(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
11 February 2010 (11.02.2010)

PCT

(10) International Publication Number
WO 2010/015634 A1

(51) **International Patent Classification**:
C12Q 1/68 (2006.01)

(21) **International Application Number**:
PCT/EP2009/060107

(22) **International Filing Date**:
4 August 2009 (04.08.2009)

(25) **Filing Language**: English

(26) **Publication Language**: English

(30) **Priority Data**:
08162082.5 8 August 2008 (08.08.2008) EP

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(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

WO 2010/015634 A1

(54) **Title**: METHOD FOR SCREENING FOR A PLANT PROTECTANT

(57) **Abstract**: The invention relates to a method for screening for a plant protectant by using metagenomic analysis of the microbial community of a habitat and a method for generating a specific fingerprint and determining the phylum, subphylum, class, order, family, genus and/or species of the microorganisms of the habitat and analyzing correspondingly the impact of a potential plant protectant.

Method for screening for a plant protectant

The invention relates to a method for screening for a plant protectant by using metagenomic analysis of the microbial community of a habitat and a method for generating a specific fingerprint and determining the phylum, subphylum, class, order, family, genus and/or species of the microorganisms of the habitat and analyzing correspondingly the impact of a potential plant protectant.

The ever-increasing world population and the dwindling supply of arable land available for agriculture fuels research towards increasing the efficiency of agriculture. The efficiency and the yield are diminished diseases and pests.

Plant disease caused by pathogens such as fungi, bacteria or viruses are a significant economic cost to plant-based industries. Losses may arise through losses of plants themselves or through reduction in growth and fruiting abilities. Traditionally, control of plant pathogens has been pursued through the application of chemicals such as fungicides. The use of chemicals is subject to a number of disadvantages.

Pesticides are expensive and frequent application may lead to pesticide resistance in agricultural pests. A further risk due to overuse of pesticides are unwanted effects of residual pesticides. In addition, most pesticides have either a broadband pesticidal activity or a pesticidal activity directed to one or a few defined pests.

Less is known about side effects of the pesticidal activity. But side effects is meanwhile of utmost importance, hence environmental issues in the agriculture and food industry have become a priority with the goal of reducing negative effects of pesticide use.

Rising economic, environmental and social costs associated with agricultural inputs, pesticide residues, government legislation and admission proceeding make new screening method for a plant protectant necessary.

Methods for identifying a compound that has a biocidal effect against a selected organism are known. According to conventional procedure the candidate active ingredients are applied to suitable test organisms in order to find novel pesticides, whereby the survival rate of the pest organisms is measured. A second approach is based on the inhibition of a characteristic enzyme, which is essential for the survival of the pest organism, by the the candidate active ingredients.

These methods are directed to the immediate relationship between the active ingredient and the pest organism, neglecting the effect on the habitat and environment as a whole.

A further problem is the fact that at the moment the majority of all microbes have not yet been cultured and therefore a simulation of environmental conditions is not possible in in vitro experiments. Additionally it has to be taken into consideration that

the microbial constitution of a habitat depends on soil composition or climactic conditions so that the effect of a pesticide will be different from location to location with regard to the ecology of the habitat as a whole.

A method for analyzing the whole microbial population of a habitat is the metagenomic analysis.

Metagenomic profiling as a result on a metagenomic analysis is used in agriculture. The application US 20040241662 disclose a method for determining the presence, absence, or amount of microbial contamination in grain or a related product by genetic fingerprinting or ribosomal genotyping for example and tailoring treatment of the grain or animal feed based upon the presence, absence, or amount of microbial contamination.

US 20060246444 disclose a method for determining an environmental condition by measuring a composition of a microbial population which has been exposed to said environmental condition and a comparison between at least two environmental conditions, namely a standard condition and an experimental condition, as a result of which the possibly detected change in a composition of a microbial population can be ascribed to the changed environmental condition. The change in the in a composition of a microbial population can be determinated on the basis of a "community fingerprint".

A process for characterizing a community of organisms by the metagenomic analysis technique is described in US 20050026188. Further, a characterization of a "test" community, which was exposed to an exogenous variable, may be compared to the characterization of a "normal" or control community.

A method for screening for a plant protectant comprising a metagenomic analysis is not known.

It is an object of the present invention to provide new method for screening for a plant protectant.

Another object is to provide a method for controlling plant pests with ordinary pesticides but keeping the environmental damages as small as possible.

Surprisingly it was found, that a method comprising a metagenomic analysis of the microbial community of a habitat related to a plant offers the possibility for screen for plant protectants with controlled and well defined impact.

As one object of the present invention, there is provided a method for screening for a plant protectant comprising the steps of:

a) generating a metagenomic analysis of the microbial community of a habitat,

b) determining the composition of the microbial community of said habitat of step a),

c) applying a formulation to be tested containing a potential plant protectant

d) generating a metagenomic analysis of the microbial community of the habitat of step a) after application of the formulation from step c),

e) determining the composition of the microbial community of said habitat of step d),

f) comparing the microbial community before and after the application of the formulation,

g) determining the alteration of the microbial community of the habitat of step a) and d),

h) selecting a formulation from step c) with a plant protectant activity in dependency of step g).

According to the present invention, the term "metagenomic analysis" is a genomic analysis of a population of microorganisms, e.g. as defined in Handelsman et al., Microbiology and Molecular Biology Reviews, December 2004, p. 669-685, Vol. 68, No. 4.

According to the invention the term "habitat" means an environment occupied by a microbial community, the physical location or type of environment in which a microbial community lives or occurs.

In one embodiment of the present invention the habitat is selected from the group of environments occupied by microbial communities consisting of: plant, preferably agricultural plant, crop plant or a part thereof, grain, soil, location where the plant is growing or is expected to grow and biofilm from the surface of plants or parts thereof or soil.

In one embodiment the plant protectant selected by the method of the invention are pesticides. The term "pesticide" as used herein refers to a substance that can be used in the control of agricultural or natural environmental pests, such as insects, fungi, bacteria, and/or viruses. Pesticides encompass naturally occurring or synthetic chemical insecticides, insect growth regulators, acaricides (miticides), nematicides, ectoparasiticides, bactericides, fungicides, and herbicides as well as plant growth regulators, plant growth promotors, resistance inducers.

In one embodiment the plant protectant of the invention is any compound that induces changes in the composition of the microbial population of the habitat. So the plant protectant effect is not necessarily an immediate consequence of the impact on a

certain pest. The effect of the plant protectant may be the consequence of the impact on environmental conditions.

Changes in the composition of the microbial population of the habitat may be determined by the ratio in which the different groups are found in the population but also the proportion of a group in the population. A ratio or proportion may, for instance, be expressed in a cell number, but also in a weight of a cell or a cell component, such as a weight of a nucleic acid, or a fluorescence intensity.

In one embodiment a change in a composition of a microbial population is understood to mean an absolute or relative increase or decrease of the proportion of a group in the population.
A group of microorganisms can be distinguished from another group on the basis of one or more specific genotypic or phenotypic characteristics. Such a group may comprise a taxonomic group such as a phylum, a family, a species or a strain, but also a group which is methodologically classified such as a phylogenetic cluster, a ribotype, an isolate, a serotype, or a morphotype.

In one embodiment the microbial populations which may be analized according to the present invention are selected from the group of microorganisms consisting of: bacteria, archaea, fungi, yeasts, protozoa and algae. Preferably, microbial populations of bacteria, yeasts and/or fungi are analized according to the invention, most preferably fungi.

In one embodiment in the method according of the invention a specific molecular identity (ID) card genetical fingerprint of the microbial community is generated by metagenomic analysis in step b) and e).
Such a specific molecular identity (ID) card is in one embodiment a specific genetical fingerprint. Genetic fingerprinting utilizes random-sequence oligonucleotide primers that hybridize with sequence-specificity to random sequences throughout the genome. Amplification results in a multitude of products. The distribution of amplification products is referred to as a genetic fingerprint. Particular patterns can be associated with microbes in the sample.

The genetic fingerprint is generated by using characteristic microbial sequences, preferably taxon-specific markers selected from the group consisting of polynucleotide marker molecules or fragments thereof as ribosomal RNA (for instance 5S, 5,8S, 9S, 12S, 16S, 18S, 23S of 25S), spacer regions between them, internal transceribed spacer, transfer RNA, genomic DNA, plasmid DNA or mitochondrial DNA.
In one embodiment 16S amplification or ITS amplification are used according to the invention.

In one embodiment total DNA of an habitat is extracted and amplified, preferably by PCR, using ITS or 16S primers.

The PCR product is purified in one embodiment either by gel extraction or by PCR purification, preferably with a PCR purification kit, for example QIAquick.
In one embodiment the PCR product is subjected to an enzymatic digestion.
Preferably the digestion enzyme is one or more selected from the group consisting of: *Hae III, Hha II, Msp I, Dra I, Alu I, EcoR I, Hinf I, Mse I, Taq I, Mva I, Rsa I, Dde I, Hpa II, Ava II, MboII, Bgl I*
The digestion product is subjected to a migration on a polyamid gel. The lanes and bands are detected and the migration patterns are conversed into a distance matrix, preferably by the so called "Symclose method".

In one embodiment the distance matrix is conversed into a dendrogram, preferably by using the unweighted pair-group method using arithmetic averages (UPGMA).

In another embodiment of the invention the sequences from the purified PCR product are incorporated in a vector and cloned for sequencing. The ratio plasmid vector and purified PCR product is between 1 to 3 and 1 to 1. Preferably the ratio is 1 to 2.
After transformation with the plasmid vector the transformed cells, for example E.coli, are selected. Following a blue and white selection, white colonies are picked, re-suspended individually in liquid LB medium containing 50 µg/ml of ampicillin and incubated for 12 to 24 h before sequencing.
For cloning, DNA transfer, expression and other genetical engineering methods, reference is made to Maniatis et al., Molecular Cloning, a Laboratory Manual (Cold Spring Harbor Press, 1989).

The sequence information may be gained by any other suitable sequencing approach that yields and differentiates sequences, which are specific for the microorganisms that assemble the microbial community of said habitat. For instance, there are techniques available that uses the PCR product or even the genomic DNA as a template for the sequencing reaction.
The sequences are compared with sequence databases for taxonomically identification.
In another embodiment, the sequence is taxonomically identified by hybridising the PCR products to species specific oligonucleotides representing known microbial species and/or genera and/or families using a southern plot or a suitable DNA-array technology.

In one embodiment the phylum, subphylum, class, order, family, genus and/or species of the microorganisms of the habitat is determinated.

Such a repartition to a phylum, subphylum, class, order, family, genus and/or species, as well as the distance matrix and the dendrogram have according to the invention the same function as the genetic fingerprint, namely the function of a molecular ID card of the habitat.

The molecular ID card based on the metagenomic analysis of the habitat

characterizes the microbial community of said habitat under the given environmental conditions in a certain moment.

Therefore the metagenomic analysis is a powerful tool in the screening for plant protectant.

In one embodiment according to step f) two molecular ID cards are compared. If the ID card of step e) shows less DNA from pest microorganisms, the tested formulation has an activity of a plant protectant.

In another embodiment, method of the invention comprises additionally the steps:

c1) applying a formulation containing a known plant protectant,

d1) generating a metagenomic analysis of the microbial community of the habitat of step a) after application of the formulation from step c1),

e1) determining the composition of the microbial community of said habitat of step d1),

f1) comparing the microbial community with the one from step f),

g1) selecting that formulation from step c) with a plant protectant which produce a microbial community with a composition having the highest identity with the composition of the microbial community of step e1).

The composition of the microbial communities are compared by comparing in one embodiment the generated molecular ID cards. A plant protectant is selected if the molecular ID cards show an identity of 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, preferably 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, more preferably 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%. An identity of molecular ID cards of for example 90% means that 90 parts from the total 100 parts of the phylum, subphylum, class, order, family, genus and/or species, as well as of the distance matrix, the dendrogram or the genetic fingerprint are identical.
In one embodiment the identity means not that the compared ID cards have to be congruent but that there is an analogy in the motifs or patterns of the ID cards.

In one embodiment the metagenomic analysis is used to screen plant protectants.

According to the invention, in one embodiment the metagenomic analysis is used to screen crop protection compounds.

In an other embodiment the metagenome analysis is used to evaluate the spectrum of microbial species which can be controlled by a given active ingredient, for example a

strobilurin.

In one embodiment those active compounds are selected in step h) from the tested potential plant protectants which have an impact selected from the group comprising:

- the composition of the microbial community is altered in a way that it comprise less classes, orders, families, genus and/or species of pests;
- the composition of the microbial community is altered in a way that the ratio of the number of species of the phylum Basidiomycota to the number of species of the phylum Ascomycota is shifted from 3:1, preferably 1,5:1, 1,6:1 1,7:1, 1,8:1, 1,9:1, 2,0:1, 2,1:1, 2,2:1, 2,3:1, 2,4:1 or 2,5:1, more preferably 2,2:1, 2,3:1 to a ratio of 6,0:1, preferably 5,5:1, 5,3:1, 5,2:1, 5,1:1 ,5,0:1 ,4,9:1, 4,8:1, 4,7:1, 4,6:1, 4,5:1, 4,2:1, 4,0:1, 3,5:1, more preferably 4,8:1.

According to the present invention, "pest" means in one embodiment efficiently the pest selected from the group consisting of: phytopathogenic fungi, including soil-borne fungi, which derive especially from the classes of the Plasmodiophoromycetes, Peronosporomycetes (syn. Oomycetes), Chytridiomycetes, Zygomycetes, Ascomycetes, Basidiomycetes and Deuteromycetes (syn. Fungi imperfecti).

According to the present invention, "pest" means in one embodiment the plant diseases selected from the group consisting of:

Albugo spp. (white rust) on ornamentals, vegetables (e. g. *A. candida*) and sunflowers (e. g. *A. tragopogonis*); *Alternaria* spp. (Alternaria leaf spot) on vegetables, rape (*A. brassicola* or *brassicae*), sugar beets (*A. tenuis*), fruits, rice, soybeans, potatoes (e. g. *A. solani* or *A. alternata*), tomatoes (e. g. *A. solani* or *A. alternata*) and wheat; *Aphanomyces* spp. on sugar beets and vegetables; *Ascochyta* spp. on cereals and vegetables, e. g. *A. tritici* (anthracnose) on wheat and *A. hordei* on barley; *Bipolaris* and *Drechslera* spp. (teleomorph: *Cochliobolus* spp.) on corn (e. g. *D. maydis*), cereals (e. g. *B. sorokiniana*: spot blotch), rice (e. g. *B. oryzae*) and turfs; *Blumeria* (formerly *Erysiphe*) *graminis* (powdery mildew) on cereals (e. g. on wheat or barley); *Botrytis cinerea* (teleomorph: *Botryotinia fuckeliana*: grey mold) on fruits and berries (e. g. strawberries), vegetables (e. g. lettuce, carrots, celery and cabbages), rape, flowers, vines, forestry plants and wheat; *Bremia lactucae* (downy mildew) on lettuce; *Ceratocystis* (syn. *Ophiostoma*) spp. (rot or wilt) on broad-leaved trees and evergreens, e. g. *C. ulmi* (Dutch elm disease) on elms; *Cercospora* spp. (Cercospora leaf spots) on corn, rice, sugar beets (e. g. *C. beticola*), sugar cane, vegetables, coffee, soybeans (e. g. *C. sojina* or *C. kikuchii*) and rice; *Cladosporium* spp. on tomatoes (e. g. *C. fulvum*: leaf mold) and cereals, e. g. *C. herbarum* (black ear) on wheat; *Claviceps purpurea* (ergot) on cereals; *Cochliobolus* (anamorph: *Helminthosporium* of *Bipolaris*) spp. (leaf spots) on corn (*C. carbonum*), cereals (e. g.

