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(54) **NOVEL MICROORGANISM AND PLANT DISEASE CONTROL AGENT USING THE MICROORGANISM**

NEUARTIGER MIKROORGANISMUS UND PFLANZENSCHÄDLINGSBEKÄMPFUNGSMITTEL MIT DEM MIKROORGANISMUS

NOUVEAU MICROORGANISME ET AGENT DE LUTTE CONTRE LES MALADIES DES PLANTES UTILISANT LE MICROORGANISME

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(56) References cited:  
**WO-A1-00/58442 WO-A1-93/15611**  
**WO-A1-98/21964 WO-A1-98/50422**  
**WO-A2-2008/009795 JP-A- 5 051 305**  
**JP-A- 8 175 919 JP-A- 2003 277 210**  
**US-A- 5 047 239 US-A- 5 589 381**

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- **DATABASE DDBJ/EMBL/GENBANK [Online] 30 March 2006 ALENIUS, H.T. ET AL.: 'DEFINITION: Bacillus subtilis subs. subtilis partial 16S rRNA gene, isolate OS-109', XP008140297 Database accession no. AM237381**
- **SEIICHI FURUYA ET AL.: 'Prevention of grapevine diseases using antagonistic microbes.' J.ASEV vol. 19, no. 2, 12 July 2008, JPN., pages 72 - 73, XP008140432**

Remarks:

The file contains technical information submitted after the application was filed and not included in this specification

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**Description**

## TECHNICAL FIELD

5 **[0001]** The present invention relates to a new species of Bacillus subtilis and a plant disease control agent using the bacterial cell and the culture of this microorganism.

## BACKGROUND ART

10 **[0002]** Recently, there has been an increased interest in biological control techniques using a biological pesticide, which has less environmental load and a higher level of safety for humans and animals compared to chemical pesticides. However, a biological pesticide has a less instantaneous effect and a less therapeutic effect compared to chemical pesticides and can control only a limited range of pathogenic fungi and also loses the efficacy of the biological pesticide itself when used in combination with a chemical pesticide. Therefore, the market share of biological pesticides has  
15 currently remained only 0.4% of the total market of pesticides.

**[0003]** The technique using a biological pesticide utilizes an organism that is a natural enemy of phytopathogenic microorganisms and pests that cause plant diseases. Bacillus subtilis has been conventionally used as a biological pesticide against the fungi causing botrytis rot in eggplants, tomatoes, or grapes, since it has antagonistic actions such as growth inhibition and a fungicidal action on phytopathogenic fungi.

20 **[0004]** For example, a method for controlling plant diseases which includes a spore fraction prepared from a culture of Bacillus bacteria such as Bacillus subtilis so as to contain 50% or more by weight of spore based on the dry weight is conventionally known as a biological pesticide using Bacillus subtilis (see Patent Literature 1). Furthermore, in order to control plant diseases caused by Phytophthora, a disease control agent is known which includes the bacterial cell or the culture of Bacillus bacteria such as Bacillus licheniformis, and moreover organic acids and salts thereof produced  
25 by the bacteria as an active ingredient (see Patent Literature 2).

**[0005]** Furthermore, a plant disease control agent including the bacterial cell or the culture of Bacillus sphaericus is known (see Patent Literature 3), and a plant disease control agent using specific strains of Bacillus subtilis as an active ingredient is also known (see Patent Literatures 4 and 5).

30 **[0006]** However, the effects of the above-mentioned conventional plant disease control agents in the case of application to a grape are mediated by antagonistic actions by Bacillus bacteria that occupy the nutrition or living area on grape tissues. Therefore, although some preventive effect is produced by spraying prior to the occurrence of diseases, the agents have no therapeutic effect such as a bactericidal action, and thus have been unable to be used after the occurrence of diseases.

35 **[0007]** Furthermore, the above-mentioned conventional plant disease control agents are effective only for the fungi causing grape botrytis rot among the pathogenic fungi of the most common three grape diseases, grape botrytis rot, downy mildew, and ripe rot. Thus, they cannot be expected to have a control effect on the fungi causing downy mildew and ripe rot other than grape botrytis rot or other pathogenic fungi for grapes.

40 **[0008]** Furthermore, although the above-mentioned conventional plant disease control agents settle onto grape leaves, the level of settlement onto pericarps was low. It was also difficult to use a chemical pesticide for safety reasons since grape pericarps are also put into the mouth, and the control of diseases such as botrytis rot and ripe rot, which also occur in fruits, has been difficult.

