia Brown Patches of Golf Green Creeping Bentgrass

A biocontrol assay was carried out to test the efficacy of *Streptomyces* sp. WYE 20 and WYE 324 in inhibiting Rhizoctonia brown patch of golf green creeping bentgrass.

Streptomyces sp. WYE 20 and WYE 324 produced by liquid culture as described in "Materials and Methods" above were used in this assay. The cell number of *Streptomyces* sp. WYE 20 or WYE 324 was adjusted to 2.0×10^5 cfu/ml. The assay was carried out on each block treated with WYE 20 or WYE 324, or control. Four blocks (1.5m × 1.5m) of each treated group and control group were prepared on a golf green. The assay was carried out in a random block design. Strain WYE 20 or WYE 324 was sprayed every 7 - 10 days in the experimental blocks from June 25, 1996 to August 23, 1996. Two and a half liters of WYE 20 or WYE 324 per block were used each time; whereas the control blocks were provided with the same amount of water. No chemical fungicides were used in the experimental period. The incidence of Rhizoctonia brown patches was periodically recorded and shown in Table XIII (Site 1) and Table XIV (Site 2).

As shown in Table XIII (Site 1) and Table XIV (Site 2), the incidence of Rhizoctonia brown patches was dramatically reduced in blocks treated with WYE 20 or WYE 324. The results showed that WYE 20 and WYE 324 were effective in inhibiting Rhizoctonia brown patches of golf green creeping bentgrass.

Table XIII (Site 1)

Treatments	Incidence of Rhizoctonia Brown Patches (%)		
	7/25/96	8/6/96	8/23/96
Control	10.18a[x]	14.6a[x]	59a[x]
WYE 20 treated	3.38b	9.2b	24b
WYE 324 treated	0.00c	5.2c	24b

[x]Means in a column followed by the same letter are not significantly different at the P = 0.05 level.

Table XIV (Site 2)

Treatments	Incidence of Rhizoctonia Brown Patches (%)		
	7/25/96	8/6/96	8/19/96
Control	5.6a[x]	9.3a[x]	28.3a[x]
WYE 20 treated	0.0b	0.8b	5.3b
WYE 324 treated	0.0b	0.0b	0.7c

[x]Means in a column followed by the same letter are not significantly different at the P = 0.05 level.

(4) Biocontrol Activity to Rhizoctonia Large Patches of a Fairway Turfgrass (*Zoysia japonica*).

A biocontrol assay was carried out to test the efficacy of biocontrol agents including *Streptomyces* sp. WYE 20 or strain IBT 678 prepared in Example VI in inhibiting Rhizoctonia large patches of fairway turfgrass (*Zoysia japonica*). The cell number of *Streptomyces* sp. WYE 20 or strain IBT 678 in a biocontrol agent was adjusted to 2.0×10^6 cfu/ml.

Soils infested with *Rhizoctonia solani* AG 2-2 were inoculated into a fairway turfgrass (*Zoysia japonica*) and then planted in pots (diameter of 25cm). Two hundred ml of the biocontrol agent were treated once per week for two weeks and three weeks in 1996 and 1997, respectively. Pots treated with the same amounts of water were used as a control group. Three pots per group were used and set in a random block arrangement. To naturally induce disease, the pots were placed near the fairway of a golf course from the beginning of September to the beginning of October, 1996 as well as from the beginning of March to the end of June, 1997, respectively. The incidence of Rhizoctonia large patches was investigated. Large patches were not detected in pots treated with the biocontrol agent containing WYE 20; whereas the disease was observed in control and IBT 678 treated pots as shown in Table XV.

Table XV

Treatments	Incidence of Large patches of *Zoysia japonica*	
	Fall 1996	Spring 1997
Pathogen + Control	+	+
Pathogen + WYE 20 Treated	-	-
Pathogen + IBT 678 Treated	Not tested	+

As shown in Table XV, the results showed that WYE 20 was effective in controlling Rhizoctonia large patches of a fairway turfgrass (*Zoysia japonica*).

(5) Biocontrol Activity Against Phytophthora Blight of Pepper Plant(*Capsicum annuum* L.)

A biocontrol assay was carried out to test the efficacy of WYE 20 or WYE 324 in the biocontrol agent of Example VI in inhibiting Phytophthora blight of pepper seedlings in pots.

Pepper seeds were submerged in sterilized water for 2 days and treated with WYE 20 or WYE 324 in the biocontrol agent of Example VI by submerging them for 18 hours (treating group). The cell numbers of *Streptomyces* sp. WYE 20 and WYE 324 were adjusted to 1.2 x 10^7 cfu/ml and 1.7 x 10^7 cfu/ml, respectively. Pepper seeds were submerged in sterilized water for 2 days and then for 18 hours. They were used as a control group. On the other hand, pepper seeds submerged in sterilized water for 2 days were replaced in the delivery medium of Example VI for 18 hours. They were used as the other control group.