C. sativus, anamorph: *B. sorokiniana*) and rice (e. g. *C. miyabeanus*, anamorph: *H. oryzae*); *Colletotrichum* (teleomorph: *Glomerella*) spp. (anthracnose) on cotton (e. g. *C. gossypii*), corn (e. g. *C. graminicola*), soft fruits, potatoes (e. g. *C. coccodes*: black dot), beans (e. g. *C. lindemuthianum*) and soybeans (e. g. *C. truncatum* or *C. gloeosporioides*); *Corticium* spp., e. g. *C. sasakii* (sheath blight) on rice; *Corynespora cassiicola* (leaf spots) on soybeans and ornamentals; *Cycloconium* spp., e. g. *C. oleaginum* on olive trees; *Cylindrocarpon* spp. (e. g. fruit tree canker or young vine decline, teleomorph: *Nectria* or *Neonectria* spp.) on fruit trees, vines (e. g. *C. liriodendri*, teleomorph: *Neonectria liriodendri*: Black Foot Disease) and ornamentals; *Davidiella tassiana, Davidiella macrospora*, *Dematophora* (teleomorph: *Rosellinia*) necatrix (root and stem rot) on soybeans; *Diaporthe* spp., e. g. *D. phaseolorum* (damping off) on soybeans; *Drechslera* (syn. *Helminthosporium*, teleomorph: *Pyrenophora*) spp. on corn, cereals, such as barley (e. g. *D. teres*, net blotch) and wheat (e. g. *D. tritici-repentis*: tan spot), rice and turf; Esca (dieback, apoplexy) on vines, caused by *Formitiporia* (syn. *Phellinus*) *punctata*, *F. mediterranea*, *Phaeomoniella chlamydospora* (earlier *Phaeoacremonium chlamydosporum*), *Phaeoacremonium aleophilum* and/or *Botryosphaeria obtusa*; *Elsinoe* spp. on pome fruits (*E. pyri*), soft fruits (*E. veneta*: anthracnose) and vines (*E. ampelina*: anthracnose); *Entyloma oryzae* (leaf smut) on rice; *Epicoccum* spp. (black mold) on wheat; *Erysiphe* spp. (powdery mildew) on sugar beets (*E. betae*), vegetables (e. g. *E. pisi*), such as cucurbits (e. g. *E. cichoracearum*), cabbages, rape (e. g. *E. cruciferarum*); *Eutypa lata* (Eutypa canker or dieback, anamorph: *Cytosporina lata*, syn. *Libertella blepharis*) on fruit trees, vines and ornamental woods; *Exserohilum* (syn. *Helminthosporium*) spp. on corn (e. g. *E. turcicum*); *Fusarium* (teleomorph: *Gibberella*) spp. (wilt, root or stem rot) on various plants, such as *F. graminearum* or *F. culmorum* (root rot, scab or head blight) on cereals (e. g. wheat or barley), *F. oxysporum* on tomatoes, *F. solani* on soybeans and *F. verticillioides* on corn; *Gaeumannomyces graminis* (take-all) on cereals (e. g. wheat or barley) and corn; *Gibberella* spp. on cereals (e. g. *G. zeae*) and rice (e. g. *G. fujikuroi*: Bakanae disease); *Glomerella cingulata* on vines, pome fruits and other plants and *G. gossypii* on cotton; Grain-staining complex on rice; *Guignardia bidwellii* (black rot) on vines; *Gymnosporangium* spp. on rosaceous plants and junipers, e. g. *G. sabinae* (rust) on pears; *Helminthosporium* spp. (syn. *Drechslera*, teleomorph: *Cochliobolus*) on corn, cereals and rice; *Hemileia* spp., e. g. *H. vastatrix* (coffee leaf rust) on coffee; *Isariopsis clavispora* (syn. *Cladosporium vitis*) on vines; *Macrophomina phaseolina* (syn. *phaseoli*) (root and stem rot) on soybeans and cotton; *Microdochium* (syn. *Fusarium*) *nivale* (pink snow mold) on cereals (e. g. wheat or barley); *Microsphaera diffusa* (powdery mildew) on soybeans; *Monilinia* spp., e. g. *M. laxa*, *M. fructicola* and *M. fructigena* (bloom and twig blight, brown rot) on stone fruits and other rosaceous plants; *Mycosphaerella* spp. on cereals, bananas, soft fruits and ground nuts, such as e. g. *M. graminicola* (anamorph: *Septoria tritici*, Septoria blotch) on wheat or *M. fijiensis* (black Sigatoka

disease) on bananas; *Peronospora* spp. (downy mildew) on cabbage (e. g. *P. brassicae*), rape (e. g. *P. parasitica*), onions (e. g. *P. destructor*), tobacco (*P. tabacina*) and soybeans (e. g. *P. manshurica*); *Phakopsora pachyrhizi* and *P. meibomiae* (soybean rust) on soybeans; *Phialophora* spp. e. g. on vines (e. g. *P. tracheiphila* and *P. tetraspora*) and soybeans (e. g. *P. gregata*: stem rot); *Phoma lingam* (root and stem rot) on rape and cabbage and *P. betae* (root rot, leaf spot and damping-off) on sugar beets; *Phomopsis* spp. on sunflowers, vines (e. g. *P. viticola*: can and leaf spot) and soybeans (e. g. stem rot: *P. phaseoli*, teleomorph: *Diaporthe phaseolorum*); *Physoderma maydis* (brown spots) on corn; *Phytophthora* spp. (wilt, root, leaf, fruit and stem root) on various plants, such as paprika and cucurbits (e. g. *P. capsici*), soybeans (e. g. *P. megasperma*, syn. *P. sojae*), potatoes and tomatoes (e. g. *P. infestans*: late blight) and broad-leaved trees (e. g. *P. ramorum*: sudden oak death); *Plasmodiophora brassicae* (club root) on cabbage, rape, radish and other plants; *Plasmopara* spp., e. g. *P. viticola* (grapevine downy mildew) on vines and *P. halstedii* on sunflowers; *Podosphaera* spp. (powdery mildew) on rosaceous plants, hop, pome and soft fruits, e. g. *P. leucotricha* on apples; *Polymyxa* spp., e. g. on cereals, such as barley and wheat (*P. graminis*) and sugar beets (*P. betae*) and thereby transmitted viral diseases; *Pseudocercosporella herpotrichoides* (eyespot, teleomorph: *Tapesia yallundae*) on cereals, e. g. wheat or barley; *Pseudoperonospora* (downy mildew) on various plants, e. g. *P. cubensis* on cucurbits or *P. humili* on hop; *Pseudopezicula tracheiphila* (red fire disease or ‚rotbrenner', anamorph: *Phialophora*) on vines; *Puccinia* spp. (rusts) on various plants, e. g. *P. triticina* (brown or leaf rust), *P. striiformis* (stripe or yellow rust), *P. hordei* (dwarf rust), *P. graminis* (stem or black rust) or *P. recondita* (brown or leaf rust) on cereals, such as e. g. wheat, barley or rye, and asparagus (e. g. *P. asparagi*); *Pyrenophora* (anamorph: *Drechslera*) *tritici-repentis* (tan spot) on wheat or *P. teres* (net blotch) on barley; *Pyricularia* spp., e. g. *P. oryzae* (teleomorph: *Magnaporthe grisea*, rice blast) on rice and *P. grisea* on turf and cereals; *Pythium* spp. (damping-off) on turf, rice, corn, wheat, cotton, rape, sunflowers, soybeans, sugar beets, vegetables and various other plants (e. g. *P. ultimum* or *P. aphanidermatum*); *Ramularia* spp., e. g. *R. collo-cygni* (Ramularia leaf spots, Physiological leaf spots) on barley and *R. beticola* on sugar beets; *Rhizoctonia* spp. on cotton, rice, potatoes, turf, corn, rape, potatoes, sugar beets, vegetables and various other plants, e. g. *R. solani* (root and stem rot) on soybeans, *R. solani* (sheath blight) on rice or *R. cerealis* (Rhizoctonia spring blight) on wheat or barley; *Rhizopus stolonifer* (black mold, soft rot) on strawberries, carrots, cabbage, vines and tomatoes; *Rhynchosporium secalis* (scald) on barley, rye and triticale; *Sarocladium oryzae* and *S. attenuatum* (sheath rot) on rice; *Sclerotinia* spp. (stem rot or white mold) on vegetables and field crops, such as rape, sunflowers (e. g. *S. sclerotiorum*) and soybeans (e. g. *S. rolfsii* or *S. sclerotiorum*); *Septoria* spp. on various plants, e. g. *S. glycines* (brown spot) on soybeans, *S. tritici* (Septoria blotch) on wheat and *S.* (syn. *Stagonospora*) nodorum (Stagonospora blotch) on cereals; *Uncinula* (syn. *Erysiphe*)

necator (powdery mildew, anamorph: *Oidium tuckeri*) on vines; *Setospaeria* spp. (leaf blight) on corn (e. g. *S. turcicum*, syn. *Helminthosporium turcicum*) and turf; *Sphacelotheca* spp. (smut) on corn, (e. g. *S. reiliana*: head smut), sorghum und sugar cane; *Sphaerotheca fuliginea* (powdery mildew) on cucurbits; *Spongospora subterranea* (powdery scab) on potatoes and thereby transmitted viral diseases; *Stagonospora* spp. on cereals, e. g. *S. nodorum* (Stagonospora blotch, teleomorph: *Leptosphaeria* [syn. *Phaeosphaeria*] *nodorum*) on wheat; *Synchytrium endobioticum* on potatoes (potato wart disease); *Taphrina* spp., e. g. *T. deformans* (leaf curl disease) on peaches and *T. pruni* (plum pocket) on plums; *Thielaviopsis* spp. (black root rot) on tobacco, pome fruits, vegetables, soybeans and cotton, e. g. *T. basicola* (syn. *Chalara elegans*); *Tilletia* spp. (common bunt or stinking smut) on cereals, such as e. g. *T. tritici* (syn. *T. caries*, wheat bunt) and *T. controversa* (dwarf bunt) on wheat; *Typhula incarnata* (grey snow mold) on barley or wheat; *Urocystis* spp., e. g. *U. occulta* (stem smut) on rye; *Uromyces* spp. (rust) on vegetables, such as beans (e. g. *U. appendiculatus*, syn. *U. phaseoli*) and sugar beets (e. g. *U. betae*); *Ustilago* spp. (loose smut) on cereals (e. g. *U. nuda* and *U. avaenae*), corn (e. g. *U. maydis*: corn smut) and sugar cane; *Venturia* spp. (scab) on apples (e. g. *V. inaequalis*) and pears; and *Verticillium* spp. (wilt) on various plants, such as fruits and ornamentals, vines, soft fruits, vegetables and field crops, e. g. *V. dahliae* on strawberries, rape, potatoes and tomatoes.

Further harmful fungi are selected from the group consisting of : Ascomycetes such as *Ophiostoma* spp., *Ceratocystis* spp., *Aureobasidium pullulans*, *Sclerophoma* spp., *Chaetomium* spp., *Humicola* spp., *Petriella* spp., *Trichurus* spp.; Basidiomycetes such as *Coniophora* spp., *Coriolus* spp., *Gloeophyllum* spp., *Lentinus* spp., *Pleurotus* spp., *Poria* spp., *Serpula* spp. and *Tyromyces* spp., Deuteromycetes such as *Aspergillus* spp., *Cladosporium* spp., *Penicillium* spp., *Trichorma* spp., *Alternaria* spp., *Paecilomyces* spp. and Zygomycetes such as *Mucor* spp., and in addition in the protection of stored products the following yeast fungi are worthy of note: *Candida* spp. and *Saccharomyces cerevisae*.

In a further embodiment the method of the invention comprises maintenance treatment of the habitat with a pesticide before proceeding the method of the invention to exclude a major pathogenic epidemic.

In a further embodiment the process of the invention comprises treating the habitat with an effective amount of a chemical formulation comprising at least one active ingredient according to step c1).

In an embodiment of the invention, the formulation applied in step c1) comprises a known plant protectant selected from the group consisting of:

- an active ingredient (B) which is an active compound selected from the group

consisting of

B1) an active compound that inhibits the mitochondrial respiration (breathing) chain at the level of the b/c_1 complex: famoxadone and strobilurins selected from the group consisting of pyraclostrobin, kresoxim-methyl, dimoxystrobin, picoxystrobin, ZJ 0712, trifloxystrobin, enestroburin, orysastrobin, metominostrobin, azoxystrobin, fluoxastrobin, metominostrobin, orysastrobin, pyribencarb, trifloxystrobin, 2-(2-(6-(3-chloro-2-methyl-phenoxy)-5-fluoro-pyrimidin-4-yloxy)-phenyl)-2-methoxyimino-N-methyl-acetamide, 3-methoxy-2-(2-(N-(4-methoxy-phenyl)-cyclopropane-carboximidoylsulfanylmethyl)-phenyl)-acrylic acid methyl ester, methyl (2-chloro-5-[1-(3-methylbenzyloxyimino)ethyl]benzyl)carbamate and 2-(2-(3-(2,6-dichlorophenyl)-1-methyl-allylideneaminooxymethyl)-phenyl)-2-methoxyimino-N-methyl-

preferably famoxadone, pyraclostrobin, kresoxim-methyl, dimoxystrobin, picoxystrobin, ZJ 0712, trifloxystrobin, orysastrobin, azoxystrobin, fluoxastrobin

B2) carboxylic amides selected from benalaxyl, benodanil, boscalid, carboxin, mepronil, fenfuram, fenhexamid, futolanil, furametpyr, metalaxyl, ofurace, oxadixyl, oxycarboxin, penthiopyrad, thifluzamid, tiadinil, 4-difluoromethyl-2-methyl-thiazol-5-carboxylic acid-(4'-bromo-biphenyl-2-yl)-amide, 4-difluoromethyl-2-methyl-thiazol-5-carboxylic acid-(4'-trifluoromethyl-biphenyl-2-yl)-amide, 4-difluoromethyl-2-methyl-thiazol-5-carboxylic acid-(4'-chloro-3'-fluoro-biphenyl-2-yl)-amide, 3-difluoromethyl-1-methyl-pyrazol-4-carboxylic acid-(3',4'-dichloro-4-fluoro-biphenyl-2-yl)-amide, 3,4-dichloro-isothiazol-5-carboxylic acid-(2-cyano-phenyl)-amide, dimethomorph, flumorph, flumetover, fluopicolide (picobenzamid), zoxamide, carpropamide, diclocymet, mandipropamid, N-(2-(4-[3-(4-chloro-phenyl)-prop-2-inyloxy]-3-methoxy-phenyl)-ethyl)-2-methanesulfonylamino-3-methyl-butyramid and N-(2-(4-[3-(4-chloro-phenyl)-prop-2-inyloxy]-3-methoxy-phenyl)-ethyl)-2-ethanesulfonylamino-3-methyl-butyramide;

preferably benalaxyl, benodanil, boscalid, carboxin, mepronil, fenfuram, fenhexamid, futolanil, furametpyr, metalaxyl, ofurace, oxadixyl, oxycarboxin, penthiopyrad, thifluzamid, tiadinil, (picobenzamid), diclocymet

