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(54) Title: FUNGICIDAL COMPOUND

(57) Abstract: The present invention relates to a polyene compound and a composition comprising the polyene compound, a process for producing the polyene compound and a method of using of the polyene compound to prevent or control phytopathogenic microorganisms on plants.

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FUNGICIDAL COMPOUND

The present invention relates to a novel compound which has pesticidal activity. The invention also relates to compositions comprising the compound, to a process for the preparation of the compound and to the use of the compound or the compositions in agriculture or horticulture for preventing or controlling phytophatogenic infestation of plants, harvested food crops, seeds or non-living materials.

BACKGROUND

Pesticides are widely used in agriculture to protect plants against damage caused by insects and fungi. Pesticides may be from chemical origin or biological orgin. Due to some negative effects of chemical pesticides on the environment, there is a growing need for pesticides such as fungicides from biological origin. Known microorganisms that produce antibiotics against fungi are actinomycetes, for instance *Streptomyces sp*. A very well-known species is *Streptomyces natalensis* that produces the antifungal compound natamycin, which is used in food and crop protection. In US 5,356,624 a *Streptomyces rimosus* strain is disclosed that was found active against several wood-degrading fungi. In WO2022/038180 new *Streptomyces sp*. are disclosed that produce several known antifungal compounds such as streptimidone, natamycin (pimaricin), or albofungin. The extracts of these bacterial strains were found active against well-known plant pests such as *Fusarium graminearum, Zymoseptoria tritici*, and *Puccinia striiformis*.

Streptomyces species are known to produce antifungal polyene compounds.

WO2004/065401 discloses a class of polyene polyketides, obtainable by *Streptomyces aizunensis* strains, which were found active against yeasts and filamentous fungi, such as *Candida* and *Aspergillus* species.

Park *et al*, Frontiers in Bioengineering and Biotechnology, 2021, Vol. 9 p. 1-10, discloses a polyene compound highly homologous neotetrafibricin, with activities against *Candida albicans* and *Fusarium oxysporum*. The polyene compound was obtained from a Streptomyces strain closely related to a *Streptomyces rubrisoli*, which was isolated from a screening of 2,400 Streptomyces strains.

Bobek et al, 2022, Int. J. of Molecular Sciences, Vol, 23, 15045, discloses several polyene antibiotics produced by hemolytic *Streptomyces* species.

Due to the development of resistance by fungi against existing fungicides, regulations by governments and societal pressure there is a continuous need to look for new compounds, such as new polyene compounds, that have fungicidal activity from biological origin.

SUMMARY

The present invention relates to a compound, wherein the compound is a polyene compound having a molecular formula of $C_{67}H_{115}NO_{25}$, wherein the polyene is further characterized by the spectrum of light absorption with has absorbance maxima at a wavelength of 235.5 nm, 301.1 nm, 315.8 nm, 330.9 nm and 348.3 nm when measured in an aqueous acetonitrile solution. The polyene having the molecular formula of $C_{67}H_{115}NO_{25}$ has a molecular mass of 1333.7758 g.

Surprisingly, it has been found that the novel compound according to the present invention has a surprising level of biological activity for preventing or controlling phytopathogenic microorganisms such as fungi. Biological activity as used herein includes fungicidal activity. Accordingly, the compound of the present invention exhibits fungicidal activity.

In a second aspect, the invention relates to a composition comprising the compound according to the present invention and a microorganism which comprises at least one nucleotide sequence encoding a protein which has at least 90% identity to at least one of the amino acid sequences according to SEQ ID NO: 26-49, preferably SEQ ID NO: 46 and 47, preferably wherein the nucleotide sequence has at least 90 % identity to at least one of the nucleotide sequences according to SEQ ID NOs: 2 to 25, preferably wherein the microorganisms comprises a nucleotide sequence which has at least 90% identity to SED NO: 22 and / or SEQ ID NO: 23. The microorganism to produce the compound according to the present invention.

In a third aspect the invention relates to a process for producing the compound, or a composition according to the present invention, comprising cultivating a microorganism in a suitable fermentation medium under conditions that allow production of the compound.

In a fourth aspect the present invention relates to a method for controlling or preventing infestation of a plant by a phytopathogenic microorganism, wherein an effective amount of the compound according to the present invention or a salt thereof, or a composition according to the invention as disclosed herein, is applied to the plant, to a part thereof or a locus thereof.

According to a fifth aspect of the invention, there is provided the use of a compound or a composition according to the present invention as a pesticide, preferably as a fungicide. According to this aspect of the invention, the use excludes methods for the treatment of the human or animal body by surgery or therapy.

DETAILED DESCRIPTION

The present invention relates to a polyene compound, having a molecular formula according to $C_{67}H_{115}NO_{25,}$ wherein the polyene is further characterized by a spectrum of light absorption as shown in Figure 1, preferably wherein the spectrum of light absorption has absorbance maxima at a wavelength of 235.5 nm, 301.1 nm, 315.8 nm, 330.9 nm and 348.3 nm when measured in an aqueous acetonitrile solution. The wavelengths of these absorbance maxima are shown in Figure 1. As used herein, an aqueous acetonitrile solution is an acetonitrile : water solution, typically an acetonitrile: water gradient used to measure the spectrum of light absorption. The polyene having a molecular formula according to $C_{67}H_{115}NO_{25}$ has a molecular mass of 1333.775 8 g.

The compound according according to the present invention is further characterized by a liquid chromatography retention time of 5.55-5.57 minutes when run under the following conditions: Kinetex Polar C18 column 100A 4.6x100mm, P.N. H17-055453. Temp: 40°C, DAD wavelength range: 250 to 260nm, Solvent gradient: Solvent A: H2O with 0.1% formic acid, Solvent B: CH3CN with 0.1% formic acid, gradient: 0min 10% B, 90% A; 1min 10% B, 90% A; 6.50min 95% B, 5% A; 8.00min 95% B, 5% A; 9.00min 10% B, 90% A; 10.00min 10% B, 90% A, Flow rate: 1.0ml/min, Injection volume: 5 uL, Total run time: 10.0min.

In one preferred embodiment, the compound according to the present invention is an isolated compound. The wording 'isolated' with reference to the compound means that the compound has been isolated from it's native environment.

A polyene is a compound comprising at least three alternating double (C=C) and single (C-C) carbon-carbon bonds. Known polyenes are for instance amphotericin B, nystatin, pimaricin, and filipin. It was surprisingly found that the polyene compound according to the present invention has a surprising level of fungicidal activity. The fungicidal activivity of the polyene compound can be similar to other known polyene compounds.

In one embodiment, the compound according to the present invention obtainable by cultivating, or is produced by a microorganism which comprises at least one nucleotide sequence encoding a protein which has at least 90% identity to at least one of the amino acid sequences according to SEQ ID NO: 26-49, preferably SEQ ID NO: 46 and 47, preferably wherein the nucleotide sequence has at least 90 % identity to at least one of the nucleotide sequences according to SEQ ID NOs: 2 to 25, preferably wherein the microorganisms comprises a nucleotide sequence which has at least 90% identity to SED NO: 22 and / or SEQ ID NO: 23. As used herein, obtainable by a microorganism is obtainable by cultivating the microorganism in a suitable fermentation broth that allows producing the compound of the invention.

Preferably, the microorganism further has at least 91% identity to the whole genome of *Streptomyces sp.* Saigon413 deposited with the Westerdijk Institute under accession number CBS149411.

In one embodiment, the microorganism further comprises a nucleotide sequence which has at least 99.5% identity to the nucleotide sequence according to SEQ ID NO: 1.

In one embodiment the microorganism is a *Streptomyces chrestomyceticus*, preferably *Streptomyces sp.* Saigon413 deposited with the Westerdijk Institute under accession number CBS149411.

In one embodiment, the microorganism disclosed herein comprises a gene which is involved in the synthesis of the compound according tot he present invention. Preferably, the microorganism comprises at least one nucleotide sequence which encodes a protein that has at least 90%, preferably at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, preferably at least 99% identity, preferably 100% identity to at least one of amino acid sequences chosen from SEQ ID NO: 26 to 49.

Preferably, the microorganism disclosed herein comprises or contains at least one, preferably at least two, preferably at least three, preferably at least four, preferably at least five, preferably at least at least six, preferably at least seven, preferably at least eight, preferably at least nine, preferably at least ten, preferably at least eleven, preferably at least twelve, preferably at least thirteen, preferably at least fourteen, preferably at least fifteen, preferably at least sixteen, preferably at least seventeen, preferably at least eighteen, preferably at least nineteen, preferably at least twenty, preferably at least twenty one, preferably at least twenty two, preferably at least twenty three, preferably all twenty four of the nucleotide sequences which encode a protein that has / have at least 90%, preferably at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, preferably at least 99% identity, preferably 100% identity to the amino sequences according to SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID

NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, and/or SEQ ID NO: 49, preferably a nucleotide sequence that encodes a protein according to the amino acid sequence(s) of SEQ ID NO: 46 and/or SEQ ID NO: 47.

Preferably, the microorganism disclosed herein comprises a gene which is involved in the synthesis of the compound according tot he present invention. Preferably, the microorganism comprises at least one nucleotide sequence which has at least 90%, preferably at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, preferably at least 99% identity, preferably 100% identity to at least one of nucleotide sequences chosen from the nucleotide sequences according to SEQ ID NO: 2 to 25.