## CITATION LIST

## 45 PATENT LITERATURE

**[0009]**

Patent Literature 1:

50 Japanese Patent Application Laid-Open No. Hei. 8-175919

Patent Literature 2:

Japanese Patent Application Laid-Open No. 2001-206811

Patent Literature 3:

Japanese Patent Application Laid-Open No. 2003-277210

55 Patent Literature 4:

Japanese Patent Application Laid-Open No. Hei. 6-133763

Patent Literature 5:

Japanese Patent Application Laid-Open No. Hei. 5-51305

see also WO 00/58442, WO98/50422, WO 98/21964 and WO 2008/009795.

## SUMMARY OF THE INVENTION

### 5 PROBLEMS TO BE SOLVED BY THE INVENTION

**[0010]** Therefore, the problem to be solved by the invention is to provide a microorganism which is expected to have both a preventive effect and a therapeutic effect and may be applied to various phytopathogenic fungi, and whose effects are not reduced even when used in combination with a chemical pesticide, and a plant disease control agent using the  
10 microorganism.

### MEANS FOR SOLVING THE PROBLEMS

**[0011]** To solve the above-mentioned problem, the microorganism according to the present invention is characterized by being the Bacillus subtilis strain KS1 (NITE BP-569).  
15

**[0012]** Furthermore, the plant disease control agent according to the present invention is characterized by including the culture and/or the microorganism bacterial cell of the above-mentioned Bacillus subtilis strain KS1 as an active ingredient.

**[0013]** Moreover, the plant disease control agent of the present invention acts effectively on the pathogenic fungus that appear on the leaves or in the fruits of grapes. By way of example, the inventive plant disease control agent is effective against the fungi causing botrytis rot, the fungi causing downy mildew, the fungi causing ripe rot, and the like.  
20

**[0014]** Furthermore, since the plant disease control agent according to the present invention has resistance to chemical pesticides, it can be used in combination with a chemical pesticide.

### 25 EFFECTS OF THE INVENTION

**[0015]** The Bacillus subtilis strain KS1 according to the present invention and the plant disease control agent that includes the culture or the microorganism bacterial cell of the Bacillus subtilis strain KS1 as an active ingredient can be expected to have a preventive effect and a therapeutic effect on a wide range of phytopathogenic fungi such as the fungi causing downy mildew, the fungi causing botrytis rot, and the fungi causing ripe rot.  
30

### BRIEF DESCRIPTION OF THE DRAWINGS

#### 35 **[0016]**

[Fig. 1] a photomicrograph showing the tips of hyphae of the fungi causing grape botrytis rot when the fungi were grown in dual culture with the Bacillus subtilis strain KS1 of the present invention.

[Fig. 2] a photomicrograph showing the tips of hyphae of the fungi causing grape ripe rot when the fungi were grown in dual culture with the Bacillus subtilis strain KS1 of the present invention.  
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### MODE FOR CARRYING OUT THE INVENTION

**[0017]** Hereinbelow, the present invention will be described in detail. The Bacillus subtilis strain KS1 according to the present invention is a bacterium discovered in grape pericarps and has features such as a high level of settlement on grape leaves. Hereinbelow, bacteriological properties of the Bacillus subtilis strain KS1 are listed.  
45

#### A. Morphology of Bacteria

#### 50 **[0018]**

- (1) Shape: bacillus
- (2) Size: 0.8 x 3 to 5  $\mu\text{m}$
- (3) Motility: no

55

#### B. Morphology of Colony (incubation at 31°C for 2 days)

#### **[0019]**

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- (1) Name of the Medium: SCD agar medium  
 (2) Shape: irregular circle  
 (3) Size: 5 mm  
 (4) Elevation: flat  
 (5) Margin Shape: undulate  
 (6) Surface Shape: wrinkled  
 (7) Texture: mucoid  
 (8) Transparency: opaque  
 (9) Luster: dull luster  
 (10) Color: light beige

C. Physiological properties

[0020]

[Table 1]