B3) azoles selected from bitertanole, bromuconazole, cyproconazole, difenoconazole, diniconazole, enilconazole, epoxiconazole, fenbuconazole, flusilazole, fluquinconazole, flutriafol, hexaconazole, imibenconazole, ipconazole, metconazole, myclobutanil, penconazole, propiconazole, prothioconazole, simeconazole, tebuconazole, tetraconazole, triadimenol, triadimefon, triticonazole, cyazofamid, imazalil, pefurazoate, prochloraz, triflumizol, benomyl, carbendazim, fuberidazole,

thiabendazole, ethaboxam, etridiazole and hymexazole; paclobutrazol, uniconazole-P;

preferably cyproconazole, epoxiconazole, fenbuconazole, fluquinconazole, flutriafol, hexaconazole, ipconazole, metconazole, propiconazole, prothioconazole, ebuconazole, tetraconazole, triadimenol, triadimefon, triticonazole, cyazofamid, imazalil, prochloraz, triflumizol, benomyl, carbendazim, thiabendazole, ethaboxam, hymexazole

B4) nitrogen-containing heterocyclic compounds selected from fluazinam, pyrifenox, 3-[5-(4-chloro-phenyl)-2,3-dimethyl-isoxazolidin-3-yl]-pyridine, bupirimat, cyprodinil, ferimzon, fenarimol, mepanipyrim, nuarimol, pyrimethanil, triforin, fludioxonil, fenpiclonil, aldimorph, dodemorph, fenpropimorph, tridemorph, iprodion, procymidon, vinclozolin, acibenzolar-S-methyl, anilazin, captan, captafol, dazomet, diclomezine, fenoxanil, folpet, fenpropidin, famoxadone, fenamidone, octhilinon, probenazol, proquinazid, pyroquilon, quinoxyfen, tricyclazol, 2-butoxy-6-iodo-3-propyl-chromen-4-one, 3-(3-bromo-6-fluoro-2-methyl-indole-1-sulfonyl)-[1,2,4]triazole-1-sulfonic acid dimethylamide, ancymidol, flurprimidol, inabenfide, tetcyclacis;

preferably pyrimethanil, fludioxonil, fenpiclonil, aldimorph, dodemorph, fenpropimorph, tridemorph, iprodion, procymidon, captan, captafol, dazomet, diclomezine, fenoxanil, probenazol, proquinazid, pyroquilon, quinoxyfen, tricyclazol,

B5) carbamates and dithiocarbamates selected from ferbam, mancozeb, metiram, metam, propineb, thiram, zineb, ziram, diethofencarb, flubenthiavalicarb, iprovalicarb, propamocarb, 3-(4-chloro-phenyl)-3-(2-isopropoxycarbonylamino-3-methyl-butyrylamino)-propionic acid methylester and N-(1-(1-(4-cyanophenyl)ethanesulfonyl)-but-2-yl) carbamic acid -(4-fluorophenyl)ester;

preferably erbam, mancozeb, metiram, metam, propineb, thiram, zineb, ziram, diethofencarb, iprovalicarb, propamocarb,

B6) guanidines selected from dodin, iminoctadine and guazatin; preferably guazatin

B7) antibiotics selected from kasugamycin, polyoxine, streptomycin, oxytetracyclin and validamycin A; preferably streptomycin, oxytetracyclin and validamycin A

B8) fentin salts; preferably fentin salts;

B9) sulfur-containing heterocyclic compounds selected from isoprothiolan and dithianon;

preferably sulfur-containing heterocyclic compounds selected from isoprothiolan and dithianon;

B10) organophosphorous compounds selected from edifenphos, fosetyl, fosetyl-aluminium, iprobenfos, pyrazophos, tolclofos-methyl, phosphoric acid and the salts thereof;

preferably edifenphos, fosetyl, iprobenfos, pyrazopho, phosphoric acid and the salts thereof;

B11) organo-chloro compounds selected from thiophanate methyl, chlorothalonil, dichlofluanid, tolylfluanid, flusulfamid, phthalide, hexachlorbenzene, pencycuron, quintozen;

preferably thiophanate methyl, chlorothalonil, dichlofluanid, flusulfamid, phthalide, quintozen;

B12) nitrophenyl derivatives selected from binapacryl, dinocap and dinobuton;

preferably binapacryl, dinocap and dinobuton

B13) inorganic active ingredients selected from Bordeaux composition, copper acetate, copper hydroxide, copper oxychloride, basic copper sulfate and sulfur;

preferably copper acetate, copper hydroxide, copper oxychloride, basic copper sulfate and sulfur;

B14) spiroxamine;

B15) cyflufenamide;

B16) cymoxanil;

B17) metrafenone;

B18) organo(thio)phosphates selected from acephate, azamethiphos, azinphos-methyl, chlorpyrifos, chlorpyrifos-methyl, chlorfenvinphos, diazinon, dichlorvos, dicrotophos, dimethoate, disulfoton, ethion, fenitrothion, fenthion, isoxathion, malathion, methamidophos, methidathion, methyl-parathion, mevinphos, monocrotophos, oxydemeton-methyl, paraoxon, parathion, phenthoate, phosalone, phosmet, phosphamidon, phorate, phoxim, pirimiphos-methyl, profenofos, prothiofos, sulprophos, tetrachlorvinphos, terbufos, triazophos and trichlorfon;

preferably metrafenone, chlorpyrifos, chlorpyrifos-methyl, chlorfenvinphos, diazinon, dichlorvos, dimethoate, disulfoton, ethion, methidathion, methyl-parathion, paraoxon, parathion, phenthoate, phosalone, phosmet, phosphamidon, phorate, phoxim, profenofos, prothiofos, sulprophos, tetrachlorvinphos, terbufos, triazophos and trichlorfon;

B19) carbamates selected from alanycarb, aldicarb, bendiocarb, benfuracarb, carbaryl, carbofuran, carbosulfan, fenoxycarb, furathiocarb, methiocarb, methomyl, oxamyl, pirimicarb, propoxur, thiodicar and triazamate;

preferably aldicarb, bendiocarb, benfuracarb, carbaryl, carbofuran, carbosulfan, fenoxycarb, furathiocarb, methiocarb, methomyl, oxamyl, pirimicarb, propoxur, thiodicar and triazamate;

B20) pyrethroids selected from allethrin, bifenthrin, cyfluthrin, cyhalothrin, cyphenothrin, cypermethrin, alpha-cypermethrin, beta-cypermethrin, zeta-cypermethrin, deltamethrin, esfenvalerate, etofenprox, fenpropathrin, fenvalerate, imiprothrin, lambda-cyhalothrin, permethrin, prallethrin, pyrethrin I and II, resmethrin, silafluofen, tau-fluvalinate, tefluthrin, tetramethrin, tralomethrin, transfluthrin and profluthrin, dimefluthrin;

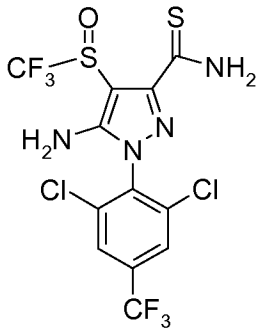
preferably bifenthrin, cyfluthrin, cyphenothrin, cypermethrin, alpha-cypermethrin, beta-cypermethrin, zeta-cypermethrin, deltamethrin, etofenprox, fenpropathrin, fenvalerate, imiprothrin, lambda-cyhalothrin, permethrin, prallethrin, pyrethrin I and II, resmethrin, tefluthrin, tetramethrin, tralomethrin, transfluthrin and profluthrin, dimefluthrin;

B21) growth regulators selected from a) chitin synthesis inhibitors that are selected from the benzoylureas chlorfluazuron, diflubenzuron, flucycloxuron, flufenoxuron, hexaflumuron, lufenuron, novaluron, teflubenzuron, triflumuron; buprofezin, diofenolan, hexythiazox, etoxazole and clofentazine; b) ecdysone antagonists that are selceted from halofenozide, methoxyfenozide, tebufenozide and azadirachtin; c) juvenoids that are selected from pyriproxyfen, methoprene and fenoxycarb and d) lipid biosynthesis inhibitors that are selected from spirodiclofen, spiromesifen and spirotetramat;

preferably flufenoxuron, hexaflumuron, teflubenzuron, triflumuron; azadirachtin, methoprene and fenoxycarb, spirodiclofen, spiromesifen and spirotetramat;

B22) nicotinic receptor agonists/antagonists compounds selected from clothianidin, dinotefuran, imidacloprid, thiamethoxam, nitenpyram, acetamiprid, thiacloprid, AKD1022, bensultap, cartap hydrochloride;

preferably clothianidin, dinotefuran, imidacloprid, thiamethoxam, nitenpyram, acetamiprid, thiacloprid, AKD1022B23) GABA antagonist compoundsselected from acetoprole, endosulfan, ethiprole, fipronil, vaniliprole, pyrafluprole, pyriprole and the phenylpyrazole compound of formula Gamma[1]



(Γ^1)

,

preferably acetoprole, endosulfan, ethiprole, fipronil, vaniliprole, pyrafluprole, pyriprole and the phenylpyrazole

B24) METI I compounds selected from fenazaquin, pyridaben, tebufenpyrad, tolfenpyrad and flufenerim;

preferably fenazaquin, pyridaben, tebufenpyrad, tolfenpyrad and flufenerim;

B25) METI II and III compounds selected from acequinocyl, fluacyprim and hydramethylnon;

preferably acequinocyl, fluacyprim and hydramethylnon;

B26) chlorfenapyr;;

B27) oxidative phosphorylation inhibitor compounds selected from cyhexatin, diafenthiuron, fenbutatin oxide and propargite;

preferably diafenthiuron, fenbutatin oxid

B28) cyromazine;

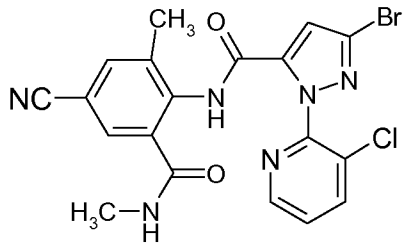
B29) piperonyl butoxide

B30) indoxacarb

B31) a compound selected from benclothiaz, bifenazate, cartap, flonicamid, pyridalyl, pymetrozine, sulfur, thiocyclam, flubendiamide, cyenopyrafen, flupyrazofos, cyflumetofen, amidoflumet, pyrifluquinazon;

preferably benclothiaz, bifenazate, cartap, flonicamid, pyridalyl, pymetrozine, sulfur, thiocyclam, flubendiamide, cyenopyrafen, flupyrazofos, cyflumetofen, amidoflumet

B.32) Anthranilamides: chloranthraniliprole, the compound of formula Gamma^2



(Γ^2)

;

B33) Acibenzolar-S-methyl;

B34) Plant bioregulators: trinexapac-ethyl, prohexadione-calcium, chlormequat chloride, mepiquat chloride, 16,17-dihydro gibberellin A_5, 1-methylcyclopropene, 2,5-norbornadiene, 3-amino-1,2,4-triazole;

preferably trinexapac-ethyl, prohexadione-calcium, chlormequat chloride, mepiquat chloride, 1-methylcyclopropene,

B35) neonicotinoid.

In a further embodiment the active ingredient (B) is an active compound that inhibits the mitochondrial respiration (breathing) chain at the level of the b/c_1 complex.

In a further embodiment of the invention the active ingredient (B) is a strobilurin selected from pyraclostrobin, kresoxim-methyl, dimoxystrobin, 2-(ortho-((2,5-Dimethylphenyl-oxymethylene)phenyl)-3-methoxy-acrylic acid methyl ester, picoxystrobin, trifloxystrobin, enestroburin, orysastrobin, metominostrobin, azoxystrobin and fluoxastrobin.

In a further embodiment of the invention the active ingredient (B) is selected from (*EZ*)-3-(2-chloro-1,3-thiazol-5-ylmethyl)-5-methyl-1,3,5-oxadiazinan-4-ylidene(nitro)amine (thiamethoxam), 5-amino-1-(2,6-dichloro-α,α,α-trifluoro-*p*-tolyl)-4-trifluoromethylsulfinylpyrazole-3-carbonitrile (fipronil) and (*EZ*)-1-(6-chloro-3-pyridylmethyl)-*N*-nitroimidazolidin-2-ylideneamine (imidacloprid).

In a further embodiment of the invention the active ingredient (B) is a plant bioregulator.

In one embodiment of the invention the plant bioregulator is selected from the group consisting of:

i) Plant bioregulators of the acylcyclohexanedione-type known to induce defence mechanisms against bacterial and fungal pathogens and against insect pests are: prohexadione and trinexapac, as free acids, esters (C1-C3) or salts (in particular: trinexapac-ethyl and prohexadione-calcium).

ii) Plant bioregulators known to reduce shoot length and leaf surface and

to increase root growth, thereby diminishing the vulnerability of plants to abiotic stress (e.g. lodging as a result of wind and severe rainfall; dehydration as a result of water shortage and high evaporative demand; cell, tissue or whole-plant death as a result of too high or too low temperatures; root anoxia as a result of intense rainfall or flooding) are: (i) Quaternary ammonium compounds, in particular chlormequat and mepiquat as chlorides, borates, sulfates, phosphates or other agriculturally useful salts; (ii) compounds with a nitrogen-containing heterocycle, in particular paclobutrazol, uniconazole-P, metconazole, tebuconazole, ancymidol, flurprimidol, inabenfide, tetcyclacis; (iii) acylcyclohexanediones, in particular prohexadione and trinexapac, as free acids, esters (C1-C3) or salts (in particular: trinexapac-ethyl and prohexadione-calcium) (iiii) derivatives of 16, 17-dihydro gibberellin A_5.

iii) Plant bioregulators diminishing the responsiveness of plants to abiotic and biotic stresses (thereby avoiding yield-reducing over-reactions), in particular ethylene modulators are:

ethylene biosynthesis inhibitors which inhibit the conversion of S-adenosyl-L-methionine into 1-aminocyclopropane-1-carboxylic acid (ACC), such as derivatives of vinylglycine, hydroxylamines, oxime ether derivatives;

ethylene biosynthesis inhibitors which block the conversion of ACC into ethylene, selected from the group consisting of: Co^{++} or Ni^{++} ions in plant-available form; phenolic radical scavengers such as n-propyl gallate; polyamines, such as putrescine, spermine or spermidine; structural analogs of ACC, such as α-aminoisobutyric acid or L-aminocyclopropene-1-carboxylic acid; salicylic acid or acibenzolar-S-methyl; structural analogs of ascorbic acid which act as inhibitors of ACC oxidase, such as prohexadione-Ca or trinexapac-ethyl; and triazolyl compounds such as paclobutrazole or uniconazole as inhibitors of cytochrome P-450-dependent monooxygenases whose main action is to block the metabolism of gibberellins;

inhibitors of the action of ethylene selected from the group consisting of: structural analogs of ethylene such as 1-methylcyclopropene or 2,5-norbornadiene and 3-amino-1,2,4-triazole or Ag^{++} ions in a weight ratio of I to II of from 20 : 1 to 0.05 : 1

Plant bioregulators can also be involved in endogenous defense mechanisms against biotic (e.g. jasmonic acid and methyl jasmonate) and abiotic stress (e.g. abscisic acid and also its synthetic analogs).