Preferably, the microorganism in the composition and/or in the process for producing the compound and/or producing the composition of the present invention comprises or contains at least one, preferably at least two, preferably at least three, preferably at least four, preferably at least five, preferably at least at least six, preferably at least seven, preferably at least eight, preferably at least nine, preferably at least ten, preferably at least eleven, preferably at least twelve, preferably at least thirteen, preferably at least fourteen, preferably at least fifteen, preferably at least sixteen, preferably at least seventeen, preferably at least eighteen, preferably at least nineteen, preferably at least twenty, preferably at least twenty one, preferably at least twenty two, preferably at least twenty three, preferably all twenty four of the nucleotide sequences which has / have at least 90%, preferably at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, preferably at least 99% identity, preferably 100% identity to the nucleotide sequences according to SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24 and/or SEQ ID NO: 25.

Preferably, the microorganism in the composition and/or producing the compound and/or producing the composition in a process according to the present invention comprises or contains a nucleotide sequence which has at least 90%, preferably at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, preferably at least 99% identity, preferably 100% identity to the nucleotide sequences according to SEQ ID NO: 22 or SEQ ID NO: 23. The microorganism in the composition and/or producing the compound and/or composition of the present invention comprises one or more of the nucleotide sequences according to SEQ ID NO: 2 to 25, preferably at least SEQ ID NO: 22 and / or SEQ ID NO: 23.

The microorganism may be a naturally occuring microorganism or a recombinant microorganism. Recombinant microorganisms can be produced by methods known to a person skilled in the art. A recombinant microorganism may be produced by transforming the microorganism with at least one of the nucleotide sequences that encode a protein of the amino acids sequences according to SEQ ID NO: 26-49, preferably at least one of the nucleotide sequences of SEQ ID NO: 2 to 25, preferably at least one of the nucleotide sequence of SEQ ID NO: 22 and / or 23, or a nucleotide sequence which have at least at least 90%, preferably at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, preferably at least 99% identity thereto.

Preferably, the microorganism as disclosed herein is a bacterium of the genus *Streptomyces sp.*, preferably the bacterium is a *Streptomyces chrestomyceticus.* Preferably, the composition comprises *Streptomyces sp.* Saigon413 deposited with the Westerdijk Institute under accession number CBS149411

Preferably, the microorganism in the composition and/or producing the compound and/or composition and / or in the process as disclosed herein is a microorganism that comprises a nucleotide sequence, preferably a 16S RNA nucleotide sequence, which has at least 99.5%, 99.6%, 99.7%, 99.8% preferably at least 99.9%, preferably at least 99.91%, 99.92%, 99.93%, 99.94%, 99.95%, 99.96% 99.97%, 99.98%, 99.99% or has 100% identity to the nucleotide sequence according to SEQ ID NO: 1.

Preferably, the microorgansim in the composition or in the process according to tthe present invention, for instance a *Streptomyces chrestomyceticus*, comprises a genome sequence which has at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least 99% identity to the whole genome of *Streptomyces chrestomyceticus* NRRL-3672 or to the whole genome of *Streptomyces sp.* Saigon413 deposited with the Westerdijk Institute under accession number CBS149411. In one embodiment the composition or process according to the present invention comprises a *Streptomyces chrestomyceticus* which is *Streptomyces sp.* Saigon413 deposited with the Westerdijk Institute under accession number CBS149411.

As used herein, the terms "percent identity," and "percent identical" refer to the relatedness of two or more nucleotide or amino acid sequences, which may be calculated by (i) comparing two optimally aligned sequences over a window of comparison, (ii) determining the number of positions at which the identical nucleic acid base (for nucleotide sequences) or amino acid residue (for proteins) occurs in both sequences to yield the number of matched positions, (iii) dividing the number of matched positions by the total number of positions in the window of comparison, and then (iv) multiplying this quotient by 100 percent to yield the percent identity. If the "percent identity" is being calculated in relation to a reference sequence without a particular comparison window being specified, then the percent identity is determined by dividing the number of matched positions over the region of alignment by the total length of the reference sequence. Accordingly, for purposes of the present invention, when two sequences (query and subject) are optimally aligned (with allowance for gaps in their alignment), the "percent identity" for the query sequence is equal to the number of identical positions between the two sequences divided by the total number of positions in the query sequence over its length (or a comparison window), which is then multiplied by 100 percent. The percent identity is typically calculated over the full length of the nucleotide or amino acid sequence.

The present invention also relates to a composition comprising the polyene compound of the present invention and a microorganism as defined herein. The microorganism is able to produce the polyene compound. The composition as disclosed herein is a composition that is a non-naturally occurring composition.

Preferably, the compound and / or composition according to the present invention has or exhibits fungicidal activity. Accordingly, the compound and / or composition according to the present invention preferably is a fungicide or fungicidal compound or fungicidal composition.

The term “compound or composition having fungicidal activity” or “fungicide” as used herein means a compound or composition that controls, modifies, or prevents the growth of fungi. The term

"fungicidally effective amount" where used means the quantity of such a compound or composition or combination of such compounds that is capable of producing an effect on the growth of fungi. Controlling or modifying effects include all deviation from natural development, such as killing, retardation and the like, and prevention includes barrier or other defensive formation in or on a plant to prevent fungal infection.

The compound and / or the composition according to the present invention may be produced in any suitable way, preferably the compound and / or the composition of the invention is produced by cultivating the microoganism as defined herein in a suitable fermentation medium that allows producing the compound and / or composition of the present invention. A composition comprising a compound according to the present invention can be a fermentation broth, preferably a fermentation broth produced by a process according to the present invention.

The compound of the present invention, or a composition comprising the compound of the present invention can be used in the agricultural sector and related fields of use, e.g., as active ingredients for controlling phytopathogenic microorganisms. The compound according to the present invention is distinguished by excellent activity at low rates of application such as from 2 to 250 ppm, for instance from 10 to 200 ppm, for instance from 20 to 100 ppm, by being well tolerated by plants and by being environmentally safe. It has very useful preventive properties and can be used for protecting numerous plants. The compound of the present invention can be used to inhibit or destroy phytopathogenic microorganisms that occur on plants or parts of plants (fruit, blossoms, leaves, stems, tubers, roots) or different crops of plants. The compound may also protect those parts of the plants that grow later.

The compound according to the present invention and / or a composition comprising the compound according to the present invention can be used as such or formulated with an auxiliary, preferably an agricultural-acceptable auxiliary. Known formulations in the art are for instance emulsifiable concentratres, coatable pastes, sprayable or dilutable solutions or suspensions, powders, dusts, granulates and encapsulations.

Accordingly, in one embodiment a composition comprising a compound according to the present invention as disclosed herein further comprises an auxiliary. Preferably the auxiliary is an agricultural-acceptable auxiliary.

Suitable auxiliaries are known in the art, and include for example solvents, liquid carriers, solid carriers or fillers, surfactants, dispersants, emulsifiers, wetters, adjuvants, solubilizers, penetration enhancers, protective colloids, adhesion agents, thickeners, humectants, repellents, attractants, feeding stimulants, compatibilizers, bactericides, anti-freezing agents, anti-foaming agents, colorants, tackifiers and binders.

Suitable solvents and liquid carriers include, for example water, organic solvents, oils of vegetable or animal origin, cyclic and aromatic hydrocarbons, alcohols, esters, fatty acids, a glycol or any other suitable liquid carrier known in the art. The solvent or liquid carrier may be water or DMSO (dimethylsulphoxide).

Suitable solid carriers include, for example, talc, titanium dioxide, pyrophyllite clay, silica, attapulgite clay, kieselguhr, chalk, diatomaxeous earth, lime, calcium carbonate, bentonite clay, fuller's earth, cotton seed hulls, wheat flour, soybean flour, pumice, wood flour, walnut shell flour and lignin.

An adjuvant may be a surface-active agent, crystallisation inhibitor, viscosity modifier, suspending agents, spray droplet modifiers, pigments, antioxidants, foaming agents, anti-foaming agents, light-blocking agents, compatibilizing agents, sequestering agents, neutralising agents and buffers, corrosion inhibitors, dyes, odorants, spreading agents, penetration aids, micronutrients, emollients, lubricants and sticking agents.

A composition as disclosed herein preferably is an agricultural-acceptable composition.

A composition comprising a compound according to the present invention as disclosed herein typically comprises 0.5 to 95 w/w% of active ingredient such as from 1% to 90 w/w%, such as from 2 to 80 w/w%, such as from 5 to 60 w/w%. The compound according to the present invention may be the sole active ingredient in a composition as disclosed herein. In one embodiment, a composition comprising a compound of the present invention further comprises at least one additional active ingredient. An active ingredient as defined herein has fungicidal and / or insecticidal and / or herbicidal activity or has activity as plant growth regulator. A compound or a composition of the present invention may be admixed with one or more additional ingredients having pesticidal activity such as fungicides, insecticides, herbicides, bactericides, acaricides, nematicides and / or the additional ingredient comprises plant growth regulators where appropriate. Pesticidal agents are referred to herein using their common name are known, for example, from "The Pesticide Manual", 19th Ed., British Crop Protection Council 2021.

