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ENDOPHYTIC FUNGI AND RHIZOBACTERIA: BENEFICIAL EFFECTS ON PLANTS

LAVRAS - MG
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Thesis presented to the Federal University of Lavras, as part of the requirements of the Post Graduate Program in Agronomy/Phytopathology, area of concentration in Phytopathology, for the degree of Doctor in Philosophy.

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Supervisor

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FUNGOS ENDOFÍTICOS E RIZOBACTÉRIA: EFEITOS BENÉFICOS NAS PLANTAS

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To my family Helena, Laís, Rita and Luiz for being my real support and for making me believe that anything can happen with hard work.
I dedicate.

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RESUMO GERAL

A preocupação com uma agricultura mais sustentável, sem causar perdas na produtividade das lavouras, revigora a pesquisa científica com atenção especial aos microrganismos benéficos. Esses microrganismos podem cooperar com o crescimento das plantas, desempenhando funções essenciais na fertilidade do solo, nutrição e saúde das plantas. O aumento do número de produtos biológicos disponíveis no mercado é uma realidade mundial e explorar as boas competências de fungos, bactérias e vírus benéficos tem sido uma proposta atraente. No entanto, para tornar isso realidade, é necessário muita pesquisa. Na primeira parte deste trabalho, seis isolados do fungo endofítico *Induratia* spp. foram obtidas e as espécies foram identificadas por meio de análise molecular. Esses isolados de *Induratia* foram capazes de produzir compostos orgânicos voláteis (COVs) com atividade antimicrobiana contra os seguintes fitopatógenos testados: *Phytophthora* sp., *Rhizoctonia solani*, *Botrytis cinerea*, *Colletotrichum lindemuthianum*, *Sclerotinia sclerotiorum* e *Fusarium verticillioides*. Os isolados de *Induratia* produziram 17 COVs diferentes, conforme determinado pela microextração em fase sólida seguida pelo método de cromatografia gasosa-espectrometria de massa (SPME-GCMS). Entre os isolados testados *in vitro*, FTB apresentou os melhores resultados contra a maioria dos patógenos e foi testado *in vivo* quanto à sua capacidade de controlar *B. cinerea* em frutos de morango armazenados em caixas hermeticamente fechadas, onde demonstrou aumentar a vida útil dos frutos. Este novo isolado de *Induratia* apresentou potencial para ser posteriormente desenvolvido em um produto comercial e uma patente foi depositada sob o número BR 10 2021 001666 3. Na segunda parte deste trabalho, exploramos a microbiota da rizosfera de feijão, aveia, colza, trigo ou solo em pousio, isolando e caracterizando funcionalmente usando meios de cultura e identificando molecularmente os isolados pelo sequenciamento da região 16S rDNA. Foram obtidos 138 isolados bacterianos e o potencial destes isolados para solubilização de fósforo (P) de fosfato de alumínio, fosfato tricálcico, fosfato de ferro, mineralização de fitato, solubilização de potássio (K), produção de sideróforos, nitrato endógeno e inibição de *Fusarium graminearum* foi testado *in vitro*. A atividade mais comum foi representada pela solubilização de P a partir de fosfato de alumínio, seguida pela solubilização de K. Isolados bacterianos exibindo solubilização de fosfato de ferro e solubilização de P mineral a partir de fitato foram encontrados apenas na rizosfera de colza e feijão. Aproximadamente, 68% dos isolados bacterianos exibiram duas ou mais atividades supostamente ligadas a promoção de crescimento. No total, foram identificados 10 gêneros: *Pseudomonas*, *Stenotrophomonas*, *Comamonas*, *Arthrobacter*, *Agromyces*, *Rhodococcus*, *Streptomyces*, *Serratia*, *Lelliotia* e *Paenisporosarcina*. Os gêneros mais comuns foram *Pseudomonas* (44,2%), seguido por *Stenotrophomonas* (23,2%). Um bioensaio com sementes de trigo foi feito com 4 isolados escolhidos por seu bom desempenho em atividades potenciais para o crescimento de plantas, e estes mostraram melhorias no crescimento da raiz e da parte aérea. Portanto, diversos isolados se mostraram potencialmente aplicáveis como biofertilizantes e agentes de biocontrole, constituindo-se em alternativas para a produção agrícola sustentável.

Palavras-chave: Atividade antimicrobiana. Compostos orgânicos voláteis. Fungos endofíticos. *Induratia*. ITS. *RPB2*. *TUB2*. *Pseudomonas*. Nutrientes essenciais. 16S rDNA. Bactérias associadas à raízes.