In a further embodiment of the invention the chemical composition comprises at least one active ingredient (B) and a component (A) which is a glucan or a glucan derivative.

Component (A) according to the present invention is a glucan or a glucan derivative.

"Glucans" are a class of homopolysaccharides which contain glucose units as monomer building blocks, wherein the glucose molecule may be linked by alpha- or beta-glycosidic bonds and may be branched or straight chain. Specific examples for suitable glucans according to the present invention are beta-glucans, more specifically beta -1,3-glucans such as, for example, laminarin and curdlan. Beta-1-3 glucans, for example, have various origins. They can be extracted from bacteria (for example *Alcaligenes faecalis* which leads to curdlan), fungi, yeasts and from various plants, particularly from algae and cereals.

"Glucan derivatives" according to the present invention are glucans that are modified, for example by sulfatation or by hydrolysis. Specific examples for suitable glucan derivatives are sulfated glucans, particularly sulfated beta -glucans, more specifically beta -1,3-glucans such as sulfated laminarin or sulfated curdlan. Furthermore, also laminaribiose, cellobiose, nigerose, laminaritriose, laminaritetrose and laminaripentose are suitable glucan derivatives according to the present invention.

Further derivates of glucans are described in US 6,979,665, Alban S. et al., Synthesis of laminarin sulfates with anticoagulant activity, Arzneim. Forsch. (1992) 42; 1005-1008; US 6,979,665; US 6,387,847; .US 6,303,587; US 6,303,587; Black et al., Appl. Chem. (1951), volume 1, pages 505 to 517; US 5,750,472 and references cited therein), US 5,750,472, FR 92 08387; which are included by reference.

According to one preferred embodiment of the present invention, component (A) is a beta -glucan, in particular a beta -1,3-glucan. Specifically, component (A) is laminarin or curdlan.

According to another preferred embodiment, component (A) is selected from sulfated glucan, Laminaribiose, Cellobiose, Nigerose, Laminaritriose, Laminaritetrose and Laminaripentose.

The active compounds of groups B1) to B17) that can be used as the active ingredient (B), their preparation and their action against harmful fungi are generally known; they are commercially available. In most of the cases, they can also be found in The Pesticide Manual, 13th Edition, British Crop Protection Council (2003) among other publications.

- benalaxyl, methyl *N*-(phenylacetyl)-*N*-(2,6-xylyl)-DL-alaninate (DE 29 03 612),
- boscalid, 2-chloro-*N*-(4′-chlorbiphenyl-2-yl)nicotinamide (EP-A 545 099);
- carboxin, 5,6-dihydro-2-methyl-*N*-phenyl-1,4-oxathiin-3-carboxamide (US 3 249 499),
- mepronil, 3′-isopropoxy-*o*-toluanilide (US 3 937 840),
- fenhexamid, N-(2,3-dichloro-4-hydroxyphenyl)-1-methylcyclohexanecarboxamide (Proc. Br. Crop Prot. Conf. – Pests Dis., 1998, Vol. 2, p. 327);
- flutolanil, α,α,α-trifluoro-3′-isopropoxy-*o*-toluanilide (JP 1104514),
- furametpyr, 5-chloro-*N*-(1,3-dihydro-1,1,3-trimethyl-4-isobenzofuranyl)-1,3-dimethyl-1*H*-pyrazole-4-carboxamide [CAS RN 123572-88-3],

- metalaxyl, methyl *N*-(methoxyacetyl)-*N*-(2,6-xylyl)-DL-alaninate (GB 15 00 581);
- ofurace, (*RS*)-α-(2-chloro-*N*-2,6-xylylacetamido)-γ-butyrolactone [CAS RN 58810-48-3];
- oxadixyl; *N*-(2,6-dimethylphenyl)-2-methoxy-*N*-(2-oxo-3-oxazolidinyl)acetamide (GB 20 58 059),
- oxycarboxin, 5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide 4,4-dioxide (US 3 399 214),
- penthiopyrad, *N*-[2-(1,3-dimethylbutyl)-3-thienyl]-1-methyl-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (JP 10130268),
- thifluzamide, *N*-[2,6-dibromo-4-(trifluoromethoxy)phenyl]-2-methyl-4-(trifluoromethyl)-5-thiazolecarboxamide;
- tiadinil, 3′-chloro-4,4′-dimethyl-1,2,3-thiadiazole-5-carboxanilide [CAS RN 223580-51-6],
- dimethomorph, 3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)-1-morpholin-4-yl-propenone (EP-A 120 321);
- flumorph, 3-(4-fluorophenyl)-3-(3,4-dimethoxyphenyl)-1-morpholin-4-ylpropenone (EP-A 860 438);
- flumetover, 2-(3,4-dimethoxyphenyl)-*N*-ethyl-α,α,α-trifluoro-*N*-methyl-*p*-toluamide [AGROW No. 243, 22 (1995)],
- fluopicolide (picobenzamid), 2,6-dichloro-N-(3-chloro-5-trifluoromethylpyridin-2-ylmethyl)benzamide (WO 99/42447);
- zoxamide, (*RS*)-3,5-dichloro-*N*-(3-chloro-1-ethyl-1-methyl-2-oxopropyl)-*p*-toluamide [CAS RN 156052-68-5];
- carpropamid, 2,2-dichloro-*N*-[1-(4-chlorophenyl)ethyl]-1-ethyl-3-methylcyclopropane-carboxamide [CAS RN 104030-54-8],
- diclocymet, 2-cyano-*N*-[(1*R*)-1-(2,4-dichlorophenyl)ethyl]-3,3-dimethyl butanamide;
- mandipropamid, (*RS*)-2-(4-chlorophenyl)-*N*-[3-methoxy-4-(prop-2-ynyloxy)phenethyl]-2-(prop-2-ynyloxy)acetamide [CAS RN 374726-62-2];
- bitertanole, prop-2-′-biphenyl]-4-yloxy)-2-ynyloxy)phenethyl]-2-*H*-1,2,4-triazole-1-ethanol (DE 23 24 020),
- bromuconazole, 1-[[4-bromo-2-(2,4-dichlorophenyl)tetrahydro-2-furanyl]methyl]-1*H*-1,2,4-triazole (Proc. 1990 Br. Crop. Prot. Conf. – Pests Dis. Vol. 1, p. 459);
- cyproconazole, 2-(4-chlorophenyl)-3-cyclopropyl-1-[1,2,4]triazol-1-ylbutan-2-ol (US 4 664 696);
- difenoconazole, 1-{2-[2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-[1,3]dioxolan-2-ylmethyl}-1H-[1,2,4]triazole (GB-A 2 098 607);
- diniconazole, (β*E*)-β-[(2,4-dichlorophenyl)methylene]-α-(1,1-dimethylethyl)-1*H*-1,2,4-triazole-1-ethanol (Noyaku Kagaku, 1983, Vol. 8, p. 575);
- enilconazole (imazalil), 1-[2-(2,4-dichlorphenyl)-2-(2-propenyloxy)ethyl]-1*H*-imidazole (Fruits, 1973, Vol. 28, p. 545);
- epoxiconazole, (2*RS*,3*SR*)-1-[3-(2-chlorophenyl)-2,3-epoxy-2-(4-

fluorophenyl)propyl]-1*H*-1,2,4-triazole (EP-A 196 038);
- fenbuconazole, α-[2-(4-chlorophenyl)ethyl]-α-phenyl-1*H*-1,2,4-triazole-1-propanenitrile (Proc. 1988 Br. Crop Prot. Conf. – Pests Dis. Vol. 1, p. 33);
- flusilazole, 1-{[bis-(4-fluorophenyl)methylsilanyl]methyl}-1H-[1,2,4]triazole (Proc. Br. Crop Prot. Conf.-Pests Dis., 1, 413 (1984));
- fluquinconazole, 3-(2,4-dichlorophenyl)-6-fluoro-2-[1,2,4]–triazol-1-yl-3H-quinazolin-4-one (Proc. Br. Crop Prot. Conf.-Pests Dis., 5-3, 411 (1992));
- flutriafol, α-(2-fluorophenyl)-α-(4-fluorophenyl)-1*H*-1,2,4-triazole-1-ethanol (EP 15 756);
- hexaconazole, 2-(2,4-dichlorophenyl)-1-[1,2,4]triazol-1-ylhexan-2-ol (CAS RN 79983-71-4);
- imibenconazole, (4-chlorophenyl)methyl *N*-(2,4-dichlorophenyl)-1*H*-1,2,4-triazole-1-ethanimidothioate ((Proc. 1988 Br. Crop Prot. Conf. – Pests Dis. Vol. 2, p. 519),
- ipconazole, 2-[(4-chlorophenyl)methyl]-5-(1-methylethyl)-1-(1*H*-1,2,4-triazol-1-yl-methyl)cyclopentanol (EP 267 778),
- metconazole, 5-(4-chlorobenzyl)-2,2-dimethyl-1-[1,2,4]triazol-1-ylmethylcyclo-pentanol (GB 857 383);
- myclobutanil, 2-(4-chlorophenyl)-2-[1,2,4]triazol-1-ylmethylpentanenitrile (CAS RN 88671–89–0);
- penconazole, 1-[2-(2,4-dichlorophenyl)pentyl]-1H-[1,2,4]triazole (Pesticide Manual, 12th Ed. (2000), p.712);
- propiconazole, 1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1*H*-1,2,4-triazole (BE 835 579);
- prothioconazole, 2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-[1,2,4]triazole-3-thione (WO 96/16048);
- simeconazole, α-(4-fluorophenyl)-α-[(trimethylsilyl)methyl]-1*H*-1,2,4-triazole-1-ethanol [CAS RN 149508-90-7],
- tebuconazole, 1-(4-chlorophenyl)-4,4-dimethyl-3-[1,2,4]triazol-1-ylmethylpentan-3-ol (EP-A 40 345);
- tetraconazole, 1-[2-(2,4-dichlorophenyl)-3-(1,1,2,2-tetrafluoroethoxy)propyl]-1*H*-1,2,4-triazole (EP 234 242);
- triadimenol, β-(4-chlorophenoxy)-α-(1,1-dimethylethyl)-1*H*-1,2,4-triazole-1-ethanol;
- triadimefon, 1-(4-chlorophenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)-2-butanone;
- triticonazole, (5*E*)-5-[(4-chlorophenyl)methylene]-2,2-dimethyl-1-(1*H*-1,2,4-triazol-1-ylmethyl)cyclopentanol (FR 26 41 277);
- cyazofamid, 4-chloro-2-cyano-*N,N*-dimethyl-5-(4-methylphenyl)-1*H*-imidazole-1-sulfonamide (CAS RN 120116-88-3],
- pefurazoate, 4-pentenyl 2-[(2-furanylmethyl)(1*H*-imidazol-1-ylcarbonyl)amino]butanoate [CAS RN 101903-30-4],
- prochloraz, N-{propyl-[2-(2,4,6-trichlorophenoxy)ethyl]}imidazole-1-carboxamide (US 3 991 071);

- triflumizole, (4-chloro-2-trifluoromethylphenyl)-(2-propoxy-1-[1,2,4]triazol-1-ylethylidene)amine (JP-A 79/119 462)
- benomyl, N-butyl-2-acetylaminobenzoimidazol-1-carboxamide (US 3 631 176);
- carbendazim, methyl (1H-benzoimidazol-2-yl)-carbamate (US 3 657 443);
- fuberidazole, 2-(2-furanyl)-1*H*-benzimidazole (DE 12 09 799),
- thiabendazole, 2-(1,3-thiazol-4-yl)benzimidazole (US 3 017 415),
- ethaboxam, *N*-(cyano-2-thienylmethyl)-4-ethyl-2-(ethylamino)-5-thiazolcarboxamide (EP-A 639 574),
- etridiazole,
- hymexazole, 5-methyl-1,2-oxazol-3-ol (JP 518249, JP 532202),
- fluazinam, 3-chloro-N-[3-chloro-2,6-dinitro-4-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2-pyridinamine (The Pesticide Manual, publ. The British Crop Protection Council, 10th ed. (1995), p. 474);
- pyrifenox, 1-(2,4-dichlorophenyl)-2-(3-pyridinyl)ethanone *O*-methyloxime (EP-A 49 854);
- bupirimate, 5-butyl-2-ethylamino-6-methylpyrimidin-4-yldimethylsulfamate [CAS RN 41483-43-6];
- cyprodinil, (4-cyclopropyl-6-methylpyrimidin-2-yl)phenylamine (EP-A 310 550);
- ferimzone, (*Z*)-2'-methylacetophenone 4,6-dimethylpyrimidin-2-ylhydrazone [CAS RN 89269-64-7];
- fenarimol, (4-chlorophenyl) (2-chlorophenyl) pyrimidin-5-ylmethanol (GB 12 18 623);
- mepanipyrim, (4-methyl-6-prop-1-ynylpyrimidin-2-yl)phenylamine (EP-A 224 339);
- nuarimol, alpha-(2-chlorophenyl)-alpha-(4-fluorophenyl)-5-pyrimidinemethanol (GB 12 18 623);
- pyrimethanil, 4,6-dimethylpyrimidin-2-ylphenylamine (DD-A 151 404);
- triforine, *N,N'*-{piperazine-1,4-diylbis[(trichloromethyl)methylene]}diformamide (DE 19 01 421);
- fludioxonil, 4-(2,2-difluorobenzo[1,3]dioxol-4-yl)-1H-pyrrole-3-carbonitrile (The Pesticide Manual, publ. The British Crop Protection Council, 10th ed. (1995), p. 482);
- fenpiclonil, 4-(2,3-dichlorophenyl)-1H-pyrrole-3-carbonitrile (Proc. 1988 Br. Crop Prot. Conf. – Pests Dis., Vol. 1, p. 65);
- aldimorph, 4-alkyl-2,5(or 2,6)-dimethylmorpholine, comprising 65-75% of 2,6-dimethylmorpholine and 25-35% of 2,5-dimethylmorpholine, comprising more than 85% of 4-dodecyl-2,5(or 2,6)-dimethylmorpholine, where "alkyl" may also include octyl, decyl, tetradecyl or hexadecyl and where the cis/trans ratio is 1:1;
- dodemorph, 4-cyclododecyl-2,6-dimethylmorpholine (DE 1198125);
- fenpropimorph, (*RS*)-*cis*-4-[3-(4-*tert*-butylphenyl)-2-methylpropyl]-2,6-dimethylmorpholine (DE 27 52 096);
- tridemorph, 2,6-dimethyl-4-tridecylmorpholine (DE 11 64 152);
- iprodione, N-isopropyl-3-(3,5-dichlorophenyl)-2,4-dioxoimidazolidine-1-carboxamide (GB 13 12 536);
- procymidone, *N*-(3,5-dichlorophenyl)-1,2-dimethylcyclopropane-1,2-dicarboximide