An additional ingredient having pesticidal activity, for instance fungicidal activity may result in an unexpected synergistic activity. The additional ingredients having pesticidal activity and / or which is a plant growth regulator may be combined with a composition of the invention and used in a method of the invention and applied simultaneously or sequentially with a composition of the invention. When applied simultaneously, these further ingredients may be formulated together with the compositions of the invention or mixed in, for example, a spray tank. As an alternative to directly admixing these further ingredients having pesticidal activity, the components may be used in separate fungicidal, insecticidal or herbicidal applications as part of a programme of fungal, insect or herbal control spread over part or all of a growing season.

The at least one additional ingredient having pesticidal activity and / or which is a plant growth regulator may be any suitable known fungicide, insecticide, herbicide and / or plant growth regulator. The at least one additional ingredient having pesticidal activity and / or plant growth regulator may be from chemical origin or biological origin, for instance from plant or microbial origin. The at least one additional ingredient having pesticidal activity in a composition as disclosed herein may be produced by the microorganism disclosed herein that is able to produce the polyene compound according to the present invention as disclosed herein above.

In addition, the compositions of the invention may also be applied with one or more systemically acquired resistance inducers (“SAR” inducer). SAR inducers are known and described in, for example, United States Patent No. US 6,919,298 and include, for example, salicylates and the commercial SAR inducer acibenzolar-S-methyl.

The compound and / or composition according to the present invention may induce resistance of a plant by a priming mechanism. Priming is a mechanism which leads to a physiological state that enables plants to respond more rapidly and/or more robustly after exposure to biotic or abiotic stress as

described for instance in review article: P. Aranega-Bou et. al. Priming of plant resistance by natural compounds. Hexanoic acid as a model.Front. Plant. Sci. 1, October 2014.

In one embodiment the composition according to the present invention further comprises cyclothiazomycin C, streptimidone and / or malonomicin. The structure of cyclothazomycin C is disclosed on p. 3 of WO2015191789 and can be produced as disclosed in Example 4 of WO2015/191789. Malonomicin can be produced as disclosed in Example I of WO2006/078939. Malonomicin is also indicated as antibiotic K16, or malomycin. Streptimidone can be synthesised following the method disclosed in Kondo, H., Oritani, T., and Kiyota, H. Synthesis and antifungal activity of the four stereoisomers of streptimidone, a glutarimide antibiotic from Streptomyces rimosus forma paromomycinus. Eur. J. Org. Chem. (20), 3459-3462 (2000). Streptimidone can also be purchased from a commercial vendor (CAS:738-72-7). In one embodiment, the active ingredients cyclothiazomycin C, streptimidone and / or malonomicin are produced by the microorganism able to produce compound according to Formula (I) according to the present invention as defined herein above.

A composition comprising a mixure of the compound of the invention and at least one additional active ingredient is preferably in a mixing ratio of from 100:1 to 1:6000, especially from 50:1 to 1:50, more especially in a ratio of from 20:1 to 1:20, even more especially from 10:1 to 1:10, very especially from 5:1 and 1:5, special preference being given to a ratio of from 2:1 to 1:2, and a ratio of from 4:1 to 2:1 being likewise preferred, above all in a ratio of 1:1, or 5:1, or 5:2, or 5:3, or 5:4, or 4:1, or 4:2, or 4:3, or 3:1, or 3:2, or 2:1, or 1:5, or 2:5, or 3:5, or 4:5, or 1:4, or 2:4, or 3:4, or 1:3, or 2:3, or 1:2, or 1:600, or 1:300, or 1:150, or 1:35, or 2:35, or 4:35, or 1:75, or 2:75, or 4:75, or 1:6000, or 1:3000, or 1:1500, or 1:350, or 2:350, or 4:350, or 1:750, or 2:750, or 4:750. Those mixing ratios are by weight. The mixtures as described above can be used in a method for controlling pests, which comprises applying a composition comprising a mixture as described above to the pests or their environment, with the exception of a method for treatment of the human or animal body by surgery or therapy and diagnostic methods practised on the human or animal body.

A composition comprising a mixture of the compound of the invention, and one or more active ingredients as described above can be applied, for example, in a single “ready-mix” form, in a combined spray mixture composed from separate formulations of the single active ingredient components, such as a “tank-mix”, and in a combined use of the single active ingredients when applied in a sequential manner, i.e., one after the other with a reasonably short period, such as a few hours or days.

In one aspect, the present invention relates to a process for producing the compound according to the present invention comprising cultivating the microorganism as disclosed herein in a suitable fermentation medium under conditions that allow production of the compound. The microorganism that is cultivated in a process as disclosed herein is a microorganism able to produce the compound according to the present invention and is further described herein above. The wording to “cultivate or cultivation” and “to fermenat or fermentation” is used interchangeably herein.

Cultivating a microorganism for producing the compound according to the present invention in a suitable fermentation medium is known to a person skilled in the art. The microorganism may be fermented under aerobic or anaerobic conditions. A microorganism belonging to *Streptomyces sp.* is typically cultivated under aerobic conditions. A suitable fermentation medium comprises nutrients, such

as a suitable carbon source such as glucose, and a suitable nitrogen source, for instance peptides, amino acids or ammonia.

In one embodiment the process further comprises producing a composition comprising the compound according to the present invention as defined herein and a microorganism able to produce the compound according to the present invention. A microorganism able to produce the compound according to the present invention is defined herein above. A microorganism able to produce the compound according to the present invention may be able to produce further active ingredients as defined herein above, for instance cyclothiazomycin C, streptimidone and / or malonomicin.

The process according to the present invention may further comprise a step of recovering the compound according to the present invention or a salt of it. The compound according to the present invention may be recovered by suitable methods known in the art, for instance via crystallization or chromatography, eg HPLC. Recovering the compound according to the present invention may further comprise a step of purifying the compound.

The process for producing a compound according to the present invention may further comprise a step of formulating the compound into a suitable formulation or composition as defined herein above.

In one further aspect, the present invention relates to a method for controlling or preventing infestation of a plant, plant propagation material and/or harvested food crops by a phytopathogenic microorganism, by treating the plant, plant propagation material and/or harvested food crops, wherein an effective amount of the compound or a composition according to the present invetion, is applied to the plant, to a part thereof or a locus thereof, plant propagation material and/or harvested food crops.

Applying an effective amount of the compound or composition of the invention in a method for controlling or preventing investation of a plant comprises applying from 0.01 g to 5 kg of the compound of the invention (active ingredient (a.i.) per hectare (ha), preferably from 0.015 g to 500 g a.i./ha, preferably from 0.020 g to 100g a.i./ha, preferably from 0.025 g to 50g a.i./ha, preferably from 0.030 g to 5 g a.i./ha, , preferably from 0.035 g to 500 mg a.i./ha.

When the compound of the invention or composition of the present invention is used for treating seed, rates of 0.0001 to 10 g of the compound of the invention per kg of seed, such as from 0.0002 to 0.1 g per kg of seed, such as from 0.0005 to 0.001 g per kg of seed are generally sufficient.

Suitably, a compound or a composition of the invention is applied either preventative, meaning prior to disease development or curative, meaning after disease development.

Phytopathogenic microorganisms that are affected by the compound of the invention are fungi and fungal vectors of disease as well as phytopathogenic bacteria and viruses. Phytopathogenic microorganisms in a method according to the present invention include the following fungi and fungal vectors of disease and phytopathogenic bacteria:

Absidia corymbifera, Albugo candida, Alternaria spp. including *A. solani, Aphanomyces spp, Ascochyta spp, Aspergillus spp.* including *A. flavus, A. fumigatus, A. nidulans, A. niger, A. terrus, Aureobasidium spp.* including *A. pullulans, Bacillus subtilis, Blastomyces dermatitidis, Blumeria graminis, Blumeriella jaapii, Botryosphaeria spp.* including *B. dothidea, B. obtusa, Botrytis spp.* including *B. cinerea, Bremia lactucae, Cadophora gregata, Candida spp.* including *C. albicans, C. glabrata, C. krusei, C. lusitaniae, C. parapsilosis, C. tropicalis, Cephaloascus fragrans, Ceratocystis spp, Cercospora spp.* including *C. arachidicola, C. beticola, C. kikuchii, C. sojina, Cercosporidium*