As described in the assay with cucumber above, *Phytophthora capsici* cultured in PDB (Potato dextrose broth: Difco) at 25 ℃ for 14-21 days was harvested and inoculated into a sterilized potting mixture (Hortus, England) to obtain an infested potting mixture with a disease incidence of 80% of Phytophthora blight for control pepper seedlings. The resulting potting mixture infested with *Phytophthora capsici* was used to determine the efficacy of WYE 20 or WYE 324 in the delivery medium in inhibiting phytophthora blight of pepper seedlings in the assay.

Disposable paper cups (diameter of 9.0cm) were used as seedling pots. Fourteen cups containing three seeds per cup were prepared in each test. Experiments were performed in a glasshouse at a temperature of 25 ℃ to 32 ℃ in daylight. In the glasshouse, pots were set in a random block arrangement. The moisture was maintained at 80% and additional water was sprayed on the top of the pots as needed.

The emergence and incidence of Phytophthora blight of pepper plants were recorded as shown in Table XVI.

Table XVI

Treatments	No. of Planted Pepper Seeds	No. of Emerged Pepper Seeds (Emergence: %)	Pepper seedlings showing Phytophthora blight (disease incidence: %)	
		14 days	14 days	18 days
No pathogen + Control	42	37(88a[x])	0(0c[x])	0(0c[x])
Phytophthora capsici + Control	42	34(81a)	28(82a)	33(97a)
Phytophthora capsici + liquid D.M. Treated	42	35(83a)	29(83a)	34(97a)
Phytophthora capsici + WYE 20 Treated	42	34(81a)	12(35b)	26(76b)
Phytophthora capsici + WYE 324 Treated	42	36(86a)	22(61c)	28(78b)

[x]Means in a column followed by the same letter are not significantly different at the P = 0.05 level.

As shown in Table XVI, there was a significant reduction in Phytophthora blight of pepper plants in the seeds treated with WYE 20 or WYE 324 as compared to the control group. The result showed that WYE 20 and WYE 324 of the present invention were effective in controlling Phytophthora blight of pepper plants.

(6) An Assay to Determine Plant Growth Enhancement Using Pepper Seedlings

A seedling assay was carried out to determine the efficacy of WYE 20 and WYE 324 in a biocontrol agent of the present invention in enhancing the growth of pepper plants.

Pepper seeds submerged in sterilized water for 2 days were treated with WYE 20 or WYE 324 in a biocontrol agent as prepared in Example V (D.M. 22) (1000 seeds per 4g biocontrol agent). The cell numbers of *Streptomyces* sp. WYE 20 and WYE 324 prior to seed treatment were adjusted to 1.2×10^7 cfu/ml and 1.7×10^7 cfu/ml, respectively. Pepper seeds were submerged in sterilized water and used as a control group (Control 1). Meanwhile, pepper seeds were treated with the delivery medium (1000 seeds per 4g D.M.22) not containing WYE 20 or WYE 324 and used as the other control group (Control 2).

The planting of the pepper seeds was carried out in the same way as the cucumber seedlings shown above.

Disposable paper cups (diameter of 9.0cm) were used as seedling pots. One hundred and twenty one cups containing one seed per cup were prepared in each test. Experiments were performed in a glasshouse at a temperature of 25 ℃ to 32 ℃ in daylight. In the glasshouse, pots were set in a random block arrangement. The moisture was maintained in a range of 40% to 60% and additional water was sprayed on the top of the pots as needed. Fifteen ml of cell culture broth of WYE 20 or WYE 324 obtained in "Materials and Methods" were inoculated into the seedling pot treated with the biocontrol agent after 4 weeks of cultivation. The same amount of water was applied to the control groups. The experiments were continued for 9 weeks and the results were shown in Table

XVII.

Table XVII

Treatments	Pepper Seedlings	Plant Height (cm) (average)
Control 1	121	23.7a[x]
Control 2	121	24.5a
WYE 20 Treated	121	27.4b
WYE 324 Treated	121	28.6b,c

[x]Means in a column followed by the same letter are not significantly different at the P = 0.05 level.

As shown in Table XVII, there was a significant enhancement in the growth of the plants from the pepper seeds treated with WYE 20 or WYE 324 as compared to those plants germinated from control seeds. This indicates that WYE 20 or WYE 324 of the present invention is highly effective in enhancing the growth of pepper plants.

(7) A Biocontrol Assay Using Pepper Seedlings in Agricultural Fields

A biocontrol assay was carried out to determine the efficacy of a biocontrol agent of the present invention for controlling Phytophthora blight and for enhancing plant growth in agricultural fields.