(US 3 903 090);
- vinclozolin, 3-(3,5-dichlorophenyl)-5-methyl-5-vinyloxazolidine-2,4-dione (DE-OS 22 07 576);
- acibenzolar-S-methyl, methyl benzo[1,2,3]thiadiazole-7-carbothionate;
- anilazine, 4,6-dichloro-*N*-(2-chlorophenyl)-1,3,5-triazine-2-amine (US 2 720 480);
- captan, 2-trichloromethylsulfanyl-3a,4,7,7a-tetrahydroisoindole-1,3-dione (US 2 553 770);
- captafol, *N*-(1,1,2,2-tetrachloroethylthio)cyclohex-4-ene-1,2-dicarboximide (Phytopathology 52, S. 754 (1962));
- dazomet, 3,5-dimethyl-1,3,5-thiadiazinane-2-thione (Bull. Soc. Chim. Fr. Vol. 15, p. 891 (1897));
- diclomezine, 6-(3,5-dichlorophenyl)-*p*-tolyl)pyridazin-3(2*H*)-one (US 4,052,395);
- fenoxanil, *N*-(1-cyano-1,2-dimethylpropyl)-2-(2,4-dichlorophenoxy) propanamide (EP-A 262 393);
- folpet, 2-trichloromethylsulfanylisoindole-1,3-dione (US 2 553 770);
- fenpropidin, (*RS*)-1-[3-(4-*tert*-butylphenyl)-2-methylpropyl]piperidine (DE 27 52 096);
- famoxadone, (*RS*)-3-anilino-5-methyl-5-(4-phenoxyphenyl)-1,3-oxazolidine-2,4-dione [CAS RN 131807-57-3];
- fenamidone, (*S*)-1-anilino-4-methyl-2-methylthio-4-phenylimidazolin-5-one [CAS RN 161326-34-7];
- octhilinone,
- probenazole, 3-allyloxy-1,2-benzothiazole 1,1-dioxide;
- proquinazid, 6-iodo-2-propoxy-3-propylquinazolin-4(3*H*)-one (WO 97/48684);
- pyroquilon, 1,2,5,6-tetrahydropyrrolo[3,2,1-*ij*]quinolin-4-on (GB 139 43 373)
- quinoxyfen, 5,7-dichloro-4-(4-fluorophenoxy)quinoline (US 5 240 940);
- tricyclazole, 5-methyl-1,2,4-triazolo[3,4-*b*]benzothiazole (GB 14 19 121);
- ferbam, iron(3+) dimethyldithiocarbamate (US 1 972 961);
- mancozeb, manganese ethylenebis(dithiocarbanate) zinc complex (US 3 379 610);
- maneb, manganese ethylenebis(dithiocarbamate) (US 2 504 404);
- metiram, zinc ammoniate ethylenebis(dithiocarbamate) (US 3 248 400);
- metam, methyldithiocarbaminic acid (US 2 791 605);
- propineb, zinc propylenebis(dithiocarbamate) polymer (BE 611 960);
- thiram, bis(dimethylthiocarbamoyl) disulfide (DE 642 532);
- zineb, zinc ethylenebis(dithiocarbamate) (US 2 457 674);
- ziram, dimethyldithiocarbamate [CAS RN 137-30-4];
- diethofencarb, isopropyl 3,4-diethoxycarbanilate (EP-A 78 663);
- flubenthiavalicarb (benthiavalicarb), isopropyl {(S)-1-[(1R)-1-(6-fluorobenzothiazol-2-yl)ethylcarbamoyl]-2-methylpropyl}carbamate (JP-A 09/323 984);
- iprovalicarb, isopropyl [(1S)-2-methyl-1-(1-p-tolylethylcarbamoyl)propyl]carbamate (EP-A 472 996);
- propamocarb, propyl 3-(dimethylamino)propylcarbamate (DE 16 43 040);
- dodine, (2,4-dichlorophenoxy)acetic acid (US 2 867 562);

- iminoctadine, bis(8-guanidinooctyl)amine (GB 11 14 155);
- guazatine, mixture of products from the amidation of iminodi(octamethylene)diamine, mainly iminoctadine [CAS RN 108173-90-6];
- kasugamycin, 1L-1,3,4/2,5,6-1-deoxy-2,3,4,5,6-pentahydroxycyclohexyl 2-amino-2,3,4,6-tetradeoxy-4-(alpha-iminoglycino)-alpha-D-arabino-hexopyranoside [CAS RN 6980-18-3];
- polyoxine, 5-(2-amino-5-*O*-carbamoyl-2-deoxy-L-xylonamido)-1-(5-carboxy-1,2,3,4-tetrahydro-2,4-dioxopyrimidin-1-yl)-1,5-dideoxy-beta-D-allofuranuronic acid and the salts thereof [CAS RN 22976-86-9];
- streptomycin, *O*-2-deoxy-2-methylamino-alpha-L-glucopyranosyl-(1□2)-*O*-5-deoxy-3-*C*-formyl-alpha-L-lyxofuranosyl-(1□4)N^1,N^3-diamidino-D-streptamine (J. Am. Chem. Soc. 69, S.1234 (1947));
- validamycin A,- fentin acetate, triphenyltin acetate (US 3 499 086);
- isoprothiolan, diisopropyl 1,3-dithiolan-2-ylidenemalonat (Proc. Insectic. Fungic. Conf. 8. Bd. 2, S. 715 (1975));
- dithianon, 5,10-Dioxo-5,10-dihydronaphtho[2,3-b][1,4]dithiin-2,3-dicarbonitril (GB 857 383);
- edifenphos, *O*-ethyl *S,S*-diphenyl phosphorodithioate (DE-A 14 93 736);
- fosetyl, fosetyl-aluminum, (aluminum) ethylphosphonate (FR 22 54 276);
- iprobenfos, *S*-benzyl *O,O*-diisopropyl phosphorothioate (Jpn. Pesticide Inf., No. 2, S. 11 (1970));
- pyrazophos, ethyl 2-diethoxyphosphinothioyloxy-5-methylpyrazolo[1,5-*a*]pyrimidine-6-carboxylate (DE 15 45 790);
- tolclofos-methyl, *O*-2,6-dichloro-*p*-tolyl *O,O*-dimethyl phosphorothioate (GB 14 67 561);
- thiophanate-methyl, 1,2-phenylenebis(iminocarbonothioyl)bis(dimethylcarbamate) (DE-OS 19 30 540);
- chlorothalonil, 2,4,5,6-tetrachloroisophthalonitrile (US 3 290 353);
- dichlofluanid, N-dichlorofluoromethylthio-*N'*,*N'*-dimethyl-*N*-phenylsulfamide (DE 11 93 498);
- tolylfluanid, *N*-dichlorofluoromethylthio-*N'*,*N'*-dimethyl-*N*-*p*-tolylsulfamide (DE 11 93 498);
- flusulfamide, 2',4-dichloro-α,α,α-trifluoro-4'-nitro-*m*-toluenesulfanilide (EP-A 199 433);
- phthalide (DE 16 43 347);
- hexachlorobenzene (C. R. Seances Acad. Agric. Fr., Vol. 31, p. 24 (1945));
- pencycuron, 1-(4-chlorobenzyl)-1-cyclopentyl-3-phenylurea (DE 27 32 257);
- quintozene, pentachloronitrobenzene (DE 682 048);
- binapacryl, (*RS*)-2-*sec*-Butyl-4,6-dinitrophenyl 3-methylcrotonat [CAS RN 485-31-4];
- dinocap, mixture of 2,6-dinitro-4-octylphenylcrotonate and 2,4-dinitro-6-octyl-phenylcrotonate, wherein “octyl” is a mixture of 1-methylheptyl, 1-ethylhexyl and 1-propylpentyl (US 2,526,660);

- dinobuton, (*RS*)-2-*sec*-Butyl-4,6-dinitrophenyl isopropyl carbonat [CAS RN 973-21-7];
- Bordeaux composition, mixture of $CuSO_4$ x $3Cu(OH)_2$ x $3CaSO_4$ [CAS RN 8011-63-0]
- copper acetate, $Cu(OCOCH_3)_2$ [CAS RN 8011-63-0];
- copper oxychloride, $Cu_2Cl(OH)_3$ [CAS RN 1332-40-7];
- basic copper sulfate, $CuSO_4$ [CAS RN 1344-73-6];
- spiroxamine, (8-tert-butyl-1,4-dioxaspiro[4.5]dec-2-yl)diethylamine (EP-A 281 842).
- cyflufenamid, (*Z*)-*N*-[α-(cyclopropylmethoxyimino)-2,3-difluoro-6-(trifluoromethyl)benzyl]-2-phenylacetamide (WO 96/19442);
- cymoxanil, 1-(2-cyano-2-methoxyiminoacetyl)-3-ethylurea (US 3 957 847);
- metrafenone, 3′-bromo-2,3,4,6′-tetramethoxy-2′,6-dimethylbenzophenone (US 5 945 567);

The compounds are named according to IUPAC, their preparation and their fungicidal actions are likewise known:

N-(4'-bromobiphenyl-2-yl)-4-difluoromethyl-2-methylthiazole-5-carboxamide, N-(4'-trifluoromethylbiphenyl-2-yl)-4-difluoromethyl-2-methylthiazole-5-carboxamide, N-(4'-chloro-3'-fluorobiphenyl-2-yl)-4-difluoromethyl-2-methylthiazole-5-carboxamide, N-(3',4'-dichloro-4-fluorobiphenyl-2-yl)-3-difluoromethyl-1-methylpyrazole-4-carboxamide (WO 03/066609),

3,4-dichloro-isothiazol-5-carboxylic acid (2-cyanophenyl) amide (WO 99/24413);

N-(2-(4-[3-(4-Chlor-phenyl)-prop-2-ynyloxy]-3-methoxy-phenyl)-ethyl)-2-methan-sulfonylamino-3-methyl-butyramid, N-(2-(4-[3-(4-Chlor-phenyl)-prop-2-ynyloxy]-3-methoxy-phenyl)-ethyl)-2-ethanesulfonylamino-3-methyl-butyramid (WO 04/49804);

3-[5-(4-chlorophenyl)-2,3-dimethylisoxazolidin-3-yl]pyridine (EP-A 10 35 122);

2-butoxy-6-iodo-3-propylchromen-4-one (WO 03/14103),

methyl 3-(4-chlorophenyl)-3-(2-isopropoxycarbonylamino-3-methylbutyrylamino)-propanoate (EP-A 1028125)

Furthermore, the commercially available compounds of groups B18) to B32) of the active ingredient (B) may be found in The Pesticide Manual, 13th Edition, British Crop Protection Council (2003) among other publications.

Thiamides of formula Γ (gamma)[1] and their preparation have been described in WO199828279.

Lepimection is known from Agro Project, PJB Publications Ltd, November 2004. Benclothiaz and its preparation have been described in EP-A1 454621. Methidathion

and Paraoxon and their preparation have been described in Farm Chemicals Handbook, Volume 88, Meister Publishing Company, 2001. Acetoprole and its preparation have been described in WO199828277. Flupyrazofos has been described in Pesticide Science 54, 1988, p. 237-243 and in US 4822779. Pyrafluprole and its preparation have been described in JP 2002193709 and in WO200100614. Pyriprole and its preparation have been described in WO199845274 and in US 6335357. Amidoflumet and its preparation have been described in US 6221890 and in JP 21010907. Flufenerim and its preparation have been described in WO2003007717 and in WO2003007718. Cyflumetofen and its preparation have been described in WO2004080180. Anthranilamides of formula Γ (gamma)[5] and their preparation have been described in WO200170671; WO200248137; WO200324222, WO200315518, WO200467528; WO200433468; and WO2005118552.

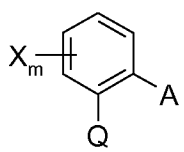
According to one embodiment of the invention, the active ingredient (B) is an active compound that inhibits the mitochondrial breathing chain at the level of the b/c_1 complex.

Active compounds that inhibit the mitochondrial breathing chain at the level of the b/c_1 complex are known as fungicides from the literature [see for example Dechema-Monographien Bd. 129, 27-38, VCH Verlagsgemeinschaft Weinheim 1993; Natural Product Reports 1993, 565-574; Biochem. Soc. Trans. 22, 63S (1993)].

A particularly important class of active compounds that inhibit the mitochondrial breathing chain at the level of the b/c_1 complex are strobilurins. Strobilurins are generally known as fungicides since a long time and have, in some cases, also been described as insecticides (EP–A 178 826; EP-A 253 213; WO 93/15046; WO 95/18789; WO 95/21153; WO 95/21154; WO 95/24396; WO 96/01256; WO 97/15552; WO 97/27189). A further example of an active compound hat inhibits the mitochondrial breathing chain at the level of the b/c_1 complex is famoxadone (5-methyl-5-(4-phenoxyphenyl)-3-(phenylamino)-2,4-oxazolidinedione).