personatum, Cladosporium spp, Clarireedia homoeocarpa, Clavibacter spp, Claviceps purpurea, Coccidioides immitis, Cochliobolus spp, Colletotrichum spp. including *C. dematium, C. lindemuthianum, C. musae, C. orbiculare, C.truncatum, Corynespora cassiicola, Cryptococcus neoformans, Diaporthe spp, Dickeya zeae, Didymella spp, Drechslera spp, Elsinoe spp, Epidermophyton spp, Eremothecium gossypiim, Erwinia spp.* including *E. amylovora, E. carotovora, Erysiphe spp.* including *E. cichoracearum, E. necator, Eutypa lata, Fusarium spp.* including *F. culmorum, F. graminearum, F. langsethiae, F. moniliforme, F. oxysporum, F.poae, F. proliferatum, F. pseudograminearum, F. sacchari, F. sambucinum, F. subglutinans, F. solani, F. sporotrichioides, F. tricinctum, F. virguliforme, Gaeumannomyces graminis, Gibberella spp.* including *G. avenacea, G. fujikuroi, G. intricans, G. moniliformis, G. zeae, Gloeodes pomigena, Gloeosporium musarum, Glomerella cingulate, Golovinomyces cichoracearum, Gymnosporangium juniperi-virginianae, Guignardia bidwellii, Gymnosporangium juniperi-virginianae, Helminthosporium spp, Hemileia spp, Histoplasma spp.* including *H. capsulatum, Hyaloperonospora parasitica, Kabatiella zeae, Laetisaria fuciformis, Leptographium lundbergii, Leveillula taurica, Lophodermium seditiosum, Microdochium majus, Microdochium nivale, Microsporum spp, Monilinia spp.* including *M. fructicola, Monographella spp* including *M. nivalis, Mucor spp, Mycosphaerella spp.* including *M. arachidis, M. fijiensis, M. graminicola, M. pomi, Nakataea oryzae, Neopseudocercosporella spp, Oculimacula spp, Oncobasidium theobromaeon, Ophiostoma spp, Pantoea stewartia, Paracoccidioides spp, Parastagonospora nodorum, Pectobacterium spp, Penicillium spp.* including *P. digitatum, P. italicum, Petriellidium spp, Peronosclerospora spp. i*ncluding *P. maydis, P. philippinensis and P. sorghi, Peronospora spp* including *P. destructor, Phaeosphaeria nodorum, Phakopsora pachyrhizi, Phellinus igniarus, Phialophora spp, Phlyctema vagabunda, Phoma spp, Phomopsis viticola, Phyllachora pomigena, Phyllosticta spp, Physoderma maydis, Phytophthora spp.* including *P. capsica, P. infestans, Plasmodiophora brassicae, Plasmopara spp.* including *P. halstedii, P. viticola, Plenodomus spp, Pleospora spp., Podosphaera spp.* including *P. leucotricha, Polymyxa graminis, Polymyxa betae, Pseudocercospora fijiensis, Pseudocercosporella herpotrichoides, Pseudomonas spp.* including *P. syringae, Pseudoperonospora spp.* including *P. cubensis, P. humuli, Pseudopeziza tracheiphila, Pseudopyrenochaeta lycopersici, Puccinia spp.* including *P. hordei, P. recondita, P. striiformis, P. triticina, Pyrenopeziza spp, Pyrenophora spp, Pyricularia spp.* including *P. oryzae, Pythium spp.* including *P. ultimum, Ralstonia solanacearum, Ramularia spp, Rathayibacter spp, Remotididymella destructiva, Rhizoctonia spp, Rhizomucor pusillus, Rhizopus arrhizus, Rhynchosporium spp, Robbsia andropogonis, Sarocladium oryzae, Scedosporium spp.* including *S. apiospermum and S. prolificans, Schizothyrium pomi, Sclerophthora macrospora, Sclerotinia spp.* including *S. sclerotiorum, Sclerotium spp, Septoria spp,* including *S. nodorum, S. tritici, Setosphaeria turcica, Sphaerotheca macularis, Sphaerotheca fusca (Sphaerotheca fuliginea), Spiroplasma kunkelii, Sporothorix spp, Stagonospora nodorum, Stagonosporopsis cucurbitacearum, Stemphylium spp, Stenocarpella macrospora, Stereum hirsutum, Streptomyces spp, Thanatephorus cucumeris, Thielaviopsis basicola, Tilletia spp, Tranzschelia discolor, Trichoderma spp.* including *T. harzianum, T. pseudokoningii, T. viride, Trichophyton spp, Typhula spp, Uncinula necator, Urocystis spp, Uromyces spp, Ustilago spp, Venturia spp.* including *V. inaequalis, Verticillium spp, Wilsonomyces carpophilus, or Xanthomonas spp*, including *X. oryzae and X. campestris, Xylella spp, Zymoseptoria tritici.*

Phytopathogenic microorganisms that are found to be surpisingly affected by the compound according to the present invention are fungi, preferably fungi belonging to *Botrytis sp., Colletotrichum sp. Fusarium sp., Microdochium sp., Mycosphaerella sp., Pythium sp., Rhizoctonia sp.,* or *Sclerotinia sp., Zymoseptoria,* preferably fungi belonging to *Botryotinia fuckeliana* (*Botrytis cinerea), Colletotrichum orbiculare (Colletotrichum lagenarium*), *Fusarium culmorum, Microdochium nivale, Mycosphaerella arachidis (Cercospora arachidicola), Pythium ultimum, Rhizoctonia solani,* or *Sclerotinia sclerotiorum, Zymospetoria tritici.*

Controlling or preventing means reducing infestation by phytopathogenic microorganisms especially fungi, to such a level that an improvement is demonstrated.

A preferred method of controlling or preventing an infestation of crop plants by phytopathogenic microorganisms, especially fungi, or insects comprises the application of the compound or composition according to the present invention is foliar application. The frequency of application and the rate of application will depend on the risk of infestation by the corresponding pathogen or insect. However, the compound or composition according to the present invention can also penetrate the plant through the roots *via* the soil (systemic action) by drenching the locus of the plant with a liquid formulation, or by applying the compounds in solid form to the soil, e.g. in granular form (soil application). In crops of water rice such granulates can be applied to the flooded rice field. The compound or composition according to the present invention may also be applied to seeds (coating) by impregnating the seeds or tubers either with a liquid formulation of the fungicide or coating them with a solid formulation.

It is also possible to use the compound or composition according to the present invention as dressing agent for the treatment of plant propagation material, e.g., seed, such as fruits, tubers or grains, or plant cuttings, for the protection against fungal infections as well as against phytopathogenic fungi occurring in the soil. The propagation material can be treated with a compound and / or a composition according to the present invention before planting: seed, for example, can be dressed before being sown. The compound and / or composition according to the present invention can also be applied to grains (coating), either by impregnating the seeds in a liquid formulation or by coating them with a solid formulation. The composition can also be applied to the planting site when the propagation material is being planted, for example, to the seed furrow during sowing. Disclosed herein are such methods of treating plant propagation material and the plant propagation material so treated.

The term “locus” as used herein means fields in or on which plants are growing, or where seeds of cultivated plants are sown, or where seed will be placed into the soil. It includes soil, seeds, and seedlings, as well as established vegetation.

The term “plants” refers to all physical parts of a plant, including seeds, seedlings, saplings, roots, tubers, stems, stalks, foliage, and fruits. Germinated plants and young plants which are to be transplanted after germination or after emergence from the soil, may also be mentioned. These young plants can be protected before transplantation by a total or partial treatment by immersion.

The term “plant propagation material” is understood to denote generative parts of the plant, such as seeds, which can be used for the multiplication of the latter, and vegetative material, such as cuttings or tubers, (for example potatoes), roots, fruits, bulbs, rhizomes or parts of plants.

The term plants involve “useful plants” or “crops”. The wording “Useful plants” and “crops” are used interchangeably herein. “Useful plants” and “crops” comprise perennial and annual crops, such as

berry plants for example blackberries, blueberries, cranberries, raspberries and strawberries; cereals for example barley, maize (corn), millet, oats, rice, rye, sorghum triticale and wheat; fibre plants for example cotton, flax, hemp, jute and sisal; field crops for example sugar and fodder beet, coffee, hops, mustard, oilseed rape (canola), poppy, sugar cane, sunflower, tea and tobacco; fruit trees for example apple, apricot, avocado, banana, cherry, citrus, nectarine, peach, pear and plum; grasses for example Bermuda grass, bluegrass, bentgrass, centipede grass, fescue, ryegrass, St. Augustine grass and Zoysia grass; herbs such as basil, borage, chives, coriander, lavender, lovage, mint, oregano, parsley, rosemary, sage and thyme; legumes for example beans, lentils, peas and soya beans; nuts for example almond, cashew, ground nut, hazelnut, peanut, pecan, pistachio and walnut; palms for example oil palm; ornamentals for example flowers, shrubs and trees; other trees, for example cacao, coconut, olive and rubber; vegetables for example asparagus, aubergine, broccoli, cabbage, carrot, cucumber, garlic, lettuce, marrow, melon, okra, onion, pepper, potato, pumpkin, rhubarb, spinach and tomato; and vines for example grapes. The term “plants” also includes wood crops, such as pine trees, or woody plants.

The term "useful plants" is to be understood as also including useful plants that have been rendered tolerant to herbicides like bromoxynil or classes of herbicides (such as, for example, HPPD inhibitors, ALS inhibitors, for example primisulfuron, prosulfuron and trifloxysulfuron, EPSPS (5-enol-pyrovyl-shikimate-3-phosphate-synthase) inhibitors, GS (glutamine synthetase) inhibitors or PPO (protoporphyrinogen-oxidase) inhibitors) as a result of conventional methods of breeding or genetic engineering.

The term "useful plants" is to be understood as also including useful plants which have been so transformed by the use of recombinant DNA techniques that they are capable of synthesising one or more selectively acting toxins, such as are known, for example, from toxin-producing bacteria, especially those of the genus Bacillus.

Any suitable plant, plant propagation material or food crop may be treated in a method according according to the present invention as defined herein. Preferably the plant, plant propagation material or food crop comprises or is potato, tomato, grape, canola/oilseed rape/colza, cucurbits, groundnut, wheat, or barley, preferably the plant is wheat, barley, corn, rice, soybean and banana.

In another aspect the invention relates to the use of a compound or a composition according to the present invention as a pesticide, preferably as a fungicide, a SAR inducer and / or as a priming agent. The features related to the compound and composition according to the present invention are as disclosed herein above. Accordingly, the present invention relates to a method for using a compound and / or composition according to the present invention as a fungicide.