GENERAL ABSTRACT

The concern with sustainable agriculture without causing losses in crop yields reinvigorates scientific research with special attention focused on beneficial microorganisms. These microorganisms may cooperate with plant growth, playing essential functions in soil fertility, plant nutrition and health. The increase in the number of biological products available in the market is a worldwide reality and the exploitation of the skills of beneficial fungi, bacteria and virus has been attractive. However, to make this a reality, a lot of investigation is needed. In the first part of this work, we isolated six strains of the endophytic fungus *Induratia* spp. and identified through phylogenetic analysis. These *Induratia* strains were able to produce volatile organic compounds (VOCs) with antimicrobial activity against the following tested plant pathogens: *Phytophthora* sp., *Rhizoctonia solani, Botrytis cinerea, Colletotrichum lindemuthianum, Sclerotinia sclerotiorum* and *Fusarium verticillioides.* The *Induratia* strains produced 17 different VOCs as determined by the solid-phase microextraction followed by gas chromatography-mass spectrometry method (SPME-GCMS). Among the *in vitro*-tested strains, FTB has shown the best results against most pathogens and was tested *in vivo* for its capacity to control *B. cinerea* on strawberry fruits stored in hermetically closed boxes, where it was shown to increase the shelf life of the fruits. This novel *Induratia* strain showed potential to be further developed into a commercial product and a patent was deposited under number BR 10 2021 001666 3. In the second part of this work, we explored the microbiota in the rhizosphere of faba bean, oat, oilseed rape, wheat or bare fallow soil to isolate, functionally characterize with cultural methods, and molecularly identify the strains by sequence analysis of the16S rDNA. A total of 138 bacterial strains were obtained and their potential for phosphorus (P) solubilisation from aluminium phosphate, tricalcium phosphate, iron phosphate, phytate mineralisation, potassium (K) solubilisation, siderophore production, endogenous nitrate and *Fusarium graminearum* inhibition was tested *in vitro*. The most common plant growth promotion (PGP) activity was represented by P solubilisation from aluminium phosphate, followed by K solubilisation. Bacterial strains displaying iron phosphate phosphate solubilisation and mineral P solubilisation from phytate were found only with oilseed rape and faba bean rhizosphere. Approximately, 68% of the bacterial strains displayed two or more activities. In total 10 genera were identified: *Pseudomonas, Stenotrophomonas, Comamonas, Arthrobacter, Agromyces, Rhodococcus, Streptomyces, Serratia, Lelliotia* and *Paenisporosarcina*. The most common genera were *Pseudomonas* (44.2%), followed by *Stenotrophomonas* (23.2%). A bioassay with wheat seeds was performed with 4 strains chosen by their good performance in the *in vitro* functional bioassays and these strains showed significant increases in root and shoot biomasses. Therefore, several strains have shown potential as biofertilizers and biocontrol agents, constituting alternatives to chemicals in sustainable agricultural crop production.

Keywords: Antimicrobial activity. Volatile organic compounds. Endophytic fungi. *Induratia*. ITS. *RPB2. TUB2*. *Pseudomonas.* Essential nutrients. 16S rDNA. Bacterial root association.

SUMMARY

FIRST PART

1 INTRODUCTION

1.1 Beneficial microorganisms in agriculture

The world's population is growing fast, and food production must increase in a sustainable manner. One way to guarantee sufficient food production is to avoid yield losses in agriculture (SAVARY et al., 2019). Some factors have favored epidemics in agroecosystems such as intensive cultivation, genetic uniformity and large-scale monocultures, as well as the uniform and indiscriminate application of chemical pesticides. In this way, plants have become more vulnerable to pathogens (SAVARY et al., 2019). Nowadays people are being more aware of the necessity to move toward a sustainable planet and are paying attention in environmental and human protection strategies to meet the standards of "Agriculture Green Development" (DAVIES; SHEN 2020). It is possible through the adoption of new technologies, such as ecofriendly environmental practices to reduce the dependence on chemical pesticides, for instance (HYDE et al., 2019; SAMARAKOON et al., 2020).

Nowadays, there are several manners to produce with food security and with less negative impacts to the environment. Microorganisms can be exploited in various ways, such as arbuscular mycorrhizal fungi used as biofertilizers, plant growth-promoting rhizobacteria contribute to plant growth by direct and indirect mechanisms, such as nutrient uptake, phytohormones; biological control of diseases directly or by induced resistance (HYDE et al., 2019; SAMARAKOON et al., 2020; OLEŃSKA et al., 2020). Moreover, beneficial microorganisms increase the availability of essential nutrients such as nitrogen (N), phosphorus (P) and iron (Fe) (MAHESHWARI et al., 2013; MAHMUD et al., 2020; THANT et al., 2018)

Microbial diversity on our planet is enormous and despite the number of studies already conducted, more than 99% of the 10^{12} estimated microbial species are still unknown (LOCEY; LENNON, 2016). Thus, beneficial microbes and their applications to enhance crop production by improving plant nutrition, stress tolerance, and protection from plant pathogens attract the scientific efforts to explore the microbiota and utilize their full potential.

1.2 Fungi

Fungal endophytes can produce secondary metabolites with antioxidant and antimicrobial activities that benefit both host plants and consumers. Protection against

pathogens, promotion of growth and maintenance of produce quality both in pre- and post-harvest are some of these benefits (KADDES et al., 2019; HYDE et al., 2019; REID et al., 2021). Isolation of endophytic fungi from plants is an important step to explore them as sources of bioactive enzymes and secondary metabolites in industrial and medical uses, such as biofertilizers and biological control agents in agriculture. Culture-based methods for studying fungi have improved aiming to increase the proportion of culturable fungal endophytes with the use of different media and smashed plant samples prior to plating (POTSHANGBAM et al., 2017). The selection of culture media is important to perform the isolation with the maximum possible diversity of cultivable endophytes (POTSHANGBAM et al., 2017). The commonly used media for isolation of endophytic fungi from plants are potato dextrose agar, water agar, Sabouraud dextrose agar, corn meal agar, malt extract agar, Czapek–Dox agar and oatmeal agar with or without antibiotic for suppressing the growth of bacteria. Endophytic fungi can be identified on the basis of their phenotype, such as colony morphology and microscopic features and by their genotype in molecular characters by phylogenetic analyses (ADHIKARI; PANDEY 2018).