Pepper seeds submerged in sterilized water for 2 days were immersed in a biocontrol agent prepared in Example VI for 3 hours. The seeds were treated with a biocontrol agent (D.M.22) (1,000 seeds per 4g) prepared in Example V. The cell numbers of *Streptomyces* sp. WYE 20 and WYE 324 prior to seed treatment were adjusted to 1.2×10^7 cfu/ml and 1.7×10^7 cfu/ml, respectively.

Pepper seeds were submerged in sterilized water for 2 days and 3 hours

again. Thus the pepper seeds were treated with the same delivery medium *not* containing WYE 20 or WYE 324 and used as a control group. Each seed was planted in seedling beds. Water was sprayed as needed and a temperature of between 20 ℃ and 35 ℃ was maintained. When seedlings were grown to 1.5 to 2.0cm in height, the pepper seedlings were transplanted into a seedling tray consisting of 25 seedling holes (5cm x 5cm, 6cm depth) containing a mixture of fine sandy potting soil and WYE 20 or WYE 324 in the delivery medium (0.1g per seedling hole). Control pepper seedlings were transplanted into seedling holes containing the same amount of sandy potting soil and the same amount of delivery medium only. These seedling pots were incubated in a greenhouse at a temperature of 18 ℃ to 35 ℃ and additional water was supplied as needed. In the greenhouse, pots were set in a random block arrangement. After 11 weeks of growth in the greenhouse, each plant was transplanted to agricultural fields. Before a week of transplantation, 10 ml culture broth of WYE 20 or WYE 324 (1.2-1.7 x 10^5cfu/ml) were added per seedling hole of treating group. The same amount of water was supplied to control seedling pots.

The disease incidence and the growth of the transplanted peppers were observed periodically and the mean was recorded as shown in Table XVIII (Field 1) and XIX (Field 2).

Table XVIII (Field 1)

Treatments	No. of Transplanted Pepper Plants	Plant Height (cm) at 62 days after transplantation	Incidence of phytophthora Blight (%)	
			62 days after transplantation	101 days after transplantation
Control	1,340	78.5a[x]	28.4a[x]	98.6a[x]
WYE 20 Treated	1,340	86.0b	23.5b	89.2b
WYE 324 Treated	1,340	90.5c	8.7c	76.1c

[x]Means in a column followed by the same letter are not significantly different at the P = 0.05 level.

Table XIX (Field 2)

Treatments	No. of Transplanted Pepper Plants	Incidence of Phytophthora Blight (%)	
		82 days after transplantation	101 days after transplantation
Control	860	26a[x]	42.4a[x]
WYE 20 Treated	860	0b	7.6b
WYE 324 Treated	860	0b	2.0c

[x]Means in a column followed by the same letter are not significantly different at the P = 0.05 level.

As shown in Table XVIII and XIX, there was a significant enhancement in the growth and reduction of Phytophthora blight in the plants from the pepper seeds treated with WYE 20 or WYE 324 as compared to those plants germinated from control seeds. This indicates that WYE 20 and WYE 324 of the present invention are highly effective in controlling Phytophthora blight and in enhancing plant growth of pepper in agricultural fields.

Having provided examples of embodiments of the present invention and preferred embodiments, it will be apparent to those skilled in the art that changes and modifications may be made without departing from the present invention and its broader aspects. Therefore, numerous variations, modifications, and embodiments are to be regarded as being within the true spirit and scope of the present invention.

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Jones and Samac, "Biological control of fungi causing alfalfa seedling damping-off with a disease-suppressive strain of *Streptomyces,"* Biological Control 7:196-204 (1996).

Lee and Rho, "Characteristics of spores formed by surface and submerged cultures of *Streptomyces albidoflavus* SMF301," J. of Gen. Microbiol. 139:3131-3137 (1993).

What is claimed is :

1. A biologically pure culture of a microorganism of *Streptomyces* sp. WYE 20 and *Streptomyces* sp. WYE 324, having the identifying characteristics of KCTC 0341BP and KCTC 0342BP, respectively.

2. Antifungal biocontrol agent including a microorganism of a biologically pure culture of *Streptomyces* sp. WYE 20 and WYE 324.

3. The microorganism of Claim 1 and 2 wherein the microorganism is able to protect susceptible plants against fungal infection.

4. The microorganism of Claim 3 wherein protection is conferred against fungal pathogens that cause seedling diseases including damping-off and root rot as well as foliar diseases including powdery mildew of cucumber, Phytophthora blight of pepper, or Rhizoctonia brown/large patches of turfgrasses.

5. Antifungal biocontrol agent of Claim 2 wherein the biocontrol agent comprises a biologically pure culture of *Streptomyces* sp. WYE 20 or WYE 324; and a delivery medium.