FIGURES

Figure 1. Spectrum of light absorption (UV-VIS) 200-500nm of polyene compound

Figure 2: Graphical representation of biosynthetic gene cluster for polyene compound of the present invention

EXAMPLES

1. Source, fermentation and isolation of polyene compound

1.1 Fermentation of *Streptomyces sp.*

Streptomyces species were ordered from culture collections disclosed in Table 2.

Streptomyces sp. Saigon413 was isolated in Vietnam before 1961. *Streptomyces sp.* Saigon413 was deposited at the Westerdijk institute under accession number CBS149411. The deposit was made by Syngenta Ltd., Jealott's Hill Research International Centre, Bracknell, Berkshire, RG42 6EY, UK under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure.

Streptomyces species were cultivated in Erlenmeyer flasks with a liquid medium consisting of (g / l) casein hydrolysate 10, glucose 40, K2HPO4 1.25, soytone 2, tryptone, 8 and incubated at 28ºC in an incubator shaking 150 rpm wirth 25 mm throw for 4 days.

1.2 Isolation of 16S rDNA, whole genome sequencing and species identification

Genomic DNA was isolated from *Streptomyces sp.* Saigon413 using the method described in Kutchma et al. (1998) Biotechniques 24(3):452-457. The 16S rRNA gene was amplified using universal 16S primers and sequenced using Sanger sequencing. The 16S rRNA of *Streptomyces sp.* Saigon413 is shown in SEQ ID NO: 1.

The species of strain *Streptomyces sp.* Saigon413 was identified by comparing the 16S rRNA sequence according to SEQ ID NO:1 with publicly available 16S rRNA sequences that were extracted using whole genome sequence assembly of genomes from *Streptomyces* species (based on The Genome Taxonomy Database GTDB (Parks, D.H., et al. (2021). GTDB: Nucleic Acids Research, 50: D785–D794) using barrnap v0.9. Based on this comparative analysis *Streptomyces sp.* Saigon413 was identified as a *Streptomyces chrestomyceticus* species. The sequence identity between the 16S rRNA sequence of *Streptomyces sp.* Saigon413 and the publicly available *S. chrestomyceticus* NRRL-3672 was 99.87%, which was determined using Muscle v3.8.31 and R package Seqinr v4.2-16.

Whole genome sequencing, using the genomic DNA from *Streptomyces* sp. Saigon413, was completed using both Pacific Biosciences and Illumina sequencing technologies. The genome was assembled using HFAP4 and polished with Pilon using the Illumina reads. Genomic DNA was also extracted from *Streptomyces rimosus* CBS 492.64, *Streptomyces rimosus* CBS 570.66, *Streptomyces rimosus* CBS 569.66, *Streptomyces chrestomyceticus* DSM 41224, *Streptomyces rimosus* subsp. r*imosus* DSM 40673, and *Streptomyces rimosus* subsp. *rimosus* DSM 41057 using a method described in Kieser *et. al.*, (2000) Practical Streptomyces Genetics. Whole genome sequencing for these strains was completed using Nanopore Sequencing technology and the genomes were assembled with Flye (Kolmogorov, M., *et. al.*, (2019), Nature Biotechnology, 37, 540).

Following assembly of the genome from *Streptomyces sp.* Saigon 413 and publicly available genomes, the average nucleotide identity (ANI) was calculated between *Streptomyces* sp. Saigon413 and closely related *Streptomyces* strains using fastANI (Jain, C., *et. al.* (2018), Nature Communications, 9, 5114) (Table 1). The highest percentage identity (ANI) of the genome of of *Streptomyces* sp. Saigon413 was 96.9 % with the genome of the publicly available strain *S. chrestomyceticus* NRRL B-3672.

Using the 16SRNA sequence identity and ANI score (%), it was also found that the strains CBS 596.66, CBS570.66 and DSM 41429 were *Streptomyce chrestomyceticus* strains and not a *Streptomyces rimosis* or *Streptomyces paromomycinus* strain as indicated by the depository institute.

In Table 2, the percentage identity of the whole genome and the 16SRNA sequence of several *Streptomyces* species to the one of *Streptomyces sp.* Saigon 413 and *S. chrestomyceticus* NRRL-B-3672 is shown.

1.3. Identification of the biosynthetic gene cluster for producing polyene compound in *Streptomyces sp.* Saigon413

Genomic DNA was extracted from *Streptomyces* sp. Saigon413. Whole genome sequencing was completed using both Pacific Biosciences and Illumina sequencing technologies. To identify genes involved in the production of the polyene compound of the present invention, the assembled genome was run AntiSMASH (version 5.1.1, Blin et. al., "antiSMASH 5.0: updates to the secondary metabolite genome mining pipeline" *Nucleic Acids Res* (2019) doi: 10.1093/nar/gkz310), a commonly used tool to assist with the identification of biosynthetic gene clusters responsible for the production of secondary metabolites.

With the identification of compound of the present invention being a polyene compound (see [Example 2), and the AntiSMASH output we were able to deduce that the polyene compound is produced by a modular type I polyketide synthase (PKS) gene cluster. The identification of a modular type I PKS gene cluster being responsible for the biosynthesis of polyene compound was based upon the analysis of biosynthetic gene clusters that have been linked to the production of characterised polyenes, such as filipin by *Streptomyces filipinensis,* amphotericin by *Streptomyces nodosus* and thailandins A and B by *Actinokineospora bangkokensis* 44EHW. Within *Streptomyces* sp. Saigon413, one large modular type I polyketide synthase biosynthetic gene cluster, (see Figure 2) was identified, and therefore was associated with the production of polyene compound.

The modular type I PKS biosynthetic gene cluster contains 14 coding sequences including eight modular type I PKS genes, a regulatory gene, and genes responsible for the biosynthesis of a precursor incorporated into polyene compound (Figure 2 and Table 1).

Table 1: Coding sequences present in type I PKS biosynthetic gene cluster responsible for the production of the polyene compound according to the present invention. Annotations provided are based upon pBLAST search using the non-redundant protein sequences on the National Centre for Biotechnology Information database

	SEQ ID		
Gene ID	**DNA**	**Translated protein**	**Annotation**
CDS_11 (ctg1_921)	SEQ ID NO: 2	SEQ ID NO: 26	ABC transporter
CDS_12 (ctg1_922)	SEQ ID NO: 3	SEQ ID NO: 27	Unknown function
CDS_13 (ctg1_923)	SEQ ID NO: 4	SEQ ID NO: 28	Unknown function
CDS_14 (ctg1_924)	SEQ ID NO: 5	SEQ ID NO: 29	Thioesterase
CDS_15 (ctg1_925)	SEQ ID NO: 6	SEQ ID NO: 30	Unknown function
CDS_16 (ctg1_926)	SEQ ID NO: 7	SEQ ID NO: 31	Histidine Kinase
CDS_17 (ctg1_927)	SEQ ID NO: 8	SEQ ID NO: 32	LuxR-like regulator
CDS_18 (ctg1_928)	SEQ ID NO: 9	SEQ ID NO: 33	Non-ribosomal peptide synthetase
CDS_19 (ctg1_929)	SEQ ID NO: 10	SEQ ID NO: 34	Polyketide synthase
CDS_20 (ctg1_930)	SEQ ID NO: 11	SEQ ID NO: 35	Polyketide synthase
CDS_21 (ctg1_931)	SEQ ID NO: 12	SEQ ID NO: 36	FkbH-like protein
CDS_22 (ctg1_932)	SEQ ID NO: 13	SEQ ID NO: 37	Acyl-CoA dehydrogenase
CDS_23 (ctg1_933)	SEQ ID NO: 14	SEQ ID NO: 38	Acyl-carrier protein
CDS_24 (ctg1_934)	SEQ ID NO: 15	SEQ ID NO: 39	3-hydroxybutyryl-CoA dehydrogenase
CDS_25 (ctg1_935)	SEQ ID NO: 16	SEQ ID NO: 40	Non-ribosomal peptide synthetase
CDS_26 (ctg1_936)	SEQ ID NO: 17	SEQ ID NO: 41	Unknown function
CDS_27 (ctg1_937)	SEQ ID NO: 18	SEQ ID NO: 42	Polyketide synthase
CDS_28 (ctg1_938)	SEQ ID NO: 19	SEQ ID NO: 43	Polyketide synthase
CDS_29 (ctg1_939)	SEQ ID NO: 20	SEQ ID NO: 44	Polyketide synthase
CDS_30 (ctg1_940)	SEQ ID NO: 21	SEQ ID NO: 45	Polyketide synthase
CDS_31 (ctg1_941)	SEQ ID NO: 22	SEQ ID NO: 46	Polyketide synthase
CDS_32 (ctg1_942)	SEQ ID NO: 23	SEQ ID NO: 47	Polyketide synthase
CDS_33 (ctg1_943)	SEQ ID NO: 24	SEQ ID NO: 48	Unknown function
CDS_43 (ctg1_944)	SEQ ID NO: 25	SEQ ID NO:49	O-methyltransferase

1.4. Deletion of genomic region including CDS_31 (SEQ ID NO: 22) and CDS_32 (SEQ ID NO: 23) from *Streptomyces* sp. Saigon413 and phenotypic analysis

To confirm the identified biosynthetic gene cluster was associated with the production of polyene compound of the present invention, a region containing two polyketide synthase genes encoded by CDS_31 and CDS_32 (SEQ ID NO: 22 and SEQ ID NO: 23) was deleted from *Streptomyces* sp. Saigon413. To generate *Streptomyces* sp. Saigon413Δ941-942, plasmid p073-031 was used. Plasmid p073-031 was prepared from pRAR017 and contained regions of homology to either side of the region to be deleted from the strain (facilitating primary and secondary crossovers).