Screening of endophytes with antagonistic activity against plant pathogenic bacteria and fungi is a tedious process and needs to be conducted with care. One of the biocontrol mechanisms of endophytic fungi is the production of volatile organic compounds (VOCs), which are molecules of low molecular weight and the ability to inhibit and kill microorganisms (KULAKIOTU; THANASSOULOPOULOS; SFAKIOTAKIS, 2004; KOITABASHI, 2005; VESPERMANN; KAI; PIECHULLA, 2007; GU et al., 2007; FIALHO et al., 2010; INSAM et al., 2010; HUANG et al., 2011). The most studied VOC-producing endophytic fungus is the genus *Induratia* (syn. *Muscodor*), which was introduced in 2001 (WORAPONG et al., 2001). First described as *Muscodor*, the genus was later shown by polyphasic taxonomy to be *Induratia* (SAMARAKOON et al., 2020).

Induratia species have been the focus of several studies and their potential to inhibit a wide range of bacteria and fungi pathogenic to humans and plants has been demonstrated. The diversity of bioactive metabolites, mainly volatiles, makes them promising biological control agents of plant pathogens. Diverse pathogens as *Pythium ultimum, Phytophthora cinnamomi, Rhizoctonia solani, Ustilago hordei, Stagnospora nodorum, Sclerotinia sclerotiorum, Aspergillus fumigatus, Botrytis cinerea, Colletrotrichum lagenarium, Colletotrichum lindemuthianum, Ceratocystis ulmi, Cercospora beticola, Geotrichum candidum, Pseudocercospora fijiensis*, *Pseudocercospora griseola*, *Phythophthora palmivora, Sclerotinia*

sclerotiorum, Verticillium dahlia, Tapiallumiae aureus, Micrococcus luteus Aspergillus ochraceus, A. sclerotiorum, A. elegans, A. foetidus, A. favus, A. tamari, A. tubingensis, A. sydowii, A. niger, A. caespitosus, A. versicolor, and *A. expansum,* were inhibited by *Induratia* species, as well as insects pests (STROBEL et al., 2001; RIGA; LACEY; GUERRA, 2008; HUANG et al., 2011; KUDALKAR et al., 2012; GUIMARAES et al., 2021; MOTA et al., 2021; GOMES et al., 2022). Nematicidal activity of *Induratia coffeana* against *Meloidogyne incognita* was observed in filtrates and by the production of volatile compounds (GUIMARAES et al., 2021). It was also observed that the metabolites produced by this genus showed antibacterial activity against *Staphylococcus aureus, Enterococcus faecalis* and *E. faecium* and reduced the pre-formed biofilm of *Staphylococcus aureus* and *S. epidermidis* (GUIMARAES et al., 2021). In addition, biofumigation with the VOCs produced by *Induratia* species has been shown to be efficient in the control of soil, seed and fruit pathogens, especially in post-harvest diseases (MERCIER; JIMENEZ, 2007; SUWANNARACH et al., 2012; GOMES et al., 2022).

The majority of the *Induratia* species produce VOC mixtures containing 1-butanol, 3-methyl, phenethyl alcohol, propanoic acid, 2-methyl, among others (KUDALKAR et al., 2012; GUIMARAES et al., 2021; GOMES et al., 2022). Kudalkar et al. (2012) observed in their GC-MS (Gas Chromatography- Mass Spectrometry) analyses that the range of VOCs varied and were characteristic of the species of *Induratia* analyzed, which may be interpreted as a chemical signature of the species. For example, in *I. sutura* the most common VOCs were 15 propanoic acid, 2-methyl and thujopsene, with anti-fungal activity; *I. alba* the most common VOCs were propanoic acid, 2-methyl, naphthalene and azulene derivatives, with anti-fungal and anti-bacterial activity; in *I. vitigena*, only naphthalene had anti-insect activity; in *I. crispa,* propanoic acid, 2-methyl and various esters, with anti-fungal and anti-bacterial activity; and in *I. fengyangensis,* derivatives of azulene and naphthalene and several others with anti-fungal and anti-bacterial activity were found (KUDALKAR et al., 2012). In *I. yucatanensis,* VOCs such as octane, 2-methyl butyl acetate, 2-pentyl furan, caryophyllene and aromadendrene were identified (MACIAS-RUBACALVA et al., 2010). It is interesting to highlight that VOC profiles may differ among strains of the same species or even in the same strain depending on the growth conditions, such as substrate, temperature, days of incubation and other environmental conditions (EZRA et al., 2003; GABLER et al., 2006; SUWANNARACH et al., 2017).

1.3 Bacteria

Methods to study the microbiota from an ecosystem can be either culture-dependent or culture-independent. Petri dishes are often used for plating the bacteria to obtain pure cultures for further analysis such as sequencing of the 16S rRNA genes for identification of bacterial strains (VESTER et al., 2014). However, there are several disadvantages to this method. Only a small fraction of these bacteria are able to grow in artificial media and therefore the community analysis is biased. It occurs because their metabolic and physiological requirements cannot be reproduced *in vitro*. The artificial homogenous medium typically select some species that can adapt to the conditions applied in the laboratory. In addition, fast-growing species efficiently overcome others. Furthermore, the difficulty of bacterial cultivation may be due to the lack of specific nutrients, oxygen level, temperature, pH and osmotic conditions (VESTER et al., 2014).

Cultivation methods have been improved to increase the proportion of culturable microorganisms, although culture-independent approaches such as metagenomics (gDNA or cDNA) using next-generation sequencing (NGS) technologies with the 16S rRNA genes of bacteria from environmental samples allow the identification of numerous new bacterial phyla (VESTER et al., 2014; CHEN et al., 2019). However, the use of PCR for amplifying the 16S rRNA genes have some limitations, such as the amount and the quality of the DNA as organisms with abundance below 0.1% will hardly be detected (HOBEL et al., 2004). Direct extraction of DNA from environmental samples involves the *in-situ* lysis of cells within the sample matrix and DNA extraction that is typically higher amount but more fragmented DNA, whereas indirect sampling involves the isolation of cells from the sample matrix before *ex-situ* lysis and the result is lower amount but with larger DNA fragments (VESTER et al., 2014).