In a further preferred embodiment of the present invention, strobilurins are used as the active ingredient (B). According to the present invention, strobilurins which have proven particularly suitable are selected from

1) compounds of formula I



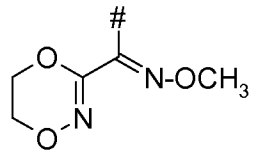
I

in which

X is halogen, C_1-C_4-alkyl or trifluoromethyl;

m is 0 or 1;

Q is $C(=CH-CH_3)-COOCH_3$, $C(=CH-OCH_3)-COOCH_3$, $C(=N-OCH_3)-CONHCH_3$, $C(=N-OCH_3)-COOCH_3$, $N(-OCH_3)-COOCH_3$, or a group Q1



Q1

wherein # denotes the bond to the phenyl ring;

A is -O-B, $-CH_2O-B$, $-OCH_2-B$, $-CH_2S-B$, -CH=CH-B, -C≡C-B, $-CH_2O-N=C(R^1)-B$, $-CH_2S-N=C(R^1)-B$, $-CH_2O-N=C(R^1)-CH=CH-B$, or $-CH_2O-N=C(R^1)-C(R^2)=N-OR^3$, where

B is phenyl, naphthyl, 5-membered or 6-membered heteroaryl or 5-membered or 6-membered heterocyclyl, containing one, two or three N atoms and/or one O or S atom or one or two O and/or S atoms, the ring systems being unsubstituted or substituted by one, two or three radicals R^a:

R^a is independently cyano, nitro, amino, aminocarbonyl, aminothiocarbonyl, halogen, C_1-C_6-alkyl, C_1-C_6-haloalkyl, C_1-C_6-alkylcarbonyl, C_1-C_6-alkylsulfonyl, C_1-C_6-alkylsulfinyl, C_3-C_6-cycloalkyl, C_1-C_6-alkoxy, C_1-C_6-haloalkoxy, C_1-C_6-alkyloxycarbonyl, C_1-C_6-alkylthio, C_1-C_6-alkylamino, di-C_1-C_6-alkylamino, C_1-C_6-alkylaminocarbonyl, di-C_1-C_6-alkylaminocarbonyl, C_1-C_6-alkylaminothiocarbonyl, di-C_1-C_6-alkylaminothiocarbonyl, C_2-C_6-alkenyl, C_2-C_6-alkenyloxy, phenyl, phenoxy, benzyl, benzyloxy, 5- or 6-membered heterocyclyl, 5- or 6-membered heteroaryl, 5- or 6-membered heteroaryloxy, $C(=NOR^a)-R^b$ or $OC(R^a)_2-C(R^b)=NOR^b$, the cyclic radicals, in turn, being unsubstituted or substituted by one, two or three radicals R^b:

R^b is independently cyano, nitro, halogen, amino, aminocarbonyl, aminothiocarbonyl, C_1-C_6-alkyl, C_1-C_6-haloalkyl, C_1-C_6-alkylsulfonyl, C_1-C_6-alkylsulfinyl, C_3-C_6-cycloalkyl, C_1-C_6-alkoxy, C_1-C_6-haloalkoxy, C_1-C_6-alkoxycarbonyl, C_1-C_6-alkylthio, C_1-C_6-alkylamino, di-C_1-C_6-alkylamino, C_1-C_6-alkylaminocarbonyl, di-C_1-C_6-alkylaminocarbonyl, C_1-C_6-alkylaminothiocarbonyl, di-C_1-C_6-alkyl-aminothiocarbonyl, C_2-

C_6-alkenyl, C_2-C_6-alkenyloxy, C_3–C_6–cycloalkyl, C_3–C_6–cycloalkenyl, phenyl, phenoxy, phenylthio, benzyl, benzyloxy, 5- or 6-membered heterocyclyl, 5- or 6-membered heteroaryl, 5- or 6-membered heteroaryloxy or C(=NOR^A)-R^B;

R^A, R^B are independently hydrogen or C_1-C_6-alkyl;

R^1 is hydrogen, cyano, C_1-C_4-alkyl, C_1-C_4-haloalkyl, C_3-C_6-cycloalkyl, C_1-C_4-alkoxy, or C_1-C_4-alkylthio;

R^2 is phenyl, phenylcarbonyl, phenylsulfonyl, 5– or 6–membered heteroaryl, 5– or 6–membered heteroarylcarbonyl or 5– or 6–membered heteroarylsulfonyl, the ring systems being unsubstituted or substituted by one, two or three radicals R^a,

C_1-C_{10}-alkyl, C_3-C_6-cycloalkyl, C_2-C_{10}-alkenyl, C_2-C_{10}-alkynyl, C_1-C_{10}-alkylcarbonyl, C_2-C_{10}-alkenylcarbonyl, C_3-C_{10}-alkynylcarbonyl, C_1-C_{10}-alkylsulfonyl, or C(=NOR^a)-R^b, the hydrocarbon radicals of these groups being unsubstituted or substituted by one, two or three radicals R^c:
R^c is independently cyano, nitro, amino, aminocarbonyl, aminothiocarbonyl, halogen, C_1-C_6-alkyl, C_1-C_6-haloalkyl, C_1-C_6-alkylsulfonyl, C_1-C_6-alkylsulfinyl, C_1-C_6-alkoxy, C_1-C_6-haloalkoxy, C_1-C_6-alkoxycarbonyl, C_1-C_6-alkylthio, C_1-C_6-alkylamino, di-C_1-C_6-alkylamino, C_1-C_6-alkylaminocarbonyl, di-C_1-C_6-alkylaminocarbonyl, C_1-C_6-alkylaminothiocarbonyl, di-C_1-C_6-alkylaminothiocarbonyl, C_2-C_6-alkenyl, C_2-C_6-alkenyloxy,

C_3-C_6-cycloalkyl, C_3-C_6-cycloalkyloxy, 5- or 6-membered heterocyclyl, 5- or 6-membered heterocyclyloxy, benzyl, benzyloxy, phenyl, phenoxy, phenylthio, 5- or 6-membered heteroaryl, 5- or 6-membered heteroaryloxy and heteroarylthio, it being possible for the cyclic groups, in turn, to be partially or fully halogenated or to have attached to them one, two or three radicals R^a; and

R^3 is hydrogen, C_1-C_6-alkyl, C_2-C_6-alkenyl, C_2-C_6-alkynyl, the hydrocarbon radicals of these groups being unsubstituted or substituted by one, two or three radicals R^c; and

2) the strobilurins (2-chloro-5-[1-(3-methyl-benzyloxyimino)-ethyl]-benzyl)-carbamic acid methyl ester, (2-chloro-5-[1-(6-methyl-pyridine-2-ylmethoxyimino)-ethyl]-benzyl)-carbamic acid methyl ester and 2-(ortho-((2,5-dimethylphenyl-oxymethylene)phenyl)-3-methoxy-acrylic acid methyl ester.

Compounds of formula I are generally known as fungicides since a long time (see references above).

The publications cited above describe synthesis routes for the preparation of strobilurins used in the method according to the invention, the disclosure of which is hereby incorporated.

According to still another preferred embodiment of the present ivnention, the active ingredient (B) is selected from the group consisting of:

Fungicide:
Anilide, preferably Isopyrazam, N-(2-bicyclopropyl-2-yl-phenyl)-3-difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic , acid amide
N-(trans-2-bicyclopropyl-2-yl-phenyl)-3-difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid amide
N-(cis-2-bicyclopropyl-2-yl-phenyl)-3-difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid amide
N-(2-(1,3-dimethyl-butyl)-phenyl)-1,3-dimethyl-5-fluoro-1H-pyrazole-4-carboxylic acid amide
3-Difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid (2',4'-difluorobiphenyl-2-yl)-amide
3-Difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid (2',4'-dichlorobiphenyl-2-yl)-amide
3-Difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid (3',4'-difluorobiphenyl-2-yl)-amide
3-Difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid (3',4'-dichlorobiphenyl-2-yl)-amide
3-Difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid (3',5'-difluorobiphenyl-2-yl)-amide
3-Difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid (3',5'-dichlorobiphenyl-2-yl)-amide
3-Difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid (3',4',5'-trifluorobiphenyl-2-yl)-amide

Fluopyram, 5-Amino-2-isopropyl-3-oxo-4-ortho-tolyl-2,3-dihydro-pyrazole-1-carbothioic acid S-allyl ester
N'-(4-(4-chloro-3-trifluoromethyl-phenoxy)-2,5-dimethyl-phenyl)-N-ethyl-N-methyl formamidine

N'-(4-(4-fluoro-3-trifluoromethyl-phenoxy)-2,5-dimethyl-phenyl)-N-ethyl-N-methyl formamidine
N'-(2-methyl-5-trifluormethyl-4-(3-trimethylsilanyl-propoxy)-phenyl)-N-ethyl-N-methyl formamidine
N'-(5-difluormethyl-2-methyl-4-(3-trimethylsilanyl-propoxy)-phenyl)-N-ethyl-N-methyl formamidine,

According to still another preferred embodiment of the present invention, the active ingredient (B) is selected from the group consisting of: PGR's: abscisic acid, Amidochlor, Ancymidol, 6-Benzylaminopurine, Brassinolide, Butralin, Choline chloride, Cyclanilide, Daminozide, Dikegulac, Dimethipin, 2,6-Dimethylpuridine, Ethephon, Flumetralin, Flurprimidol, Fluthiacet, Forchlorfenuron, Gibberellic acid, Inabenfide, indole-3-acetic acid, Maleic hydrazide, Mefluidide, naphthaleneacetic acid, N-6 benzyladenine, Paclobutrazol, Prohydrojasmon, Thidiazuron, Triapenthenol, Tributyl phosphorotrithioate, 2,3,5-tri-iodobenzoic acid, Uniconazole,

According to still another preferred embodiment of the present invention, the active ingredient (B) is selected from the group consisting of: fungicides: azoxystrobin, Dimoxystrobin, Kresoxim-methyl, Orysastrobin, Pyraclostrobin, Trifloxystrobin, Bixafen, Boscalid, Isopyrazam, Metalaxyl, Penthiopyrad, 3-Difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid (2',4',5'-trifluorobiphenyl-2-yl)-amide , N-(2-bicyclopropyl-2-yl-phenyl)-3-difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid amide, Dimethomorph, Fluopicolide, Difenoconazole, Epoxiconazole, Fluquinconazole, Flusilazole, Flutriafol, Metconazol, Myclobutanil, Propiconazole, Prothioconazole, Tebuconazole, Tetraconazole, Triticonazole, Prochloraz, Carbendazim , Fluazinam, Cyprodinil, Pyrimethanil, Fludioxonil, Dodemorph, Fenpropimorph, Tridemorph, Fenpropidin, Iprodione, Vinclozolin, Famoxadone, Probenazole, Captan, Folpet, 5-ethyl-6-octyl-[1,2,4]triazolo[1,5-a]pyrimidine-7-ylamine, Mancozeb, Maneb, Metiram, Thiram, Dithianon, Fosetyl, Fosetyl-aluminium, Chlorothalonil, Thiophanate Methyl, Cymoxanil, Metrafenone, Spiroxamine,most preferred: Azoxystrobin, Dimoxystrobin, Kresoxim-methyl, Orysastrobin, Pyraclostrobin, Trifloxystrobin, Bixafen, Boscalid, Isopyrazam, Metalaxyl, Penthiopyrad, 3-Difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid (2',4',5'-trifluorobiphenyl-2-yl)-amide , Dimethomorph, Difenoconazole, Epoxiconazole, Fluquinconazole, Metconazol, Propiconazole, Prothioconazole, Tebuconazole, Triticonazole, Prochloraz, Carbendazim, Cyprodinil, Pyrimethanil, Fenpropimorph, Tridemorph, Iprodione, 5-ethyl-6-octyl-[1,2,4]triazolo[1,5-a]pyrimidine-7-ylamine, Mancozeb, Maneb, Metiram, Dithianon, Chlorothalonil, Thiophanate Methyl, Cymoxanil, Metrafenone.According to still another preferred embodiment of the present ivnention, the active ingredient (B) is selected from the group consisting of: N-(3',4',5'-trifluorobiphenyl-2-yl)- 3-difluoromethyl-1-methyl-1H-pyrazole-4-carboxamide,

N-[2-(4'-trifluoromethylthio)-biphenyl]-3-difluoromethyl-1-methyl-1H-pyrazole-4-carboxamide,
N-[2-(1,3-dimethylbutyl)-phenyl]-1,3-dimethyl-5-fluoro-1H-pyrazole-4-carboxamide,
N-(2-bicyclopropyl-2-yl-phenyl)-3-difluoromethyl-1-methyl-1H-pyrazole-4-carboxamide,
N-(cis-2-bicyclopropyl-2-yl-phenyl)-3-difluoromethyl-1-methyl-1H-pyrazole-4-carboxamide,
N-(trans-2-bicyclopropyl-2-yl-phenyl)-3-difluoromethyl-1-methyl-1H-pyrazole-4-carboxamide,
N-[1,2,3,4-tetrahydro-9-(1-methylethyl)-1,4-methanonaphthalen-5-yl]-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide.

In one embodiment the habitat is a plant, selected from the families Aceraceae, Anacardiaceae, Apiaceae, Asteraceae, Brassicaceae, Cactaceae, Cucurbitaceae, Euphorbiaceae, Fabaceae, Malvaceae, Nymphaeaceae, Papaveraceae, Rosaceae, Salicaceae, Solanaceae, Arecaceae, Bromeliaceae, Cyperaceae, Iridaceae, Liliaceae, Orchidaceae, Gentianaceae, Labiaceae, Magnoliaceae, Ranunculaceae, Carifolaceae, Rubiaceae, Scrophulariaceae, Caryophyllaceae, Ericaceae, Polygonaceae, Violaceae, Juncaceae or Poaceae and preferably from a plant selected from the group of the families Apiaceae, Asteraceae, Brassicaceae, Cucurbitaceae, Fabaceae, Papaveraceae, Rosaceae, Solanaceae, Liliaceae or Poaceae. Preferred are crop plants such as plants advantageously selected from the group of the genus peanut, oilseed rape, canola, cotton, sunflower, sugar cane, safflower, olive, sesame, hazelnut, almond, avocado, bay, pumpkin/squash, linseed, soybeans, pistachio, borage, maize, wheat, rye, oats, sorghum and millet, triticale, rice, barley, cassava, potato, sugar beet, egg plant, alfalfa, and perennial grasses and forage plants, oil palm, vegetables (brassicas, root vegetables, tuber vegetables, pod vegetables, fruiting vegetables, onion vegetables, leafy vegetables and stem vegetables), buckwheat, Jerusalem artichoke, broad bean, vetches, lentil, dwarf bean, lupin, clover, potato, tomato, lettuce, onions and Lucerne.

In one prefered embodiment, the plant is selected from the families Aceraceae, Anacardiaceae, Apiaceae, Asteraceae, Brassicaceae, Cactaceae, Cucurbitaceae, Euphorbiaceae, Fabaceae, Malvaceae, Nymphaeaceae, Papaveraceae, Rosaceae, Salicaceae, Solanaceae, Arecaceae, Bromeliaceae, Cyperaceae, Iridaceae, Liliaceae, Orchidaceae, Gentianaceae, Labiaceae, Magnoliaceae, Ranunculaceae, Carifolaceae, Rubiaceae, Scrophulariaceae, Caryophyllaceae, Ericaceae, Polygonaceae, Violaceae, Juncaceae or Poaceae and preferably from a plant selected from the group of the families Apiaceae, Asteraceae, Brassicaceae, Cucurbitaceae, Fabaceae, Papaveraceae, Rosaceae, Solanaceae, Liliaceae or Poaceae.