Plasmid p073-031 was used to transform *E. coli* ET12567/pUZ8002 using a standard electroporation method, and then introduced into *Streptomyces* sp. Saigon413 by mycelial conjugation (T. Kieser *et. al.,* Practical Streptomyces Genetics, 2000, John Innes Foundation, Norwich). Thiostrepton resistant

colonies were patched on ISP-4 agar media supplemented with 40 µg/ml thiostrepton and 25 µg/ml nalidixic acid. These patches were initially incubated for 6 days at 28 °C allowing plasmid replication. After 6 days at 28°C, strains were re-patched on ISP-4 agar media supplemented with 40 µg/ml thiostrepton and incubated at 37°C for further 6 days to force primary integration. After 6 days at 37°C, the obtained strains were transferred onto ISP-4 solid agar media without selection and incubated for 15 days at 28°C to allow a second crossover.

After 15 days of growth, strains were collected in 20 % glycerol. 100 µl of the cell suspension was used to inoculate fresh plates, as well as to make serial dilutions up to 10^{-10}. 100 µl of 10^{-8} to 10^{-10} were then plated onto ISP-4 agar plate. Plates were incubated at 28°C until single colonies were observed.

Single colonies were double patched on non-selective and thiostrepton selective ISP-4 agar media. Sensitive patches (representing secondary recombination) were then screened via PCR using gDNA isolated with FastSpin kit for soil (MP Biomedicals).

To confirm the region containing both SEQ ID NO: 22 and SEQ ID NO: 23 has been removed from the strain, a primer pair binding to the outside of the deleted region was used. Sanger sequencing of the PCR product and alignment to the *Streptomyces* sp. Saigon413 genome, confirmed deletion of the genomic region containing SEQ ID NO: 22 and SEQ ID NO: 23. Additionally, full genome analysis using Illumina-PCR-free sequencing confirmed that no other alterations had been made to the genome.

Cultivation of *Streptomyces* sp. Saigon413Δ941-942 and analysis of extracts from the strain confirmed that the polyene compound was no longer produced by the strain. Confirming SEQ ID NO: 22 and SEQ ID NO: 23 are essential for production of the polyene compound.

2. Characterisation of polyene compound according to the present invention

2.1. Purification of the polyene compound

A spray dried sample from a culture of *Streptomyces sp.* Saigon 413 was washed with water. The solid residue was extracted twice with isopropanol and the isopropanol was removed. The resulting solid was purified by preparative reverse phase (C18) HPLC using an acetonitrile:water gradient. Further purification was conducted by preparative reverse phase HPLC using a Zorbax C8 column and eluting with an acetonitrile:water gradient.

The polyene compound was detected by UV-VIS in the acetonitrile:water solution (Figure 1).

2.2. Other polyene type compounds

Filipin complex isolated from *Streptomyces filipinensis* (CAS: 11078-21-0) was purchased from a commercial vendor. It is a macrolide antibiotic and used as a dye in microscopy. The sample is a mixture of 8 isomers, with Filipin III as a main component. Filipin III has a mass of 654.83 and a molecular composition of C35H58O11

Amphotericin B is a macrolide antibiotic isolated from *Streptomyces nodosum* (CAS1397-89-3) was purchased from a commercial vendor. It has a mass of 924.08 and a molecular composition of C47H73NO17.

2.3 Liquid Chromatography and High-Resolution Mass Spectrometry

Spectra were recorded on an Orbitrap ID-X Tribrid Mass Spectrometer from Thermo Scientific equipped with an OptaMax NG Heated Electrospray Source (Spray Voltage: Static, Polarity Ion (V): 3400 (Positive ion mode) & 2400 (Negative ion mode), Sheath Gas (Arb): 40, Aux Gas (Arb): 5, Sweep Gas (Arb): 1, Ion Transfer Tube Temperature: 350 °C, Vaporizer Temperature: 350 °C). The Scan Parameters were as follows;

Experiment 1: MS OT (Orbitrap Resolution: 50,000, Scan Range (m/z): 200 to 2000, RF Lens (%): 60, AGC Target: Standard, Maximum Injection Time Mode: Auto, Microscans: 1, Data Type: Profile, Polarity: Both),

Experiment 2: tMS2 OT CID (MSn Level (n): 2, Isolation Window (m/z): 1.0, Activation Type: CID, CID Collision Energy (%): 30, Detector Type: Orbitrap, Orbitrap Resolution: 30,000, RF Lens (%): 60, Polarity: Positive),

Experiment 3: tMS2 OT HCD (MSn Level (n): 2, Isolation Window (m/z): 1.0, Activation Type: HCD, HCD Collision Energy (%): 30, Detector Type: Orbitrap, Orbitrap Resolution: 30,000, RF Lens (%): 60, Polarity: Positive), Experiment 4: tMS3 OT HCD (MSn Level (n): 3, Isolation Window (m/z): 1.6, Activation Type: HCD, HCD Collision Energy (%): 30, MS2 Isolation Window (m/z): 2, MS2 Activation Type: HCD, MS2 HCD Collision Energy (%): 30, Detector Type: Orbitrap, Orbitrap Resolution: 30,000, RF Lens (%): 60, Polarity: Positive).

The mass spectrometer was connected to a Vanquish Flex UHPLC from Thermo Scientific using a Vanquish Split Sampler FT, Vanquish Binary Pump F, Vanquish Column Compartment H, Vanquish Diode Array Detector FG and Vanquish Charged Aerosol Detector.

Liquid Chromatography Conditions included: Waters ACQUITY UPLC C18 column 1.7µm 3.0x50mm, P.N. 186004660. Temp: 40°C, DAD wavelength range: 250 to 260nm, Solvent gradient: Solvent A: H2O with 0.1% formic acid, Solvent B: CH3CN with 0.1% formic acid, gradient: 0 min 10% B, 90% A; 4.00min 90% B, 10% A; 4.25min 90% B, 10% A; 4.50min 10% B, 90% A; 5.00min 10% B, 90% A, Flow rate: 1.0ml/min, Injection volume: 2 uL, Total run time: 5.0min.

A purified fermentation broth as described under section 1.3 was injected.

The key peaks of the mass spectrum observed were:

Negative ion: C67H114NO25 $[M-H]^-$ Expected: 1332.7685, Observed: 1332.7679

Positive ion: C67H114NO24 $[M-H2O+H]^+$ Expected: 1316.7725, Observed: 1316.7709

Positive ion: C67H115NO24 $[M-H2O+2H]^{2+}$ Expected: 658.8899, Observed: 658.8895

Positive ion: C67H113NO23 $[M-2(H2O)+2H]^{2+}$ Expected: 649.8846, Observed: 649.8843

Positive ion: C67H111NO22 $[M-3(H2O)+2H]^{2+}$ Expected: 640.8793, Observed: 640.8790

In a second experiment, Liquid Chromatography Conditions included: Kinetex Polar C18 column 100A 4.6x100mm, P.N. H17-055453. Temp: 40°C, DAD wavelength range: 250 to 260nm, Solvent gradient: Solvent A: H2O with 0.1% formic acid, Solvent B: CH3CN with 0.1% formic acid, gradient: 0min 10% B, 90% A; 1min 10% B, 90% A; 6.50min 95% B, 5% A; 8.00min 95% B, 5% A; 9.00min 10% B, 90% A; 10.00min 10% B, 90% A, Flow rate: 1.0ml/min, Injection volume: 5 uL, Total run time: 10.0min.

Under these conditions, the polyene compound had a retention time of 5.55-5.57 minutes.

The observed mass of the polyene compound was the same as in the previous Liquid chromatography run (results not shown).

2.4 Molecular composition and mass

The molecular composition and mass of polyene compound was determined using the results of liquid chromatography and high-resolution mass spectrometry as disclosed in 2.3. The polyene compound has the following composition.

Molecular composition $C_{67}H_{115}NO_{25}$ and exact mass of 1333.7758.

2.5 Solubility

The solubility of a compounds was determined in DMSO:

Compound	solvent (pH)	solubility (ppm)
Polyene of compound	DMSO	>10'000
Philipin complex	DMSO	>10'000
Amphotericin B	DMSO	>10'000

The presence of the polyene compound with a mass of 1333.7758 g in the fermentation broth of several Streptomyces species, was determined by isolating and purifying according to the method described in section 2. The results in Table 2 show that the polyene compound with a molecular formula of $C_{67}H_{115}NO_{25}$ was produced by *Streptomyces chrestomyceticus* species.