Conventional agriculture has resulted in intensification of cropping practices and the use of big amounts of chemical pesticides and fertilizers that damage the environment and affect humans negatively (DONLEY, 2019; RAY et al., 2020). According to Donley (2019) the largest agricultural producers and exporters such as Brazil, United States, European Union, and China are also the world’s largest pesticide users. Although chemical pesticides are indispensable for high-quality crops, they cause a rapid emergence and evolution of resistant pathogens (FISCHER et al., 2018). Therefore, there is a need to embrace agricultural practices that do not depend on greater use of chemical pesticides and fertilizers to meet the growing demand for food.

The soil and plant roots are inhabited by a plethora of beneficial microorganisms forming a complex community - the microbiome, that can offer benefits to plants, including plant growth promotion, nutrient use efficiency, and control of pests and pathogens (BULGARELLI et al., 2015; LUNDBERG et al., 2012; BACKER et al., 2018; RAY et al., 2020). There is a need to explore the functional potential of beneficial microorganisms associated with soil in order to produce healthy crops (RAY et al., 2020). Plant growth-promoting microorganisms play a critical role in growth and improvement of plant resistance to biotic pathogens and tolerance to abiotic stresses. Also, they can play crucial roles in adhering and desorbing inorganic nutrients, as well as breaking down organic residues and incorporating them into the soil (RAY et al., 2020). Plant growth promoting bacteria benefit plants by different mechanisms (Table 1).

Table 1. Microorganisms and their respective plant growth promotion (PGP) activity.
N: nitrogen; endo: endogenous; Fe: iron; Zn: zinc; K: potassium; P: phosphorus.

Microorganism	PGP activity	Reference
Microbacterium sp.	N endo, K, Zn	Reid et al., 2021
Arthrobacter sp.	N endo, P, K, Zn	Reid et al., 2021
Pseudoarthrobacter sp.	N endo, P, Zn	Reid et al., 2021
Streptomyces sp.	N endo, Fe	Reid et al., 2021
Bacillus sp.	N endo, P, K, Fe, Zn	Reid et al., 2021
Paenibacillus sp.	N endo, P, K, Fe	Reid et al., 2021
Bosea sp.	N endo, P, K	Reid et al., 2021
Burkholderia sp.	N endo, P, K	Reid et al., 2021; Istina et al.,2015
Paraburkholderia sp.	N endo, P, K	Reid et al., 2021
Brevundimonas sp.	N endo, Zn	Reid et al., 2021
Caulobacter sp.	N endo, P, K	Reid et al., 2021
Acidovorax sp.	N endo, K, Fe, Zn	Reid et al., 2021
Variovorax sp.	N endo, P, K, Fe, Zn	Reid et al., 2021
Massilia sp.	N endo	Reid et al., 2021
Pseudomonas sp.	N endo, P, K, Fe, Zn; production of phytohormones and siderophores;	Reid et al., 2021
Allorhizobium sp.	N endo, P, K, Fe, Zn	Reid et al., 2021
Kaistia sp.	N endo	Reid et al., 2021
Mesorhizobium sp.	N endo, P, Zn	Reid et al., 2021
Luteibacter sp.	N endo	Reid et al., 2021
Rhodanobacter sp.	N endo	Reid et al., 2021
Sphingomonas sp.	N endo	Reid et al., 2021
Lysobacter sp.	N endo, P, K, Zn	Reid et al., 2021
Stenotrophomonas sp.	N endo, Fe, Zn	Reid et al., 2021
Azotobacter sp.	P	Kumar et al., 2014

Microorganism	PGP activity	Reference
Leifsonia sp.	N endo, K	Reid et al., 2021
Chryseobacterium sp.	K	Chen et al., 2006
Pseudomonas trivalis	K	Arwidsson et al., 2010
Enterobacter sp.	K	Shahid et al., 2012
Serratia marcescens	K	Chen et al., 2006
Agrobacterium sp.	P	Babalola and Glick, 2012
Arpergillus sp.	P, siderophores production; k;	Doilom et al., 2020; Srinivasan, et al., 2012; Haas et al., 2008; Kasana et al.,2017
Talaromyces sp.	P	Doilom et al., 2020
Gongronella sp.	P	Doilom et al., 2020
Penicillium sp.	P	Doilom et al., 2020
Trichoderma sp.	P, siderophores production	Bononi et al., 2020; Gloshi et al., 2018
Rhizoctonia sp.	P	Jacobs et al., 2002
Arthrobotrys sp.	P	Duponnois et al., 2006
Alternaria sp.	siderophores production;	Haas et al., 2008
Cochliobolus sp.	siderophores production;	Haas et al., 2008
Epicghloe sp.	siderophores production;	Haas et al., 2008
Fusarium sp.	siderophores production;	Haas et al., 2008
Neurospora sp.	siderophores production;	Haas et al., 2008
Magnaporte sp.	siderophores production;	Haas et al., 2008
Fomitopsis sp.	K;	Kasana et al.,2017
Aspergillus candidus	K	Shin et al., 2006
Aspergillus flavus	K	Shin et al., 2006
Trichoderma sp.	K	Akintokun et al., 2007
Fusarium oxysporium	K	Akintokun et al., 2007