1	Gram staining	(+)	22	L-xylose	(-)	44	Lactose	(-)
2	Sporulation	(+)	23	Adonitol	(-)	45	Melibiose	(-)
3	Catalyse	(+)	24	Methyl- $\beta$ -D-xylopyranoside	(-)	46	Sucrose	(+)
4	$\beta$ -galactosidase	(+)	25	Galactose	(-)	47	Trehalose	(+)
5	Arginine dihydrolase	(-)	26	Glucose	(+)	48	Inulin	(-)
6	Lysine decarboxylase	(-)	27	Fructose	(+)	49	Melezitose	(-)
7	Ornithine decarboxylase	(-)	28	Mannose	(+)	50	Raffinose	(-)
8	Citrate utilization	(-)	29	Sorbose	(+)	51	Starch	(-)
9	H <sub>2</sub> S production	(-)	30	Rhamnose	(-)	52	Glycogen	(-)
10	Urease	(-)	31	Dulcitol	(-)	53	Xylitol	(-)
11	Tryptophan deaminase	(-)	32	Inositol	(+)	54	Gentiobiose	(-)
12	Indole production	(-)	33	Mannitol	(+)	55	D-turanose	(+)
13	Acetoin production	(+)	34	Sorbitol	(+)	56	D-lyxose	(-)
14	Gelatin liquefaction	(+)	35	Methyl- $\alpha$ -D-mannopyranoside	(-)	57	D-tagatose	(-)
15	Nitrate reduction	(+)	36	Methyl- $\alpha$ -D-glucopyranoside	(+)	58	D-fucose	(-)
16	Fermentation/oxidation		37	N-acetylglucosamine	(+)	59	L-fucose	(-)
17	Glycerol	(+)	38	Amygdalin	(-)	60	D-arabitol	(-)
18	Erythritol	(-)	39	Arbutin	(-)	61	L-arabitol	(-)
19	D-arabinose	(-)	40	Esculin	(+)	62	Gluconate	(-)
20	L-arabinose	(+)	41	Salicin	(-)	63	2-ketogluconate	(-)
21	Ribose	(+)	42	Cellobiose	(-)	64	5-ketogluconate	(-)
22	D-xylose	(+)	43	Maltose	(+)			

(+) Positive (-) :Negative ( $\pm$ ) :False-positive

[0021] The base sequence of rDNA corresponding to the 16S subunit is as follows.

[SEQ ID NO: 1]

5  
 10  
 15  
 20  
 25  
 30  
 35  
 40  
 45  
 50  
 55

**GACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGGACAGATGG  
 GAGCTTGCTCCCTGATGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAA  
 CCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGAT  
 GGTGTTTTGAACCGCATGGTTCGAACATAAAAGGTGGCTTCGGCTACCAC  
 TTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAATGGCTCAC  
 CAAGGCGACGATGCGTAGCCGAACCTGAGAGGGTGATCGGCCACACTGGG  
 ACTGAGACACGGCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCC  
 GCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTT  
 TCGGATTGTAAGCTCTGTTGTTAGGGAAGAACAAGTACCGTTCGAATAG  
 GGGGCGACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGC**

**[0022]** This bacterial strain was identified as Bacillus subtilis on the basis of the above-mentioned bacteriological properties and SEQ ID NO: 1, the base sequence of 16SrDNA, and designated Bacillus subtilis strain KS1. Incidentally, this Bacillus subtilis strain KS1 was deposited with the National Institute of Technology and Evaluation, Patent Microorganisms Depository (2-5-8 Kazusakamatari, Kisarazu-shi, Chiba) on May 13, 2008 and deposited under Accession No. "NITE P-569." The strain was converted to an international deposit under the Budapest Treaty on May 20, 2009 and received Accession No. "NITE BP-569."

**[0023]** Incidentally, the Bacillus subtilis strain KS1 according to the present invention is not limited to the base sequence that is completely identical to the base sequence of 16SrDNA shown in SEQ ID NO: 1 described above. The inventive Bacillus subtilis strain KS1 also includes those that even when the strains are composed of a base sequence in which one or several bases of the base sequence are deleted, substituted, or other bases are added thereto, have similar traits to the Bacillus subtilis strain KS1 regarding a plant disease control action, especially a grape disease control action.

**[0024]** The culture medium that can be used in the present invention is not particularly limited as long as the strain of the present invention can grow in culture. For example, glucose, sucrose, casein, yeast extract, meat extract, and the like are used as a carbon source, and yeast extract, meat extract, polypeptone, peptone, trypsin, and the like are used as a nitrogen source. Sodium, potassium, magnesium, iron, calcium, and the like are also added as other nutrients.