Table 2. Identification of polyene compound $C_{67}H_{115}NO_{25}$ in the fermentation broth of *Streptomyces sp.* Saigon 413 and several other *Streptomyces* strains and 16SRNA and whole genome % identity relative to *Streptomyces sp.* Saigon 413

Strain ID	**Species**	**16S RNA sequence identity (%)**	ANI (%) vs. NRRL B3672	**ANI (%)** vs. CBS149411	**Polyene** $C_{67}H_{115}NO_{25}$
CBS 149411	*Streptomyces sp.* Saigon 413	100	96.90	100	yes
CBS 492.64	*Streptomyces sp.*	99.48	90.02	90.04	No
CBS 569.66	*S. chrestomyceticus (S. rimosis)*	99.94	96.63	99.74	Yes
CBS 570.66	*S. chrestomyceticus (S. rimosis)*	99.94	96.65	99.74	Yes
CBS 612.68	*S. albofaciens*	98.82	89.91	89.92	No
CBS 938.68	*S. rimosus*	98.89	89.88	89.81	No
DSM 40673	*S. rimosus*	99.02	89.86	89.78	No
DSM 41057	*S. rimosus*	98.89	90.00	89.96	No
DSM 41224	*S. chrestomyceticus*	99.87	99.97	96.96	Yes
DSM 41429	*S. chrestomyceticus (S. paromomycinus)*	99.87	95.88	95.95	Yes
DSM-41561	*S. rimosus*	99.02	89.79	89.84	No
NRRL B-2626	*S. rimosus*	98.89	89.82	89.75	No
NRRL B-3672	*S. chrestomyceticus*	99.87	100	96.9	Yes

3. Activity of polyene compound to control fungal disease

3.1 Fungicidal activity in liquid culture assays

Mycelia fragments or conidia suspensions of a fungus, prepared either freshly from liquid cultures of the fungus or from cryogenic storage, were directly mixed into nutrient broth.

A stock solution of the polyene compound according to the present invention, Filipin complex or Amphotericin B were produced in DMSO (max. 10 mg/ml), which was diluted with water plus 0.025% Tween20 to produce a 10x concentrated sample and 10 µl of this solution was pipetted into a microtiter plate (96-well format). The nutrient broth containing the fungal spores/mycelia fragments was then added to give an end concentration of the tested compound. The test plates were incubated in the dark at 24°C and 96% rh. The inhibition of fungal growth was determined photometrically after 2 – 7 days, depending on the pathosystem, and percent antifungal activity relative to the untreated check was calculated.

The effect of the polyene compound of the present invention, Filipin complex or Amphotericin B was tested against the following fungi under the conditions as outlined above and specifically herein below:

Botryotinia fuckeliana (*Botrytis cinerea)* (BOTRCI)/ liquid culture (Gray mould)
Conidia of the fungus from cryogenic storage were directly mixed into nutrient broth (Vogels broth). The inhibition of growth is determined photometrically 3-4 days after application.

Colletotrichum orbiculare (*Colletotrichum lagenarium*) (COLLLA) / liquid culture (Anthracnose)
Conidia of the fungus from cryogenic storage were directly mixed into nutrient broth potato dextrose broth PDB). The inhibition of growth was measured photometrically 3-4 days after application.

Fusarium culmorum FUSACU /liquid culture (root rot/foot rot/culm rot/head bligh of cerals)
Conidia of the fungus from cryogenic storage were directly mixed into nutrient broth (PDB). The inhibition of growth was measured photometrically 3-4 days after application.

Microdochium nivale (MONGNI) liquid culture (foot rot/head bligh/snow mould of cerals)
Conidia of the fungus from cryogenic storage were directly mixed into nutrient broth (PDB). The inhibition of growth was measured photometrically 4-5 days after application.

Mycosphaerella arachidis *(Cercospora arachidicola)* MYCOAR / liquid culture (early leaf spot of groundnut)
Conidia of the fungus from cryogenic storage were directly mixed into nutrient broth (PDB). The inhibition of growth was determined photometrically 4-5 days after application.

Pythium ultimum PYTHUL / liquid culture (damping-off of seedlings)
Mycelia fragments and oospores of a newly grown liquid culture of the fungus are directly mixed into nutrient broth (PDB). The inhibition of growth was measured photometrically 2-3 days after application.

Rhizoctonia solani RHIZSO / liquid culture (Rice Sheath Blight)

Mycelia fragments of a newly grown liquid culture of the fungus are directly mixed into nutrient broth (PDB). The inhibition of growth was determined photometrically 3-4 days after application.

Sclerotinia sclerotiorum SCLESC / liquid culture (white mould)

Mycelia fragments of a newly grown liquid culture of the fungus are directly mixed into nutrient broth (PDB). The inhibition of growth was determined photometrically 3-4 days after application.

The results in Table 3 show that the compound according to the present invention has fungicidal activity.

Zymoseptoria tritici **(Mycosphaerella graminicola, Septoria tritici), SEPTTR**

Conidia of the fungus from cryogenic storage were directly mixed into nutrient broth (PDB potato dextrose broth). A DMSO solution of the test compounds was placed into a microtiter plate (96-well format) and the nutrient broth containing the fungal spores was added to it. The test plates were incubated at 24 C and the inhibition of growth was determined photometrically after 72 hrs.

Table 3. Control of fungal development by polyene compound of the present invention, filipin complex and amphotericin in liquid culture.

	concentration	Target fungal pathogen									
compound	in ppm	BOTRCI	COLLLA	FUSACU	MONGNI	MYCOAR	PYTHUL	RHIZSO	SCLESC	SEPTTR	SEPTTR (R)
Polyene compound with MW=1333.7758	100	100	100	100	100	100	70	100	100	100	100
	33.3	100	100	100	100	100	20	100	100	100	100
	11.1	100	100	100	100	100	0	100	100	100	100
	3.7	100	100	100	100	100	0	100	70	100	100
	1.2	100	100	100	100	100	0	70	50	100	100
	0.4	90	100	100	100	90	0	20	20	100	100
Filipin complex	100	100	100	100	100	100	100	100	100	100	100
	33.3	100	100	100	100	100	100	100	100	100	100
	11.1	100	100	100	100	100	70	100	100	100	100
	3.7	100	100	100	100	100	0	0	70	100	100
	1.2	100	100	20	100	0	0	0	0	100	100
	0.4	0	0	0	0	0	0	0	0	0	0
Amphotericin B	100	100	100	100	100	0	100	100	100	100	100
	33.3	100	100	100	100	0	100	100	100	100	100
	11.1	100	100	100	100	0	100	100	100	100	100
	3.7	100	100	90	100	0	70	50	100	100	100
	1.2	100	100	70	100	0	0	0	100	100	100
	0.4	100	90	20	70	0	0	0	100	100	100

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BP/A/II/12
page 14

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PROCEDURE OF PATENT PROCEDURE

INTERNATIONAL FORM

Syngenta Ltd **Jealott's Hill Research International Centre** **Bracknell, Berkshire** **RG42 6EY** **UK** *name and address of depositor*	RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT issued pursuant to Rule 7.1 by the INTERNATIONAL DEPOSITARY AUTHORITY identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITORY AUTHORITY:
***Streptomyces* sp. Saigon413**	**CBS 149411**

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:

☐ a scientific description

☒ a proposed taxonomic designation *(mark with a cross where applicable)*

III. RECEIPT AND ACCEPTANCE

This International Depositary accepts the microorganism identified under I above, which was received by it on **09-09-2022** *(date dd-mm-yyyy of the original deposit)* [1]

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depository on **not applicable** *(date dd-mm-yyyy of the original deposit)* and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on **not applicable** *(date dd-mm-yyyy of receipt of request for conversion)*

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: **Westerdijk Fungal Biodiversity Institute (CBS)** Address: **Uppsalalaan 8** **P.O. Box 85167** **3508 AD UTRECHT** **The Netherlands**	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): **Dr M. Groenewald** **Dr G.J.M. Verkley** Date *(dd-mm-yyyy)*: **19-09-2022**

1 Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired.

CLAIMS

1. A compound, wherein the compound is a polyene compound characterized by a molecular formula according to $C_{67}H_{115}NO_{25}$, wherein the polyene is further characterized by the spectrum of light absorption with absorbance maxima at a wavelength of 235.5 nm, 301.1 nm, 315.8 nm, 330.9 nm and 348.3 nm when measured in an aqueous acetonitrile solution.

2. The compound according to claim 1, wherein the compound is obtainable by cultivating a microorganism which comprises at least one nucleotide sequence encoding a protein which has at least 90% identity to at least one of the amino acid sequences according to SEQ ID NO: 26-49, preferably SEQ ID NO: 46 and 47, preferably wherein the nucleotide sequence has at least 90 % identity to at least one of the nucleotide sequences according to SEQ ID NOs: 2 to 25, preferably wherein the microorganism comprises a nucleotide sequence which has at least 90% identity to SED NO: 22 and / or SEQ ID NO: 23.

3. The compound according to claim 2, wherein the microorganism has at least 91% identity to the whole genome of *Streptomyces sp.* Saigon413 deposited with the Westerdijk Institute under accession number CBS149411.

4. The compound according to any one of the claims 2 to 3, wherein the microorganism comprises a nucleotide sequence which has at least 99.5% identity to the nucleotide sequence according to SEQ ID NO: 1.

5. The compound according to any one of the claims 2 to 4, wherein the microorganism is a *Streptomyces chrestomyceticus*, preferably *Streptomyces sp.* Saigon413 deposited with the Westerdijk Institute under accession number CBS149411.

6. A composition comprising the compound according to any one of the claims 1 to 5 and the microorganism as defined in any one of the claims 2 to 5.

7. The composition according to claim 6, further comprising at least one additional ingredient having pesticidal activity and / or at least one plant growth regulator, preferably wherein the additional ingredient comprises cyclothiazomycin C, streptimidone and / or malonomicin.

8. The composition according to claim 6 or 7, further comprising an auxiliary, preferably an agricultural acceptable carrier.