Soil nitrogen levels depends on organic matter degradation, synthetic fertilizer applications and biological nitrogen fixation (BNF) via nitrogenase enzyme activity

(MAHMUD et al., 2020). Nitrogen is a key component in protein synthesis, which is essential to life. Even though the soil contains nitrogen naturally, cultivated plants have a dependence on nitrogenous fertilizers and it represents the most expensive cost in crop production due to the high price of nitrogen (GOOD; SHRAWAT; MUENCH, 2004). Plant growth promoting bacteria (PGPB) can enhance nitrogen availability to plants indirectly by increasing the root surface and morphology and directly through mineralization, nitrification and fixation, converting complex nitrogen forms into simple ones that plants can use (MASCLAUX-DAUBRESSE, 2010; CALVO et al., 2019; MAHMUD et al., 2020). The symbiotic relationship between soil bacteria (diazotrophic bacteria) such as *Rhizobium, Bradyrhizobium, Mesorhizobium, Sinorhizobium* and leguminous plant roots originates a new differentiated special organ named nodule. The bacteria living in nodules fix atmospheric nitrogen gas by biological reduction through the action of the protein complex called nitrogenase, composed of enzymes dinitrogenase and dinitrogenase reductase with metal co-factors of Mo (molybdenum) and reactive compound NH3 (ammonia). The ammonia is assimilated by plants via glutamine synthase to form glutamine, whereas the bacteria derive plant carbohydrates, mainly malate for food and an energy source for nitrogen fixation. The establishment of this beneficial interaction depends on factors such as plant species, genotype, bacterial strains and environmental factors (MAHMUD et al., 2020; THIEBAUT et al., 2022). Nearly 50% of the total nitrogen in crop fields is the contribution of BNF by diazotrophic bacteria (RAMÍREZ-PUEBLA et al., 2019). There are also non-nodular diazotrophic bacteria that live inside plant tissues and were identified in association with non-legume plants, including the genera *Azospirillum, Azorhizobium, Azoarcus, Bacillus, Burkholderia, Citrobacter, Enterobacter, Gluconacetobacter, Herbaspirillum, Klebsiella* and *Pseudomonas* (SANTI et al., 2013). These non-nodular diazotrophic bacteria also are considered plant growth–promoting rhizobacteria (PGPR) as they produce growth regulators and are involved in the modulation of phytohormone and defense responses as well as production of antioxidants, osmotic adjustment and resistance against biotic and tolerance to abiotic stresses (THIEBAUT et al., 2022).

Phosphorus (P) is a structural component of nucleic acids and one of the major macronutrients for plant growth. It is essential in photosynthesis, respiration, signal transduction, biosynthesis of several cell components and it is directly involved in shoot and root development and contribute to disease resistance (AHEMAD et al., 2009; RAZAQ; ZHANG; SHEN, 2017). Phosphorus is abundant in soil and according to Tiessen (2008), the concentration on Earth is around 1200 mg P kg^{-1}. However, most soils over the world are P-

deficient, considering that less than 5% of the total P soil available to plants (MOLLA et al., 1984; ALORI et al., 2017). The use of fertilizers, sometimes exaggerated, results in negative effects on composition and functions of rhizosphere microorganisms (TAO et al., 2008; REID et al., 2021). For example, large amounts of P accumulated in Brazilian soils through fertilization was demonstrated by Pavinato et al. (2020). One way to make soluble phosphorus available to plants is through the production of organic acid by phosphate solubilizing microorganisms, as well as through the processes of acidification, chelation and exchange reactions (BEHERA et al., 2016). Phosphate solubilizing microorganisms can help in minimizing the excessive use of fertilizers and enhance soil fertility (ADHIKARI; PANDEY, 2019). Bacterial strains are screened for phosphate hydrolysation by the production of phosphatases on different phosphate sources. The phosphate solubilizing efficiency of microorganisms can be easily screened based on visual observation of a clear zone around the colonies, which is an indicator of phosphate hydrolysation (PIKOVSKAYA, 1948). Acid phosphatase enzymes contribute in the process of hydrolysation of phosphate by catalyzing the hydrolysis of phosphatic compounds. These enzymes catalyse the hydrolysis of a wide variety of phosphomonoesters and catalyze transphosphorylation reactions by transferring a phosphoryl group to alcohol in the presence of certain phosphate acceptors (BEHERA et al., 2017). Phosphatase production varies with the substrates used in the following order calcium > iron > aluminium phosphate in acidic medium and iron > calcium > aluminium phosphate in alkaline medium. Also, phosphatase production is greatly influenced by growth parameters such as pH, temperature, agitation velocity (BEHERA et al., 2017; ADHIKARI; PANDEY, 2019). Therefore, it is crucial to assess the optimal conditions of these parameters under laboratory conditions.

Potassium (K) is the third most important macronutrient after N and P and essential for all organisms, including plants. The function of K in plants includes regulation of turgor, charge balance, movement and protein synthesis (LEIGH; WYN-JONES, 1984). It is also involved in growth, development and metabolism, and K deficiency results in poor development of roots and reduced plant growth and yield (LEIGH; WYN-JONES, 1984). Potassium is an abundant element in soil, however, less than 2% of K in soil can be directly absorbed by plants because most of K is fixed in silicate minerals, especially those under intensive agriculture (ZHANG; KONG, 2014). One of the natural ways in which K can be available to plants is through the hydrolysation and release of K from insoluble minerals by K-solubilizing bacteria (KSB). Among the soil bacterial, *B. mucilaginosus, B. edaphicus* and *B. circulans* have been labeled

as effective K solubilisers (MEENA et al., 2015). These bacteria release K by excreting organic acids such as citric, oxalic, tartaric, succinic, and α-ketogluconic acids, because these acids dissolve rock K or chelate the primary mineral ions as silicon to release K in the soil solution (PRIYADHARSINI; MUTHUKUMAR, 2016). Also, the release of K is possible due to chelation, decomposition of organic matter and crop residues, exchange reactions and complexation (MEENA et al., 2015; ETESAMI; EMAMI; ALIKHANI, 2017; SARIKHANI et al., 2018; SATTAR et al., 2019). Plant growth-promoting rhizobacteria also influence the activity of mycorrhizal fungi symbiosis resulting in improved K uptake by plants. These interactions among beneficial microorganisms are important in sustainable agriculture as it relies on biological processes to achieve improve plant growth and yield (PRIYADHARSINI; MUTHUKUMAR, 2016).