Preferred are crop plants and in particular plants mentioned herein above as host plants such as the families and genera mentioned above for example preferred the species *Anacardium occidentale, Calendula officinalis*, *Carthamus tinctorius*,

Cichorium intybus, *Cynara scolymus*, *Helianthus annus*, *Tagetes lucida, Tagetes erecta, Tagetes tenuifolia*; *Daucus carota*; *Corylus avellana, Corylus colurna*, *Borago officinalis*; *Brassica napus*, *Brassica rapa* ssp., *Sinapis arvensis Brassica juncea, Brassica juncea var. juncea, Brassica juncea var. crispifolia, Brassica juncea var. foliosa, Brassica nigra, Brassica sinapioides, Melanosinapis communis*, *Brassica oleracea*, Arabidopsis thaliana, *Anana comosus, Ananas ananas, Bromelia comosa*, *Carica papaya*, *Cannabis sative*, *Ipomoea batatus, Ipomoea pandurata, Convolvulus batatas, Convolvulus tiliaceus, Ipomoea fastigiata, Ipomoea tiliacea, Ipomoea triloba, Convolvulus panduratus*, *Beta vulgaris, Beta vulgaris var. altissima*, *Beta vulgaris var. vulgaris, Beta maritima, Beta vulgaris var. perennis, Beta vulgaris var. conditiva, Beta vulgaris var. esculenta*, *Cucurbita maxima, Cucurbita mixta, Cucurbita pepo, Cucurbita moschata*, *Olea europaea*, *Manihot utilissima, Janipha manihot,, Jatropha manihot., Manihot aipil, Manihot dulcis, Manihot manihot, Manihot melanobasis, Manihot esculenta*, *Ricinus communis*, *Pisum sativum, Pisum arvense, Pisum humile*, *Medicago sativa, Medicago falcata, Medicago varia, Glycine max Dolichos soja, Glycine gracilis, Glycine hispida, Phaseolus max, Soja hispida, Soja max*, *Cocos nucifera, Pelargonium grossularioides, Oleum cocoas*, *Laurus nobilis*, *Persea americana, Arachis hypogaea*, *Linum usitatissimum, Linum humile, Linum austriacum, Linum bienne, Linum angustifolium, Linum catharticum, Linum flavum, Linum grandiflorum, Adenolinum grandiflorum, Linum lewisii, Linum narbonense, Linum perenne, Linum perenne var. lewisii, Linum pratense, Linum trigynum*, *Punica granatum, Gossypium hirsutum, Gossypium arboreum, Gossypium barbadense, Gossypium herbaceum, Gossypium thurberi*, *Musa nana, Musa acuminata, Musa paradisiaca, Musa* spp., *Elaeis guineensis*, *Papaver orientale, Papaver rhoeas, Papaver dubium*, *Sesamum indicum*, *Piper aduncum, Piper amalago, Piper angustifolium, Piper auritum, Piper betel, Piper cubeba, Piper longum, Piper nigrum, Piper retrofractum, Artanthe adunca, Artanthe elongata, Peperomia elongata, Piper elongatum, Steffensia elongata, , Hordeum vulgare, Hordeum jubatum, Hordeum murinum*, *Hordeum secalinum, Hordeum distichon Hordeum aegiceras, Hordeum hexastichon., Hordeum hexastichum, Hordeum irregulare, Hordeum sativum, Hordeum secalinum, Avena sativa, Avena fatua, Avena byzantina, Avena fatua var. sativa, Avena hybrida, Sorghum bicolor, Sorghum halepense, Sorghum saccharatum, Sorghum vulgare, Andropogon drummondii, Holcus bicolor, Holcus sorghum, Sorghum aethiopicum, Sorghum arundinaceum, Sorghum caffrorum, Sorghum cernuum, Sorghum dochna, Sorghum drummondii, Sorghum durra, Sorghum guineense, Sorghum lanceolatum, Sorghum nervosum, Sorghum saccharatum, Sorghum subglabrescens, Sorghum verticilliflorum, Sorghum vulgare, Holcus halepensis, Sorghum miliaceum millet*, *Panicum militaceum, Zea mays, Triticum aestivum, Triticum durum, Triticum turgidum, Triticum hybernum, Triticum macha, Triticum sativum* or *Triticum vulgare, Cofea spp., Coffea arabica, Coffea canephora, Coffea liberica, Capsicum annuum, Capsicum annuum var. glabriusculum, Capsicum*

frutescens, Capsicum annuum, Nicotiana tabacum, Solanum tuberosum, *Solanum melongena, Lycopersicon esculentum, Lycopersicon lycopersicum., Lycopersicon pyriforme, Solanum integrifolium, Solanum lycopersicum Theobroma cacao* or *Camellia sinensis*.

Anacardiaceae such as the genera Pistacia, Mangifera, Anacardium e.g. the species *Pistacia vera* [pistachios, Pistazie], *Mangifer indica* [Mango] or *Anacardium occidentale* [Cashew]; Asteraceae such as the genera Calendula, Carthamus, Centaurea, Cichorium, Cynara, Helianthus, Lactuca, Locusta, Tagetes, Valeriana e.g. the species *Calendula officinalis* [Marigold], *Carthamus tinctorius* [safflower], *Centaurea cyanus* [cornflower], *Cichorium intybus* [blue daisy], *Cynara scolymus* [Artichoke], *Helianthus annus* [sunflower], Lactuca sativa, *Lactuca crispa, Lactuca esculenta, Lactuca scariola L. ssp. sativa, Lactuca scariola L. var. integrata, Lactuca scariola L. var. integrifolia, Lactuca sativa subsp. romana*, *Locusta communis, Valeriana locusta* [lettuce], *Tagetes lucida, Tagetes erecta or Tagetes tenuifolia* [Marigold]; Apiaceae such as the genera Daucus e.g. the species *Daucus carota* [carrot]; Betulaceae such as the genera Corylus e.g. the species *Corylus avellana* or *Corylus colurna* [hazelnut]; Boraginaceae such as the genera Borago e.g. the species *Borago officinalis* [borage]; Brassicaceae such as the genera Brassica, Melanosinapis, Sinapis, Arabadopsis e.g. the species *Brassica napus*, *Brassica rapa* ssp. [canola, oilseed rape, turnip rape], *Sinapis arvensis Brassica juncea, Brassica juncea var. juncea, Brassica juncea var. crispifolia, Brassica juncea var. foliosa, Brassica nigra, Brassica sinapioides, Melanosinapis communis* [mustard], *Brassica oleracea* [fodder beet] or *Arabidopsis thaliana*; Bromeliaceae such as the genera Anana, Bromelia e.g. the species *Anana comosus, Ananas ananas* or *Bromelia comosa* [pineapple]; Caricaceae such as the genera Carica e.g. the species *Carica papaya* [papaya]; Cannabaceae such as the genera Cannabis e.g. the species *Cannabis sative* [hemp], Convolvulaceae such as the genera Ipomea, Convolvulus e.g. the species *Ipomoea batatus, Ipomoea pandurata, Convolvulus batatas, Convolvulus tiliaceus, Ipomoea fastigiata, Ipomoea tiliacea, Ipomoea triloba* or *Convolvulus panduratus* [sweet potato, Man of the Earth, wild potato], Chenopodiaceae such as the genera Beta, i.e. the species *Beta vulgaris, Beta vulgaris var. altissima*, *Beta vulgaris var. Vulgaris, Beta maritima, Beta vulgaris var. perennis, Beta vulgaris var. conditiva* or *Beta vulgaris var. esculenta* [sugar beet]; Cucurbitaceae such as the genera Cucubita e.g. the species *Cucurbita maxima, Cucurbita mixta, Cucurbita pepo* or *Cucurbita moschata* [pumpkin, squash]; Elaeagnaceae such as the genera Elaeagnus e.g. the species *Olea europaea* [olive]; Ericaceae such as the genera Kalmia e.g. the species *Kalmia latifolia, Kalmia angustifolia, Kalmia microphylla, Kalmia polifolia*, *Kalmia occidentalis, Cistus chamaerhodendros* or *Kalmia lucida* [American laurel, broad-leafed laurel, calico bush, spoon wood, sheep laurel, alpine laurel, bog laurel, western bog-laurel, swamp-

laurel]; Euphorbiaceae such as the genera Manihot, Janipha, Jatropha, Ricinus e.g. the species *Manihot utilissima, Janipha manihot,, Jatropha manihot., Manihot aipil, Manihot dulcis, Manihot manihot, Manihot melanobasis, Manihot esculenta* [manihot, arrowroot, tapioca, cassava] or *Ricinus communis* [castor bean, Castor Oil Bush, Castor Oil Plant, Palma Christi, Wonder Tree]; Fabaceae such as the genera Pisum, Albizia, Cathormion, Feuillea, Inga, Pithecolobium, Acacia, Mimosa, Medicajo, Glycine, Dolichos, Phaseolus, Soja e.g. the species *Pisum sativum, Pisum arvense, Pisum humile* [pea], *Albizia berteriana, Albizia julibrissin, Albizia lebbeck, Acacia berteriana, Acacia littoralis, Albizia berteriana, Albizzia berteriana, Cathormion berteriana, Feuillea berteriana, Inga fragrans, Pithecellobium berterianum, Pithecellobium fragrans, Pithecolobium berterianum, Pseudalbizzia berteriana, Acacia julibrissin, Acacia nemu, Albizia nemu, Feuilleea julibrissin, Mimosa julibrissin, Mimosa speciosa, Sericanrda julibrissin, Acacia lebbeck, Acacia macrophylla, Albizia lebbek, Feuilleea lebbeck, Mimosa lebbeck, Mimosa speciosa* [bastard logwood, silk tree, East Indian Walnut], *Medicago sativa, Medicago falcata, Medicago varia* [alfalfa] *Glycine max Dolichos soja, Glycine gracilis, Glycine hispida, Phaseolus max, Soja hispida* or *Soja max* [soybean]; Geraniaceae such as the genera Pelargonium, Cocos, Oleum e.g. the species *Cocos nucifera, Pelargonium grossularioides* or *Oleum cocois* [coconut]; Gramineae such as the genera Saccharum e.g. the species *Saccharum officinarum;* Juglandaceae such as the genera Juglans, Wallia e.g. the species *Juglans regia, Juglans ailanthifolia, Juglans sieboldiana, Juglans cinerea, Wallia cinerea, Juglans bixbyi, Juglans californica, Juglans hindsii, Juglans intermedia, Juglans jamaicensis, Juglans major, Juglans microcarpa, Juglans nigra* or *Wallia nigra* [walnut, black walnut, common walnut, persian walnut, white walnut, butternut, black walnut]; Lauraceae such as the genera Persea, Laurus e.g. the species laurel *Laurus nobilis* [bay, laurel, bay laurel, sweet bay], *Persea americana Persea americana, Persea gratissima* or *Persea persea* [avocado]; Leguminosae such as the genera Arachis e.g. the species *Arachis hypogaea* [peanut]; Linaceae such as the genera Linum, Adenolinum e.g. the species *Linum usitatissimum, Linum humile, Linum austriacum, Linum bienne, Linum angustifolium, Linum catharticum, Linum flavum, Linum grandiflorum, Adenolinum grandiflorum, Linum lewisii, Linum narbonense, Linum perenne, Linum perenne var. lewisii, Linum pratense* or *Linum trigynum* [flax, linseed]; Lythrarieae such as the genera Punica e.g. the species *Punica granatum* [pomegranate]; Malvaceae such as the genera Gossypium e.g. the species *Gossypium hirsutum, Gossypium arboreum, Gossypium barbadense, Gossypium herbaceum* or *Gossypium thurberi* [cotton]; Musaceae such as the genera Musa e.g. the species *Musa nana, Musa acuminata, Musa paradisiaca, Musa* spp. [banana]; Onagraceae such as the genera Camissonia, Oenothera e.g. the species *Oenothera biennis* or *Camissonia brevipes* [primrose, evening primrose]; Palmae such as the genera Elacis e.g. the species *Elaeis guineensis* [oil plam]; Papaveraceae such as the genera Papaver e.g. the species *Papaver orientale, Papaver rhoeas, Papaver dubium*

[poppy, oriental poppy, corn poppy, field poppy, shirley poppies, field poppy, long-headed poppy, long-pod poppy]; Pedaliaceae such as the genera Sesamum e.g. the species *Sesamum indicum* [sesame]; Piperaceae such as the genera Piper, Artanthe, Peperomia, Steffensia e.g. the species *Piper aduncum, Piper amalago, Piper angustifolium, Piper auritum, Piper betel, Piper cubeba, Piper longum, Piper nigrum, Piper retrofractum, Artanthe adunca, Artanthe elongata, Peperomia elongata, Piper elongatum, Steffensia elongata*. [Cayenne pepper, wild pepper]; Poaceae such as the genera Hordeum, Secale, Avena, Sorghum, Andropogon, Holcus, Panicum, Oryza, Zea, Triticum e.g. the species *Hordeum vulgare, Hordeum jubatum, Hordeum murinum*, *Hordeum secalinum, Hordeum distichon Hordeum aegiceras, Hordeum hexastichon., Hordeum hexastichum, Hordeum irregulare, Hordeum sativum, Hordeum secalinum* [barley, pearl barley, foxtail barley, wall barley, meadow barley], *Secale cereale* [rye], Avena sativa, *Avena fatua, Avena byzantina, Avena fatua var. sativa, Avena hybrida* [oat], *Sorghum bicolor, Sorghum halepense, Sorghum saccharatum, Sorghum vulgare, Andropogon drummondii, Holcus bicolor, Holcus sorghum, Sorghum aethiopicum, Sorghum arundinaceum, Sorghum caffrorum, Sorghum cernuum, Sorghum dochna, Sorghum drummondii, Sorghum durra, Sorghum guineense, Sorghum lanceolatum, Sorghum nervosum, Sorghum saccharatum, Sorghum subglabrescens, Sorghum verticilliflorum, Sorghum vulgare, Holcus halepensis, Sorghum miliaceum* millet, *Panicum militaceum [Sorghum, millet], Oryza sativa, Oryza latifolia* [rice], *Zea mays* [corn, maize] *Triticum aestivum, Triticum durum, Triticum turgidum, Triticum hybernum, Triticum macha, Triticum sativum* or *Triticum vulgare* [wheat, bread wheat, common wheat], Proteaceae such as the genera Macadamia e.g. the species *Macadamia intergrifolia* [macadamia]; Rubiaceae such as the genera Coffea e.g. the species Cofea spp., *Coffea arabica, Coffea canephora* or *Coffea liberica* [coffee]; Scrophulariaceae such as the genera Verbascum e.g. the species *Verbascum blattaria, Verbascum chaixii, Verbascum densiflorum, Verbascum lagurus, Verbascum longifolium, Verbascum lychnitis, Verbascum nigrum, Verbascum olympicum, Verbascum phlomoides, Verbascum phoenicum, Verbascum pulverulentum* or *Verbascum thapsus* [mullein, white moth mullein, nettle-leaved mullein, dense-flowered mullein, silver mullein, long-leaved mullein, white mullein, dark mullein, greek mullein, orange mullein, purple mullein, hoary mullein, great mullein]; Solanaceae such as the genera Capsicum, Nicotiana, Solanum, Lycopersicon e.g. the species *Capsicum annuum, Capsicum annuum var. glabriusculum, Capsicum frutescens* [pepper], *Capsicum annuum* [paprika], *Nicotiana tabacum, Nicotiana alata, Nicotiana attenuata, Nicotiana glauca, Nicotiana langsdorffii, Nicotiana obtusifolia, Nicotiana quadrivalvis, Nicotiana repanda, Nicotiana rustica, Nicotiana sylvestris* [tobacco], *Solanum tuberosum* [potato], *Solanum melongena* [egg-plant] (*Lycopersicon esculentum, Lycopersicon lycopersicum., Lycopersicon pyriforme, Solanum integrifolium* or *Solanum lycopersicum* [*tomato*]; Sterculiaceae such as the genera Theobroma e.g. the species *Theobroma cacao*

[cacao]; Theaceae such as the genera Camellia e.g. the species *Camellia sinensis*) [tea].

In a further embodiment of the invention the plant is a plant with a the transgenic modification.

The present examples illustrate the basic invention without being intended as limiting the subject of the invention.

The content of all of the references, patent applications, patents and published patent applications cited in the present patent application is herewith incorporated by reference.

Examples

Wheat was grown in the field trial at Gommersheim, Rhineland-Palatinate, Germany on loamy sand at a regular seeding rate with a regular seeding time and a regular fertilizer regime.

The trial consisted of two different treatments in six replicates: untreated control and treated with F500® fungicide. F500® was applied as Headline® with an application rate of 1L of Headline® per HA. The untreated control was sprayed with a spray solution, which contained the blank formulation of Headline® (product with all formulation agents but without F500®). Application rate of the blank formulation was 1L/HA, accordingly. Spray volume was 400 L spray solution per HA in both cases. Application was made at developmental stage 39/49 (BBCH scale), May 24, 2006. All plots received maintenance treatment with Opus™ fungicide at BBCH 31 and 5 days before Headline treatment to exclude a major pathogenic epidemic.