6. Antifungal biocontrol agent of Claim 5 wherein the delivery medium consists of 40 to 65 w/w% of wheat bran, 1 to 5 w/w% of chitosan, 30 to 55 w/w% of wood sawdust, 1 to 3 w/w% of chitin, and 1 to 3 w/w% of Pharmamedia on the basis of the total weight of the delivery medium.

7. Antifungal biocontrol agent of Claim 6 wherein the delivery medium further consists of 0.2 to 3.5 w/w% of sporulation medium.

8. Antifungal biocontrol agent of Claïm 6 and 7 wherein the biologically pure culture of *Streptomyces* sp. WYE 20 or WYE 324 comprises 10^5-10^{10} colony forming unit per gram of delivery medium.

9. Antifungal biocontrol agent of Claim 5 wherein the delivery medium consists of 1.0 to 3.0 w/w% of pectin and 0.1 to 0.6 w/w% of colloidal chitin in water.

10. Antifungal biocontrol agent of Claim 9 wherein the biologically pure culture of *Streptomyces* sp. WYE 20 or WYE 324 comprises 10^5-10^{10} colony forming unit per gram of delivery medium.

11. A method of manufacturing antifungal biocontrol agent comprises an incubation process for the production of a biologically pure culture of *Streptomyces* sp. WYE 20 or WYE 324; and

a downstream process.

12. The incubation process of Claim 11 wherein the process comprises incubation at 130 to 300 rpm at 25 ℃ to 33 ℃ for 3 to 7 days.

13. The downstream process of Claim 11 wherein the process comprises lyophilization of harvested pure culture of *Streptomyces* sp. WYE 20 or WYE 324.

14. The downstream process of Claim 11 wherein the process comprises incorporation of harvested pure culture to a proper delivery medium.

15. A method of manufacturing antifungal biocontrol agent wherein the method comprises the steps of:

preparation of delivery medium consisting of 40 to 65 w/w% of wheat bran, 1 to 5 w/w% of chitosan, 30 to 55 w/w% of wood sawdust, 1 to 3 w/w% of chitin, and 1 to 3 w/w% of Pharmamedia on the basis of the total weight of the delivery medium;

autoclaving the resulting delivery medium;

incorporation of *Streptomyces* sp. WYE 20 (KCTC 0341BP) or *Streptomyces* sp. WYE 324 (KCTC 0342BP) into the delivery medium;

incubating incorporated cells of *Streptomyces* sp. WYE 20 (KCTC 0341BP) or *Streptomyces* sp. WYE 324 (KCTC 0342BP) at 25 ℃ to 33 ℃ for 5 to 14 days; and

aseptically drying the resulting product.

16. A method of manufacturing antifungal biocontrol agent of Claim 15 wherein the method comprises aseptically blending the resulting dried product.

17. The preparation of delivery medium of Claim 15 wherein the preparation comprises palletizing the delivery medium; and

coating the resulting pellet with 0.2 to 3.5 w/w% of sporulation medium.

18. The incorporation of Claim 15 wherein *Streptomyces* sp. WYE 20 or

WYE 324 comprises 10^5-10^{10} colony forming unit per gram of delivery medium.

19. The autoclaving of Claim 15 wherein the autoclaving comprises at 121 ℃ for 30 to 40 minutes.

20. A method of manufacturing antifungal biocontrol agent wherein the method comprises the steps of:

preparation of delivery medium consisting of 1.0 to 3.0 w/w% of pectin, 0.1 to 0.6 w/w% of colloidal chitin, and the remaining water; and

autoclaving the resulting delivery medium; and

incorporation of *Streptomyces* sp. WYE 20 (KCTC 0341BP) or *Streptomyces* sp. WYE 324 (KCTC 0342BP) into the delivery medium.

21. The incorporation of Claim 20 wherein *Streptomyces* sp. WYE 20 or WYE 324 comprises 10^5-10^{10} colony forming unit per gram of delivery medium.

22. A method of use of antifungal biocontrol agent of Claim 2 to Claim 10 wherein the use comprises coating, mixing, spraying or in-furrow application to plant seeds, potting mixtures, growing plants, or soils.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR 98/00015

A. CLASSIFICATION OF SUBJECT MATTER

IPC[6]: C 12 N 1/20; A 01 N 63/00 // (C 12 N 1/20; C 12 R 1:465)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC[6]: C 12 N 1/20; A 01 N 63/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPIL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	US 4 595 589 A (TAHVONEN) 17 June 1986 (17.06.86), claims.	1-3,22
A	EP 0 408 811 A1 (KEMIRA OY) 23 January 1991 (23.01.91), claims.	1-3,22
A	WO 93/18 135 A1 (RESEARCH CORPORATION TECHNOLGIES, INC.) 16 September 1993 (16.09.93), abstract.	1-3,22

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 April 1998 (16.04.98)	28 April 1998 (28.04.98)

Name and mailing address of the ISA/ AT	Authorized officer
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INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/KR 98/00015

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