Iron (Fe) is an important cofactor for several enzymes that plays a vital role in cellular reactions and it is involved in many biological processes such as tricarboxylic acid cycle, electron transfer chain, oxidative phosphorylation, nitrogen fixation, biosynthesis of aromatic compounds and generation of metabolic products including porphyrins, toxins, antibiotics, pigments and siderophores (FARDEAU et al., 2011). Bacteria can acquire Fe through the secretion of siderophores, which are secondary metabolites of low-weight, high-affinity iron chelating molecules produced in response to iron deficiency. They are produced mainly in environments where the ferric form of iron is insoluble and inaccessible at physiological pH (7.35–7.40) and iron is delivered to cells via specific receptors (SAHA et al., 2015; KRAMER et al., 2020). Bacteria with siderophore-producing activity have been reported to promote the growth of several plant species and increase their yield by enhancing the Fe uptake. They also act as biocontrol agents against pathogens because siderophores can tightly bind iron and reduce its availability to pathogens, and enhance bioremediation of heavy metals (SAHA et al., 2015). Depending on the oxygen ligands for Fe, there are three siderophore categories: hydroxamates, catecholates and carboxylates. Hydroxamate is the most common group of siderophores found in nature that are produced by microorganisms including bacteria and fungi, for instance *Pseudomonas fluorescens* secretes ferribactin, and *Trichoderma* spp. and *Fusarium* spp. produce coprogens and fusigen. The catecholate group supplies two oxygen atoms for chelation with iron in order to form a hexadentate octahedral complex and is mostly produced by certain bacteria such as *Escherichia coli*, *Salmonella typhimurium,* and *Klebsiella pneumoniae.* The carboxylate type of siderophores binds to iron through carboxyl and hydroxyl groups and it is produced mostly by bacteria like *Rhizobium* and *Staphylococcus* and fungi such as Mucorales

(SAHA et al., 2015). Siderophores act in the biological control of certain phytopathogens such as *Fusarium oxysporum*, responsible for the *Fusarium* wilt of pepper, which is controlled by *Pseudomonas* (YU et al., 2011). *Pseudomonas* species enhance plant growth by producing pyoverdine siderophores (GAMALERO; GLICK, 2011). Moreover, siderophores can be useful in heavy metal remediation of contaminated soils by the rapid accumulation of heavy metals due to the indiscriminate application of chemical fertilizers, pesticides and wastewater irrigation (RAJKUMAR et al., 2010; HESSE et al., 2018).

In view of the demand for more biological products in the agricultural market, new studies need to be conducted in this field. The objective of this thesis was to explore the functional potential of bacteria and fungi, from isolation to practical uses, as beneficial organisms. In the first chapter, endophytic fungi were isolated and selected to control post-harvest gray mold losses caused by *B. cinerea* in packaged strawberries. In the second chapter, bacterial strains from rhizosphere and soil were isolated to assess the functional plant growth promotion traits including their capacity to solubilise nutrients and antifungal activity potential.

2 SCOPE OF THIS THESIS

This thesis consists of a general introduction succeeded by two experimental studies presented in the form of scientific manuscripts written in English and a patent application written in Portuguese. This thesis is composed of studies on genetic and functional diversity of beneficial microorganisms spanning rhizobacteria and endophytic fungi. The first chapter deals with endophytic fungi in the control of post-harvest rot disease in strawberries was done in Brazil. The model product developed may be applied on other fruits and vegetables as long as they are in air tight environment. The second was carried out in England at Rothamsted Research and focused on rhizobacteria from different crops and soil. Their potential to solubilise nutrients and control fusarium was evaluated.

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SECOND PART - MANUSCRIPTS AND PATENT

MANUSCRIPT 1

Manuscript prepared according to the standard of Journal of BioControl

Identification and characterization of *Induratia* strains and their biocontrol potential

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ABSTRACT: The increase in biological products available on the market is a worldwide reality. The genus *Induratia* is well known to produce volatile organic compounds with antimicrobial activity. In this study, we isolated six strains of the endophytic fungus *Induratia* sp. Phylogenetic relationships based on ITS, *Tub*2 and *rpb*2 DNA sequence revealed close affinities with *I. coffeana* and a novel *Induratia* species. These *Induratia* strains were able to produce volatile organic compounds with antimicrobial activity against the following tested plant pathogens: *Phytophthora* sp., *Rhizoctonia solani*, *Botrytis cinerea*, *Colletotrichum lindemuthianum, Sclerotinia sclerotiorum* and *Fusarium verticillioides.* The *Induratia* strains produced 17 different volatile organic compounds as determined by the solid-phase microextraction followed by gas chromatography-mass spectrometry method. Among the strains tested *in vitro, I. coffeana* FTB have shown the best results against most pathogens and was tested *in vivo* for its capacity to control *B. cinerea* on strawberry fruits stored in hermetically closed boxes, where it was shown to increase the shelf life up to 6 days more. This novel *Induratia* strain showed potential to be further developed into a commercial product and a patent was deposited under the number BR 10 2021 001666 3.