**[0025]** Culture in the present invention may be performed under aerobic conditions, for example with aeration-agitation, by shaking culture, by a solid culture method, or the like. Although the culture condition is not particularly limited, temperatures ranging from 30 to 37°C, pH ranging from 6.5 to 7.5, and time periods ranging from 12 to 48 hours are suitable.

**[0026]** The Bacillus subtilis strain KS1 cultured as described above may be used without being separated from the culture, or used after the separation of bacterial cells by centrifugation. Furthermore, in the case of using this strain as a plant disease control agent, it may be formulated with various additives such as a surfactant (for example, sorbitan monolaurate commonly known as Tween20) and a spreader (for example, SABUMAHJI from Syngenta Japan) and used as a granule, an emulsifiable concentrate, a water dispersible powder, a flowable, and the like.

**[0027]** The bacterial cell or the culture thus prepared of the Bacillus subtilis strain KS1 may be applied to a plant body or soil, thereby controlling plant diseases. The control agent of the present invention has a significant effect on the control of grape diseases caused by, for example, the fungi causing grape botrytis rot, the fungi causing grape ripe rot, the fungi causing grape white root-rot, the fungi causing grape downy mildew, and the like. In addition, the control agent also has an effect on the control of the fungi causing botrytis rot and the fungi causing anthracnose, which infect plants other than a grape, such as strawberries and cucumbers.

**[0028]** The application method of the control agent is selected as appropriate depending on the form of the formulation, the type of the plant, and the type and degree of the disease. For example, a method in which a liquefied control agent is used for ground application of the liquid formulation or aerial application of the liquid formulation, a method of directly spraying or applying the control agent onto plant leaves, moreover, a method by immersion into a solution of the control agent, or the like may be selected.

**[0029]** Although the application amount varies depending on, for example, the type of diseases and plants for application, for example, the control agent is applied at a concentration of  $1.5 \times 10^8$  cells/mL (containing 0.02% Tween20) and using 5 liters per are of the field.

## Examples

[0030] Hereinbelow, the present invention will be described in detail on the basis of Examples.

5 Example 1: Example of culture

[0031] A portion of the Bacillus subtilis strain KS1 according to the present invention taken by means of a platinum loop was inoculated on a broth medium (10 g of meat extract, 10 g of peptone, 5 g of sodium chloride, and 1 L of water), and incubated with shaking at 150 rpm for 12 hours at 37°C to obtain the culture medium. The bacterial cells of strain KS1 were harvested from this culture medium by centrifugation. Then, the harvested bacterial cells of strain KS1 were adjusted with water to 1.3 to 1.5 x 10<sup>8</sup> cells/mL and Tween20 (at a final concentration of 0.02%) was added as a surfactant, thereby formulating a flowable.

15 Example 2: Application to pathogenic bacteria, test 1

[0032] In this example, a test to determine the control effect of Bacillus subtilis strain KS1 on the fungi causing grape downy mildew was performed. The flowable prepared in Example 1 described above was sprayed onto ten-year-old Koshu grapes (espalier) planted in the experimental field of the Institute of Enology and Viticulture, Yamanashi University, and the effect was examined. Because completely pesticide-free farming has been performed since 2005 (Heisei 17) in this experimental field, grape downy mildew frequently occurs every year. Thus, the above-mentioned flowable was sprayed evenly on young shoots of grapes once per week, every week from May 11 to August 17 in 2007 (Heisei 19). The application rate was approximately five liters per are of the field. In parallel, as a comparative example to this example, a commercially available microbial pesticide, a Botokiller water dispersible powder, (registered trademark, Idemitsu Kosan Co., Ltd.) was sprayed at the same concentration and at the same frequency. An untreated area where no agents were sprayed was also compared.

[0033] Incidence of downy mildew was examined on August 21, 2007 (Heisei 19). Disease incidence (%) was calculated on the basis of the number of leaves that had developed symptoms of downy mildew, and also, disease severity (cm<sup>2</sup>) was calculated on the basis of the total area with symptoms in the experimental field. These results are shown in Table 2.

30 [Table 2]

	Disease incidence (%)	Disease severity (cm <sup>2</sup> )
<b><i>B. subtilis</i> KS1</b>	10	4
<b>Botokiller water dispersible powder</b>	50	301
<b>Untreated area</b>	60	299

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[0034] As is clear from the results in Table 2 described above, Bacillus subtilis strain KS1 was found to have a control effect, particularly a very high level of preventive effect on the fungi causing grape downy mildew.