9. A process for producing the compound according to any one of the claims 1 to 5, or a composition according to any one of the claims 6 to 8, comprising cultivating a microorganism which comprises at least one nucleotide sequence encoding a protein which has at least 90% identity to at least

one of the amino acid sequences according to SEQ ID NO: 26-49, preferably SEQ ID NO: 46 and 47, preferably wherein the nucleotide sequence has at least 90 % identity to at least one of the nucleotide sequences according to SEQ ID NOs: 2 to 25, preferably wherein the microorganism comprises a nucleotide sequence which has at least 90% identity to SED NO: 22 and / or SEQ ID NO: 23 in a suitable fermentation medium under conditions that allow production of the compound and / or composition, and optionally recovering the compound and / or composition.

10. A method for controlling or preventing infestation of a plant, plant propagation material and/or harvested food crops by a phytopathogenic microorganism, by treating the plant, plant propagation material and/or harvested food crops, wherein an effective amount of the compound according to any one of the claims 1 to 5, or the composition according to any one of the claims 6 to 8 is applied to the plant, to a part thereof or a locus thereof, the plant propagation material and/or harvested food crops.

11. The method according to claim 10, wherein the effective amount comprises 0.001 g to 5 kg of the compound of claim 1 per hectare.

12. The method according to claim 10, wherein the plant propagation material is seed and the effective amount comprises 0.0001 to 50 g of the compound of claim 1 per kg of seed.

13. The method according to any one of the claims 10 to 12, wherein the phytopathogenic microorganism is a fungus, preferably a fungus belonging to *Botrytis, Colletotrichum. Fusarium, Microdochium, Mycosphaerella, Pythium, Rhizoctonia,* or *Sclerotinia, Zymoseptoria,* preferably fungi belonging to *Botryotinia fuckeliana* (*Botrytis cinerea), Colletotrichum orbiculare (Colletotrichum lagenarium*), *Fusarium culmorum, Microdochium nivale, Mycosphaerella arachidis (Cercospora arachidicola), Pythium ultimum, Rhizoctonia solani,* or *Sclerotinia sclerotiorum. Zymoseptoria tritici.*

14. The method according to any one of the claims 10 to 13, wherein the plant comprises wheat, barley, corn, rice, soybean and/or banana.

15. Use of a compound according to any one of the claims 1 to 5, or a composition according to any one of the claims 6 to 8 as a pesticide, preferably as a fungicide, a SAR inducer and / or as a priming agent.

FIGURES

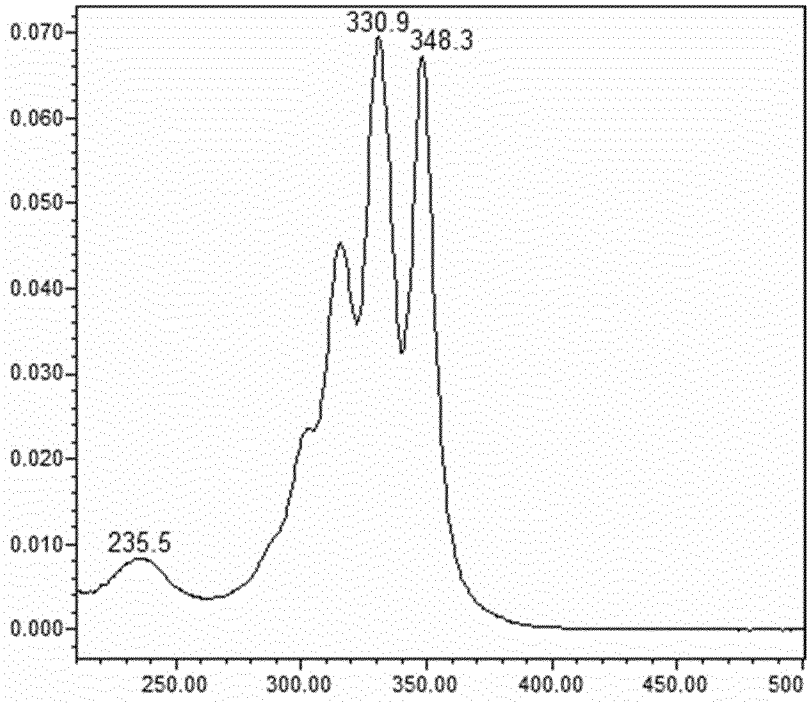

Fig. 1

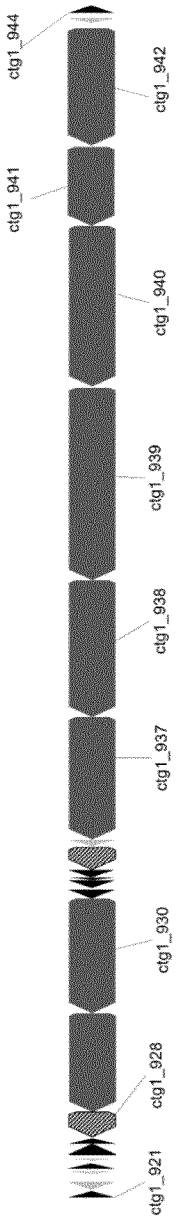
ctg1_944
ctg1_942
ctg1_941
ctg1_940
ctg1_939
ctg1_938
ctg1_937
ctg1_930
ctg1_928
ctg1_921

Fig 2

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/077837

A. CLASSIFICATION OF SUBJECT MATTER
INV. A01N63/28 A01P3/00 C12N1/20
ADD.

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)
A01N C12R A01P C12N

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2004/065401 A1 (ECOPIA BIOSCIENCES INC [CA]; BACHMANN BRIAN O [US] ET AL.) 5 August 2004 (2004-08-05)	1-5
Y	claims examples table 6	1-15

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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 application or patent 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
17 December 2024	10/01/2025

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Galley, Carl

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PARK HEUNG-SOON ET AL: "Screening and Isolation of a Novel Polyene-Producing Streptomyces Strain Inhibiting Phytopathogenic Fungi in the Soil Environment", FRONTIERS IN BIOENGINEERING AND BIOTECHNOLOGY, vol. 9, 12 July 2021 (2021-07-12), XP093132997, CH ISSN: 2296-4185, DOI: 10.3389/fbioe.2021.692340	1-5
Y	abstract figure 2 figure 5 compounds I-NTF table 1	1-15

X	BOBEK JAN ET AL: "Polyenic Antibiotics and Other Antifungal Compounds Produced by Hemolytic Streptomyces Species", INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES, vol. 23, no. 23, 30 November 2022 (2022-11-30), page 15045, XP093133000, Basel, CH ISSN: 1422-0067, DOI: 10.3390/ijms232315045	1-5
Y	abstract Section 1.2 table 1	1-15

X	WO 2010/107294 A1 (UNIV PUTRA MALAYSIA [MY]; SEOW HENG FONG [MY] ET AL.) 23 September 2010 (2010-09-23)	1-9,15
Y	claims page 18; examples figure 6 compound J5	1-15

Y	CN 102 533 600 A (SOUTH CHINA SEA INST OCEANOLOG) 4 July 2012 (2012-07-04) claims abstract	1-15

Y	CN 1 434 115 A (YUNNAN INST OF MICROBIOLOGY [CN]) 6 August 2003 (2003-08-06) abstract claims	1-15

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/077837

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SINHA ARKADEEP ET AL: "The complete genomic sequence ofStreptomyces spectabilisNRRL-2792 and identification of secondary metabolite biosynthetic gene clusters", JOURNAL OF INDUSTRIAL MICROBIOLOGY & BIOTECHNOLOGY, BASINGSTOKE, GB, vol. 46, no. 8, 13 June 2019 (2019-06-13), pages 1217-1223, XP036864648, ISSN: 1367-5435, DOI: 10.1007/S10295-019-02172-8 [retrieved on 2019-06-13]	1-9,15
Y	claims examples	1-15

X	SECO E M ET AL: "Starter Unit Choice Determines the Production of Two Tetraene Macrolides, Rimocidin and CE-108, in Streptomyces diastaticus var. 108", CHEMISTRY & BIOLOGY, CURRENT BIOLOGY, LONDON, GB, vol. 11, no. 3, 1 March 2004 (2004-03-01), pages 357-366, XP025940223, ISSN: 1074-5521, DOI: 10.1016/J.CHEMBIOL.2004.02.017 [retrieved on 2004-03-22]	1-9,15
Y	abstract page 364, paragraph bridging - page 365	1-15

Y	WO 2023/017016 A1 (SYNGENTA CROP PROTECTION AG [CH]) 16 February 2023 (2023-02-16) claims examples	7

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2024/077837

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2024/077837

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2004065401 A1	05-08-2004	CA 2453071 A1	03-04-2004
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		JP 2007525148 A	06-09-2007
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CN 102533600 A	04-07-2012	CN 102533600 A	04-07-2012
		US 2013316408 A1	28-11-2013
		WO 2013097424 A1	04-07-2013
CN 1434115 A	06-08-2003	NONE	
WO 2023017016 A1	16-02-2023	AR 126729 A1	08-11-2023
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		CL 2024000369 A1	16-08-2024
		CO 2024001394 A2	15-02-2024
		CR 20240062 A	19-03-2024
		EP 4384011 A1	19-06-2024
		JP 2024531932 A	03-09-2024
		US 2024334934 A1	10-10-2024
		WO 2023017016 A1	16-02-2023