KEY WORDS: Gray mold, Volatile organic compounds, Biological product, Endophytic fungi, Phylogeny.

1 INTRODUCTION

Microorganisms play a crucial role in agriculture. Even though some of them are pathogenic to plants, others can control pests and plant diseases and they have been showing great potential (Hyde *et al.* 2019, Samarakoon *et al.* 2020). Due their potential, bioproducts available in the market are increasing every year.

The capacity of several fungi to produce organic volatile compounds with antibiotic action has been recognized in many studies in the last years (Morath *et al.* 2012; Kaddes *et al.* 2019). The endophytic genus *Induratia* was introduced in 2001 (Worapong *et al.* 2001) and they are promising in biocontrol due to their antibiotic volatiles that are able to inhibit a large variety of plant pathogens and notably efficient in controlling post-harvest diseases (Strobel 2011). A recent study showed that *Induratia* sp. have similarities with the Xylarialean genus *Emarcea*. By using polyphasic taxonomic the genus *Muscodor* was transferred to *Induratia* and their sexual state was reported (Samarakoon *et al.* 2020).

The control of diseases in post-harvest using fungicides is difficult because these end products are directly consumed. Among the post-harvest diseases, grey mold caused by *Botrytis cinerea* occurs in many succulent fruits, including strawberries (Bautista-Baños *et al.* 2003). According to Anvisa (2017), strawberry fruits contain high levels of toxic residues that result from the inadequate use of pesticides in the field. Even these high concentrations of pesticides are not enough to inhibit *B. cinerea* in post-harvest. Strawberries are marketed in packages wrapped with a plastic film that favors the growth and development of post-harvest fungi and for this reason its shelf-life is estimated in approximately five days (Henrique and Cereda, 1999). This sealed environment also favors the application of *Induratia* species as biofumigants. Other authors have successfully demonstrated the activity of *Induratia* species against *B. cinerea* in grapes (Gabler *et al.* 2006; Mercier *et al.* 2010).

Developing products with a fungus that does not produce spores easily is a challenge, but commercial products formulated with *Induratia* for usage in agriculture are already a reality. Products such as ARABESQUE™, ANDANTE™, GLISSADE™, all based on *M. albus* strain QST 20799 are available for different purposes. However, there are more challenges to be solved to expand its use (Strobel 2011). Among these, formulation, storage and dosage are essential to lead to successful results (Kaddes *et al.* 2019). Understanding the mechanisms of action will help the safe utilization of *Induratia* in disease control (Strobel 2011; Kaddes *et al.* 2019).

In this scenario, we report the identification, antimicrobial activity, analysis of volatiles and a formulation based on *Induratia* sp. that effectively controls post-harvest gray mold caused by *B. cinerea* in packaged strawberries.

2 MATERIAL AND METHODS

Microorganisms and culture conditions

The pathogens *Rhizoctonia solani, Botrytis cinerea, Colletotrichum lindemuthianum, Sclerotinia sclerotiorum, Fusarium verticillioides, Rhizopus stolonifer* and *Phytophthora* sp. were obtained from the Coleção Micológica of Lavras (CML) at the Federal University of Lavras and *Pectobacterium carotovorum* was obtained from the working collection of hte Bacteriology laboratory at the Federal University of Lavras. These microorganisms were routinely grown PDA at 25 °C.

According to Worapong (2002) and Zhang (2010), strains of the genus *Induratia* do not inhibit the development of other strains of the same genus. Based on that, *Induratia* sp. FTB previously isolated by our research group was used to select new strains of this genus from leaves, stems and petioles of the parasitic plant *Struthanthus flexicaulis* collected in Lavras, Minas Gerais province. The tissues were washed with neutral detergent and sterile distilled water and then dried at room temperature. The samples were cut in fragments of 1 cm, surface-sterilized with 70% alcohol for 30 s, 2.5% hypochlorite for 3 min, 70% alcohol for 30 s and washed three times with sterilized distilled water. These surface-sterilized fragments of *S. flexicaulis* were plated on plates containing stain FTB of *Induratia* grown for 3 days on MEA (Malt extract and Agar). Petri dishes were incubated at 25 °C and examined daily for 15 days. Once fungus hyphae were observed, they were to fresh MEA plates to obtain pure cultures. The purified cultures were stored in mineral oil, glycerol 40% and distilled water, separately.

Characterization of *Induratia* strains

To determine the growth at different temperatures, fresh mycelial plugs (5 days of growth on MEA) with 5 mm diameter of the *Induratia* strains (Table 1) were placed at the center of Petri dishes (90 mm diameter) containing MEA. The plates were incubated in 12-h photoperiod at 15, 20, 23, 25, 28, 30, 35 ºC and after incubation for 14 days, radial mycelial growth was assessed.

Table 1. *Induratia* strains from *S. flexicaulis* used in this study.