40 Example 3: Application to pathogenic fungi, test 2

[0035] In this example, a test to determine a control effect of Bacillus subtilis strain KS1 on the fungi causing botrytis rot and the fungi causing ripe rot was performed. First, the pathogenic fungi to be examined (the fungi causing botrytis rot and the fungi causing ripe rot) were precultured on a PDA medium (one containing potato extract and glucose in agar) at 25°C for about 5 days. Next, a disk with a diameter of 6 mm was hollowed out of the grown-up mycelium using a cork borer and placed in the center of a new petri dish containing the PDA medium. Furthermore, dual culture was performed by linearly smearing Bacillus subtilis strain KS1 on the end of the same petri dish, and interaction between the fungal lawn of the examined fungi and Bacillus subtilis strain KS1 was monitored.

[0036] Figs. 1 and 2 are photomicrographs (200x) showing the tips of hyphae of the examined fungi after 3 days of dual culture at 25°C. These photographs show the state in which the tips of hyphae of both the fungi causing botrytis rot and the fungi causing ripe rot swelled and ruptured and the growth of the pathogenic fungi was inhibited. One of the mechanisms of this growth inhibition is presumed to result from fungicidal substances produced by Bacillus subtilis strain KS1. As is clear from this example, it was confirmed that Bacillus subtilis strain KS1 had a control effect, particularly a therapeutic effect due to a fungicidal action, on the fungi causing botrytis rot or the fungi causing ripe rot, which are among the main pathogenic fungi for grapes.

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## Example 4: Drug resistance test

[0037] In this example, a test for resistance of *Bacillus subtilis* strain KS1 to chemical pesticides was performed. First, the YBS medium (one containing yeast extract and meat extract in agar) containing the chemical pesticide of interest was prepared in a petri dish. The concentration (ppm) of the chemical pesticide at this time was the same as a general application concentration. Next, *Bacillus subtilis* strain KS1 was smeared on the surface of the above-mentioned medium containing the chemical pesticide and incubated at 37°C for 2 days. As a control area, a test for resistance of *Bacillus subtilis* isolated from a Botokiller water dispersible powder to chemical pesticides was performed in parallel. In this case, the suspension of the Botokiller water dispersible powder was spread on the YBS medium and the bacteria that appeared after overnight incubation at 37°C were considered as the bacteria constituting a Botokiller water dispersible powder. In the resistance test, the bacteria constituting a Botokiller water dispersible powder were smeared on the surface of the medium containing a chemical pesticide and incubated at 37°C for 2 days, as is the case with the above-mentioned *Bacillus subtilis* strain KS1.

[0038] After 2 days of incubation at 37°C, the *Bacillus subtilis* strain KS1 and the bacteria constituting a Botokiller water dispersible powder were macroscopically observed to see whether they had grown or not. When the growth of the bacteria was observed (when the bacteria had grown greatly), they were considered to be resistant to the chemical pesticide. Table 3 indicates the results of resistance tests on 11 chemical pesticides that are currently commonly used in viticulture.

[Table 3]

Chemical pesticides used in viticulture	Concentration (ppm)	<i>B. Subtilis</i> KS1	<i>B. subtilis</i> used in a Botokiller water dispersible powder
Azoxystrobin	150	+	+
Benomyl	200	+	+
Carbaryl	850	+	+
Diethofencarb	50	+	+
Fenhexamid	100	+	+
Fenitrothin	400	+	+
Imidacloprid	100	+	+
Maneb	100	+	-
Permerhrin	100	+	+
Procymidone	20	+	+
Thiophanate-methyl	100	+	+
<b>+ Good growth - No growth</b>			

[0039] According to the test results, the *Bacillus subtilis* strain KS1 of the present invention showed resistance to all the tested chemical pesticides and also showed resistance to Maneb (product name: Maneb-Dithane M water dispersible powder) that is widely used especially for downy mildew, ripe rot, and the like. Therefore, the possibility of effective combined application of the strain KS1 with chemical pesticides is suggested. Incidentally, the bacteria constituting a Botokiller water dispersible powder showed no resistance to the above-mentioned Maneb.

## INDUSTRIAL APPLICABILITY

[0040] The *Bacillus subtilis* strain KS1 of the present invention is effective against a wide variety of grape diseases such as the fungi causing downy mildew, the fungi causing botrytis rot, and the fungi causing ripe rot. This wide control effect means that the KS1 strain can decrease the number or the application amount of agents that are currently applied to control grape diseases (chemical pesticides and biological pesticides). Since the KS1 strain produces an effect such as a contribution to the reduction of labor or cost in viticulture in addition to environmental conservation, it has an industrial applicability as a biological pesticide.