Wheat flag leaves were sampled for the analysis of the phyllospheric metagenome, when plants in the untreated plots showed indications of senescence. In each plot 10 leaves were collected in a sterile plastic bag and placed immediately on dry ice. Samples were stored in a freezer at -20°C before analysis.

Total DNA was extracted and fungal specific sequences were amplified using primers ITS-1F and ITS-4 designed from an Internal Transcribed Spacer (ITS) region of the tandem associated ribosomal DNA (White et al. 1990, Gardes & Bruns, 1993). The DNA was bulked for treated and untreated samples, respectively, prior to amplification. The amplification products were analyzed using agarose gel electrophoresis and purified using a QIAquick® PCR purification kit (QIAgene). Purified PCR products were used for cloning the incorporated sequences with a TOP TA Cloning® kit (Invitrogen) for sequencing. Following a blue and white selection, 1500 white colonies were picked for each of the two modalities (treated, untreated), re-suspended individually in liquid LB medium containing 50 µg/ml of ampicillin and incubated for 12 to 24 h before sequencing. Sequences were submitted for alignment

to the NIH sequence database (http://www.ncbi.nlm.nih.gov) using blastn algorithm. Sequences were considered taxonomically identified only, if matches show a minimum overlap of 320 nucleotides and a minimum similarity of 90% according to the expected minimum length of the amplification product of 400 nucleotides. If the sequence showed a minimum overlap of 396 nucleotides and a minimum similarity of 99%, sequences were considered to be identified at the species level.

Table 1: Number of identified sequences and level of identification of cloned sequences

Level of identification	Total	untreated	Headline (F500®)
Species	2015 (68.2%)	970 (65.6%)	1045 (70.8%)
Genus	250 (8.4%)	152 (11.6%)	98 (6.6%)
Not informative	670 (22.7%)	344 (23.3%)	326 (22.1%)
Not identified	19 (0.6%)	12 (0.8%)	7 (0.5%)
Total	2954	1478	1476

Out of the 3000 clones that were picked in total for sequencing 2954 revealed useful sequences used in further analysis (table 1), 1478 and 1476 for the untreated and F500® treated samples, respectively. 19 sequences (0.6%) did not match to any sequence at the above described criteria. 670 sequences (22.7%) were aligned to database sequences of unknown or taxonomically not described origin, and hence, were not informative.

Table 2: Distribution of identified clones

Phylum	Level of identification	Total	Untreated	Headline (F500®)
Ascomycota	Species	475	295	180
	Genus	121	84	37
	Total	*596*	*379*	*217*
Basidiomycota	Species	1540	675	865
	Genus	129	68	61
	Total	*1669*	*743*	*926*

Table 2 shows the distribution of the identified sequences to the phyla *Ascomycota* and *Basidiomycota* for treated and untreated samples. In general more than 70 % of the cases the identified *species* or *genera* belong to the *Basidiomycetes*. If taken all submitted sequences into account it is still more than 50%. The vast majority of the identified Basidiomycetes belong to yeasts of the *genera Sporobolomyces*, *Cryptococcus* and *Dioszegia* (table 3, 1554 clones representing 52.6 % of all submitted sequences). However, in total 11 different *Basidiomycota genera* were found.

In contrast, only 20.2 % of all clones (26.3 % of the identified sequences) could be assigned to the *Ascomycota*. Although less dominating, the *Ascomycetes* were more diverse with 16 *genera* identified. Nevertheless, only two *genera*, *Davidiella* and *Lewia*, represented the majority of the sequences assigned to the *Ascomycetes* (table 4, 405 clones representing 67.9% of the sequences assigned to that *phylum*).

In general, the biodiversity for the fungal population is greater in the untreated control than in the F500® treated samples (tables 3 and 4). There are 14 Ascomycetes and 9 Baidiomycetes identified only in the untreated samples whereas 1 Ascomycete species and 4 Basidiomycete species were found only in the F500® treated samples.

A number of primary or secondary pathogens were observed within the *Ascomycota* like the black head mold causing species of the genera *Alternaria*, *Epicoccum*, *Davidiella*, Cladosporium or the Septoria blotch causing *Mycosphaerella graminicola*. The number of identified clones of these pathogens are significantly reduced by the treatment with F500® (table 3, http://www.apsnet.org/online/common/names/wheat.asp). In contrast, the number of clones representing *Davidealla* species was not reduced.

Table 3: Number of identified clones (sequences) in the phylum *Ascomycota*

Genus	Organism name	Species Level			Genus Level			Total # of clones per genus
		Untreated	F500®	Σ	Untreated	F500®	Σ	
	ascomycete sp.	-	-	-	1	2	3	
?	*ascomycete* sp. HK-S242	5	-	5	-	-	-	8
	Lewia infectoria	76	1	77	-	-	-	
	lewia sp.	-	-	-	21	1	22	
Lewia	*Lewia* sp. 04MIT A1	1	-	1	-	-	-	100
Alternaria	*Alternaria* sp.	-	-	-	5	-	5	5
Pyrenophora	*Pyrenophora tritici-repentis*	2	-	2	-	-	-	2
	Epicoccum sp. 6/97-74	3	-	3	-	-	-	
	Epicoccum sp.	-	-	-	2	-	2	
Epicoccum	*Epicoccum nigrum*	1	-	1	-	-	-	6
?	uncultured *Leptosphaeriaceae*	2	-	2	1	-	1	3
Phaeosphaeria	*Phaeosphaeria* sp.	-	-	-	1	-	1	1
Didymella	*Didymella* sp.	-	-	-	2	-	2	2
?	uncultured *dothideomycete*	-	-	-	1	-	1	1
Ajellomyces	*Ajellomyces* sp.	-	-	-	25	13	38	38
Hypocrea	*Trichoderma* sp.	-	-	-	2	1	3	3
	Davidiella tassiana	138	162	300	-	-	-	
Davidiella	*Davidiella macrospora*	3	2	5	-	-	-	305
	Mycosphaerella graminicola	37	7	44	-	-	-	
Mycosphaerella	*Mycosphaerella sp.*	-	-	-	1	1	2	46
	Cladosporium magnusianum	2	-	2	-	-	-	
	Cladosporium cladosporioides	-	1	1	-	-	-	
	Cladosporium sp. 4/97 17	2	3	5	-	-	-	
Cladosporium	*Cladosporium* sp.	-	-	-	2	2	4	12
?	uncultured *Mycosphaerellaceae*	-	-	-	15	17	32	32
	Ascochyta sp. CBS 110130	18	1	19	-	-	-	
	Ascochyta sp. 02MIN3F2	2	3	5	-	-	-	
	Ascochyta skagwayensis	3	-	3	-	-	-	
Ascochyta	*Ascochyta sp.*	-	-	-	5	-	5	32
Total *Ascomycota*		295	180	475	84	37	121	596

Table 4: Number of identified clones (sequences) in the phylum *Basidiomycota*

Genus	Organism name	Species Level			Genus Level			Total # of Clones per Genus
		Untreated	F500®	∑	Untreated	F500®	∑	
Entyloma	*Entyloma ficariae*	2	1	3	-	-	-	12
	Entyloma sp.	-	-	-	9	-	9	
Tilletiopsis	*Tilletiopsis* sp. TUB 012103	6	3	9	-	-	-	10
	Tilletiopsis sp.	-	-	-	1	-	1	
Sporobolomyces	*Sporobolomyces roseus*	178	502	680	-	-	-	707
	Sporobolomyces sp. AS 2.2108	1	1	2	-	-	-	
	Sporobolomyces sp.	-	-	-	11	12	23	
	Sporobolomyces sp. JCM 11360	2	-	2	-	-	-	
Sporidiobolus	*Sporidiobolus* sp. CBS 5541	-	4	4	-	-	-	4
Udeniomyces	*Udeniomyces pannonicus*	22	22	44	-	-	-	45
	Udeniomyces sp.	-	-	-	-	1	1	
Cryptococcus	*Cryptococcus* sp. CBS 681.93	7	2	9	-	-	-	411
	Cryptococcus sp. HB 1222	44	1	45	-	-	-	
	Cryptococcus victoriae	122	162	284	-	-	-	
	Cryptococcus macerans	1	1	2	-	-	-	
	Cryptococcus sp.	-	-	-	34	33	67	
	Cryptococcus foliicola	1	-	1	-	-	-	
	Cryptococcus flavescens	1	-	1	-	-	-	
	Cryptococcus festucosus	1	-	1	-	-	-	
	Cryptococcus heimaeyensis	1	-	1	-	-	-	
Bullera	*Bullera globispora*	1	-	1	-	-	-	1
Dioszegia	*Dioszegia hungarica*	265	148	413	-	-	-	435
	Dioszegia sp.	-	-	-	9	13	22	
Sporobolomyces	*Sporobolomyces sp.*	-	-	-	1	-	1	1
Funalia	*Funalia trogii*	-	2	2	-	-	-	2
?	uncultured *basidiomycete* yeast	2-	16	36	3	1	4	41
	basidiomycete sp.	-	-	-	-	1	1	
Total B*asidiomycota*		675	865	1540	68	61	129	1669

There was a higher number of sequences belonging to the Basidiomycetes in the F500® treated samples than in the untreated ones (table 4). *Sporobolomyces* which include species causing black head mold in wheat showed a higher number of sequences following F500® treatment.
The tables show the differential profile of sequences representing different identified fungal species for the untreated leaf control and the F500® treated samples by means of metagenome analysis.

African Journal of Biotechnology; Vol. 2 (4), April 2003, pp. 82-85; PCR identification of Fusarium genus based on nuclear ribosomal-DNA sequence data
Kamel A. Abd-Elsalam 1, 3*, Ibrahim N. Aly2, Mohmed A. Abdel-Satar2, Mohmed S. Khalil1 and Joseph A. Verreet3
ITS1 and ITS2 and the inverting 5.8S coding rDNA were amplified by PCR using the

primers ITS1 and ITS4 as described by White et al. (1990).

White TJ, Bruns T, Lee S, Taylor JW (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: A Guide to Methods and Applications, eds. Innis, MA, Gelfand DH, Sninsky JJ, White TJ. Academic Press, Inc., New York, pp. 315-322.

Gardes, M. and T.D. Burns. 1993. ITS primers with enhanced specificity of basidiomycetes: application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113-118.

Claims

1. A method for screening for a plant protectant comprising the steps of:

a) generating a metagenomic analysis of the microbial community of a habitat,

b) determining the composition of the microbial community of said habitat of step a),

c) applying a formulation to be tested containing a potential plant protectant

d) generating a metagenomic analysis of the microbial community of the habitat of step a) after application of the formulation from step c),

e) determining the composition of the microbial community of said habitat of step d),

f) comparing the microbial community before and after the application of the formulation,

g) determining the alteration of the microbial community of the habitat of step a) and d),

h) selecting a formulation from step c) with a plant protectant activity in dependency of step g).

2. The method according to claim 1 whereby the habitat is selected from the group of environments occupied by microbial communities consisting of: plant, preferably agricultural plant, crop plant or a part thereof, grain, soil, location where the plant is growing or is expected to grow and biofilm from the surface of plants or parts thereof or soil.

3. The method according to claim 1 comprising generating in step b) and e) a molecular identity card.

4. The method according to claim 1 comprising generating in step b) and e) a specific fingerprint .

5. The method according to claim 1 comprising generating a specific fingerprint by using 16S amplification or ITS amplification.

6. The method according to claim 1 comprising determining the phylum, subphylum, class, order, family, genus and/or species of the microorganisms of the habitat.

7. The method according to claim 1 comprising additionally a step:

c1) applying a formulation containing a known plant protectant,

d1) generating a metagenomic analysis of the microbial community of the habitat of step a) after application of the formulation from step c1),

e1) determining the composition of the microbial community of said habitat of step d1),

f1) comparing the microbial community with the one from step f),

g1) selecting that formulation from step c) with a plant protectant which produce a microbial community with a composition having the highest identity with the composition of the microbial community of step e1).

8. The method according to claim 1 whereby active compounds are selected in step h) from the tested potential plant protectants which have an impact selected from the group comprising:

i) the composition of the microbial community is altered in a way that it comprise less classes, orders, families, genus and/or species of pests;

ii) the composition of the microbial community is altered in a way that the ratio of the number of species of the phylum Basidiomycota to the number of species of the phylum Ascomycota is increased, preferably the ratio is doubled.

9. Use of the metagenomic analysis to screen plant protectants.

10. Use according to claim 9 to screen crop protection compounds.

11. Use of the metagenome analysis to evaluate the spectrum of microbial species which can be controlled by a given active ingredient

12. Use of the metagenome analysis to evaluate the spectrum of microbial species which can be controlled by a mixture of given active ingredients

13. Use of the metagenome analysis to evaluate the spectrum of microbial species which can be controlled by a given crop protection product

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2009/060107

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68

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)
C12Q

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 practical, search terms used)
EPO-Internal, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 6 057 490 A (RYALS JOHN ANDREW [US] ET AL) 2 May 2000 (2000-05-02) the whole document	1-13
Y	WO 2004/053147 A (TNO [NL]; VAN DER VOSSEN JOSEPHUS MAURIT [NL]; SCHUREN FRANK HENRI JOH) 24 June 2004 (2004-06-24) cited in the application the whole document	1-13

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"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
15 September 2009	22/09/2009

Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2009/060107

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LYNCH J M ET AL: "Microbial diversity in soil: ecological theories, the contribution of molecular techniques and the impact of transgenic plants and transgenic microorganisms" BIOLOGY AND FERTILITY OF SOILS, vol. 40, no. 6, December 2004 (2004-12), pages 363-385, XP002507685 ISSN: 0178-2762 the whole document	1-13
A	YANG Y -H ET AL: "Effects of agricultural chemicals on DNA sequence diversity of soil microbial community: A study with RAPD marker" MICROBIAL ECOLOGY, vol. 39, no. 1, January 2000 (2000-01), pages 72-79, XP002507686 ISSN: 0095-3628 the whole document	1-13
A	PAUL DEBARATI ET AL: "Bacterial community structure of a pesticide-contaminated site and assessment of changes induced in community structure during bioremediation" FEMS MICROBIOLOGY ECOLOGY, vol. 57, no. 1, July 2006 (2006-07), pages 116-127, XP002507687 ISSN: 0168-6496 the whole document	1-13
A	HANDELSMAN JO: "Metagenomics: Application of genomics to uncultured microorganisms" MICROBIOLOGY AND MOLECULAR BIOLOGY REVIEWS, vol. 68, no. 4, December 2004 (2004-12), pages 669-685, 668, XP002507688 ISSN: 1092-2172 cited in the application the whole document	1-13

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2009/060107

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 6057490 A	02-05-2000	US 6232525 B1	15-05-2001
WO 2004053147 A	24-06-2004	AU 2003296049 A1	30-06-2004
		EP 1570068 A1	07-09-2005
		JP 2006509506 T	23-03-2006
		NL 1022152 C2	18-06-2004
		US 2006246444 A1	02-11-2006