## SEQUENCE LISTING

[0041]

&lt;110&gt; UNIVERSITY OF YAMANASHI

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5 <140> EP09794161.1

<141> 2009-07-03

<150> JP2008-181449

<151> 2008-07-11

10

<160> 1

<170> PatentIn version 3.1

15

<210> 1

<211> 499

<212> DNA

<213> *Bacillus subtilis* KS1

20

<400> 1

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25 aactccggga aaccggggct aataccgat ggttgttga accgatggt tcgaacataa 180  
aaggtggctt cggctaccac ttacagatgg acccgggcg cattagctag ttggtgaggt 240  
aatggctcac caaggcgacg atgcgtagcc gaacctgaga gggatgatcgg ccacactggg 300  
30 actgagacac ggcccagact cctacgggag gcagcagtag ggaatcttcc gcaatggacg 360  
aaagtctgac ggagcaacgc cgcgtgagtg atgaaggttt tcggattgta aagctctggt 420  
gttaggggaag aacaagtacc gttcgaatag ggggacgacct tgacgggtacc taaccagaaa 480  
35 gccacggcta actacgtgc 499

### Claims

40

1. An isolated microorganism being the *Bacillus subtilis* strain KS1 (NITE BP-569) belonging to *Bacillus subtilis*.
2. A plant disease control agent, comprising a culture and/or a microorganism bacterial cell of the *Bacillus subtilis* strain KS1 (NITE BP-569) as an active ingredient.
- 45 3. The plant disease control agent according to claim 2, wherein the culture and/or the microorganism bacterial cell of the *Bacillus subtilis* strain KS1 acts on a pathogenic fungus that appears on leaves or in fruits of grapes.
4. The plant disease control agent according to claim 2 or 3, wherein the culture and/or the microorganism bacterial cell of the *Bacillus subtilis* strain KS1 acts on a fungus causing botrytis rot, a fungus causing downy mildew, and a fungus causing ripe rot.
- 50 5. The plant disease control agent according to claim 2, wherein the culture and/or the microorganism bacterial cell of the *Bacillus subtilis* strain KS1 has resistance to chemical pesticides.

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**Patentansprüche**

1. Isolierter Mikroorganismus, nämlich der Stamm Bacillus subtilis KS1 (NITE BP-569), der zu *Bacillus subtilis* gehört.
- 5 2. Pflanzenschutzmittel, das eine Kultur und/oder eine Mikroorganismus-Bakterienzelle des Stammes Bacillus subtilis KS1 (NITE BP-569) als einen aktiven Bestandteil enthält.
3. Pflanzenschutzmittel nach Anspruch 2, bei dem die Kultur und/oder die Mikroorganismus-Bakterienzelle des Stammes Bacillus subtilis KS1 auf einen pathogenen Pilz wirkt, der auf Blättern oder in Früchten von Trauben auftritt.
- 10 4. Pflanzenschutzmittel nach Anspruch 2 oder Anspruch 3, bei dem die Kultur und/oder die Mikroorganismus-Bakterienzelle des Stammes Bacillus subtilis KS1 auf einen Botrytis-Fäule verursachenden Pilz, einen falschen Mehltau verursachenden Pilz und einen Reifefäule verursachenden Pilz wirkt.
- 15 5. Pflanzenschutzmittel nach Anspruch 2, bei dem die Kultur und/oder die Mikroorganismus-Bakterienzelle des Stammes Bacillus subtilis KS1 gegenüber chemischen Pestiziden resistent ist.

**Revendications**

- 20 1. Un microorganisme isolé étant la souche Bacillus subtilis KS1 (NITE BP-569) appartenant à *Bacillus subtilis*.
2. Un agent de lutte contre les maladies des plantes, comprenant une culture et/ou une cellule bactérienne du microorganisme de la souche Bacillus subtilis KS1 (NITE BP-569) en tant que principe actif.
- 25 3. L'agent de lutte contre les maladies des plantes selon la revendication 2, dans lequel la culture et/ou la cellule bactérienne du microorganisme de la souche Bacillus subtilis KS1 agit sur un champignon pathogène qui apparaît sur les feuilles ou dans les fruits de vigne.
- 30 4. L'agent de lutte contre les maladies des plantes selon la revendication 2 ou la revendication 3, dans lequel la culture et/ou la cellule bactérienne du microorganisme de la souche Bacillus subtilis KS1 agit sur un champignon causant la pourriture grise, un champignon causant le mildiou, et un champignon causant le coître.
- 35 5. L'agent de lutte contre les maladies des plantes selon la revendication 2, dans lequel la culture et/ou la cellule bactérienne du microorganisme de la souche Bacillus subtilis KS1 a une résistance aux pesticides chimiques.

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Fig. 1

Bacteria causing grape botrytis rot

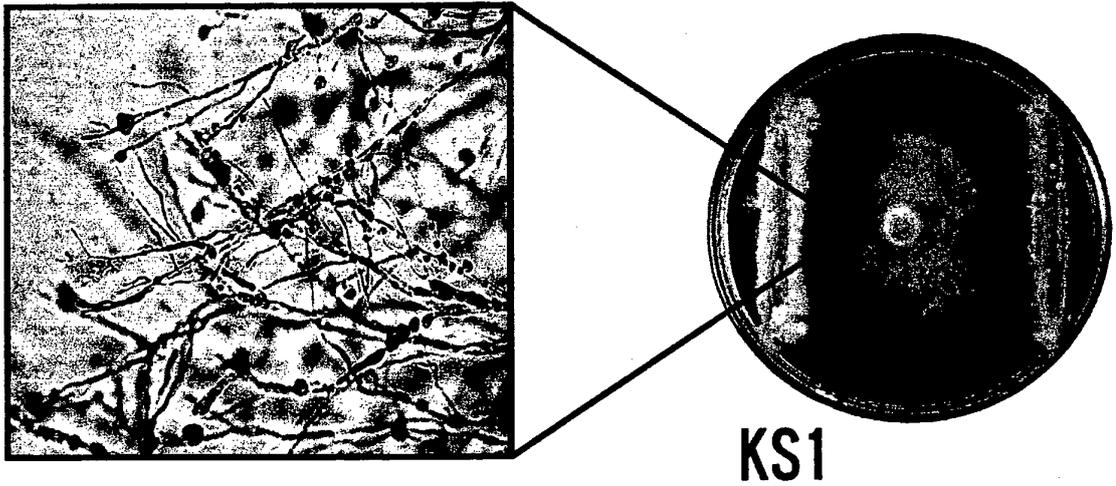
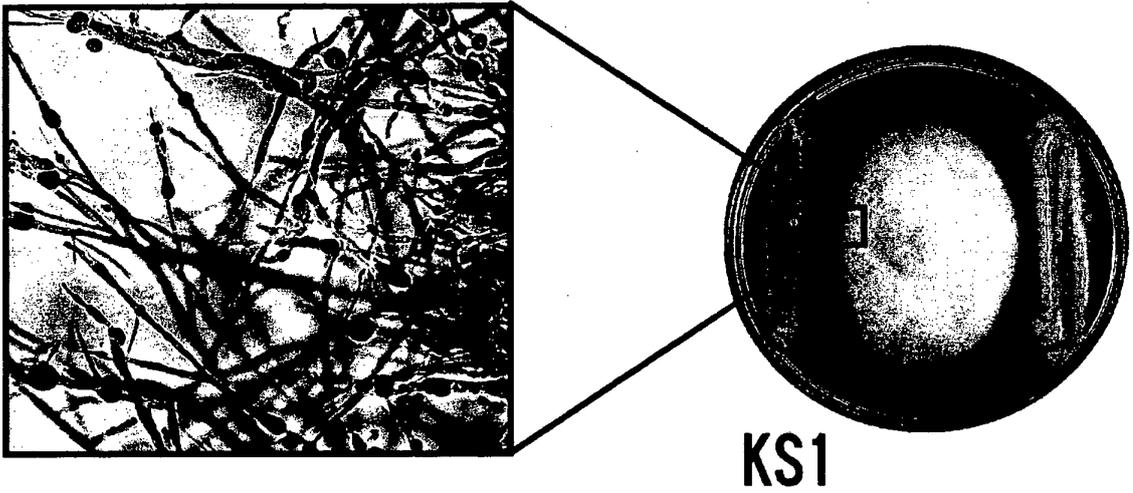


Fig. 2

Bacteria causing grape ripe rot



**REFERENCES CITED IN THE DESCRIPTION**

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