Strain	Species	Tissue
P3 1T	*Induratia sp. new*	Petiole
C3 5T	*Induratia coffeana*	Stem
FTB	*Induratia coffeana*	Leaf
P2 2T	*Induratia coffeana*	Petiole
P2 1T	*Induratia coffeana*	Petiole
F1 5T	*Induratia coffeana*	Leaf

Fungal DNA extraction and phylogenetic analysis

Genomic DNA was extracted according to modified CTAB method (Murray & Thompson, 1980). A ten-day-old culture of *Induratia* strains grown on malt extract (ME) was used. Approximately 0.1 g of mycelium was ground using liquid nitrogen and 100 mg of polyvinylpyrrolidone. The macerate was transferred to a 1.5 mL microcentrifuge tube and 750 µL of 2% CTAB buffer and 7.5 µL of β-mercaptoethanol (0.2% final concentration) were added. The samples were homogenized in a vortex for 2 min. Then, the tubes were incubated in a water bath at 65 °C for 15 min. An aliquot of 500 µL of chloroform: isoamyl alcohol, 24: 1 was added and shaken in a vortex for 1 min. After this, centrifuged for 10 min at 13.000 rpm. The supernatant was recovered and transferred to a new tube. An aliquot of 600 µL of ice-cold isopropanol and 300 µL 3M sodium acetate, pH 5.2 were added and then shaken by inversion and left at -20 °C for 10 min. After centrifugation at 13,000 rpm for 10 min, the supernatant was discarded and 100 µL of 70% ice-cold ethanol was added and shaken by inversion. The samples were centrifuged at 13,000 rpm for 10 min again and after this, the supernatant was discarded and the pellet dried in an oven at 60 °C until the ethanol residues were eliminated. The precipitate was resuspended in 100µL of 1x TE (Tris-EDTA) and 5µL of RNAse A (0.5mg. mL^{-1} final volume) was added and incubated in a water bath at 37 °C for 15 min. After adding 10 µL of 3M sodium acetate, pH 5.2 and 250 µL of ice-cold absolute ethanol, the homogenate was shaken by inversion and incubated in a freezer for 15 min and then centrifuged at 10.000 rpm for 10 min. The supernatant was discarded and 500 µL of 70% ice-cold ethanol were added and shaken by inversion. After centrifuging at 10.000 rpm for 5min, the supernatant was carefully discarded and the pellet dried in an oven at 60 °C until the ethanol residues were

eliminated. Finally, 50 μL of 1x TE were added and left overnight in the refrigerator to resuspend the DNA gradually.

Three fragments were amplified by polymerase chain reaction (PCR): The Internal Transcribed Spacer (ITS) of the rDNA with primers ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (CCTCCGCTTATTGATATGC) (White *et al.* 1990), the RNA polymerase second largest subunit (*rpb2*) by using the primers 5f (GAYGAYMGWGATCAYTTYGG) and 7cr (CCCATRGCTTGYTTRCCCAT) (Liu *et al.* 1999), and *β-tubulin* (*Tub2*) was amplified using the primers T1 AACATGCGTGAGATTGTAAGT and T22 (TCTGGATGTTGTTGGGAATCC) (O'Donnell and Cigelnik 1997). The reaction was carried out in a 50 μL containing from 50 to 100 ng of fungal DNA. The mixture contained 2 μL of the DNA template, 1.4 μL of each primer, 5 μL of $MgCl_2$, 1 μL of dNTP, 0.25 μL GoTaq polymerase, 10 μL of Buffer and 28.95 μL of ultra-pure water and PCR was performed in a Multigene^{TM} OptiMax Thermal Cycler. For ITS the polymerase chain reaction conditions were: initial denaturing at 94 °C for 5 min followed by 35 cycles of denaturing at 94 °C for 30 s, annealing at 54 °C for 1 min and extension at 72 °C for 1 min and 30 s, followed by a 5 min at 72 °C for final extension. For *RPB2* the PCR conditions were: initial denaturing at 95 °C for 5 min followed by 30 cycles of denaturing at 95 °C for 1 min, annealing at 50 °C for 2 min and extension at 72°C for 2 min, followed by a 10 min at 72 °C for final extension. For *TUB2* the PCR conditions were: 95 °C for 2 min; 30 cycles of 95 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min; and a final extension for 10 min at 72 °C. The amplified fragment was visualized in 1% agarose gel electrophoresis. The PCR products were purified and sequenced by the sanger method at Macrogen, Korea.

Consensus sequences were obtained from a bidirectional DNA sequence using the SeqAssem ver. 07/2008 (Hepperle 2004). Sequences from the type and reference strains of *Induratia* species available in GenBank were added to the analyses (Table 2). Multiple sequence alignments were performed using MUSCLE, as implemented in MEGA X (Kumar *et al.* 2018). Phylogenetic analyses of each gene separately and combined data set (ITS-*rpb2*-*Tub2*) were performed with maximum parsimony (MP) and maximum likelihood (ML) using Mega X with 1,000 bootstrap replications. According to Abadi (2019) choosing one criterion over another for the most suitable model for phylogeny is not crucial step, even so Table 3 shows the most suitable substitution models inferred using MEGA X, and parameters of matrices used to generate the phylogenies. *Emarcea castanopsidicola* and *E. eucalyptigena* were used as outgroup taxa.

Table 2. *Induratia* strains used in phylogeny with GenBank accessions.

Species	Strains code[a]	GenBank accession no.[b]			References
		ITS	***rpb2***	***Tub2***	
I. alba	9–6	HM034857	KC243321	HM034844	Zhang et al. (2010)
I. brasiliensis	LGMF 1256T	KY924494	MF510171	N/A	Pena et al. (2019)
I. camphorae	NFCCI 3236T	KC481681	N/A	N/A	Meshram et al. (2017)
I. cinnanomi	BCC 38842T	GQ848369	N/A	N/A	Suwannarach et al. (2010)
I. coffeana	COAD 1900	KP862879	KP862880	N/A	Hongsanan et al. (2015)
I.coffeana	COAD 1842T	KM514680	KP862881	N/A	Hongsanan et al. (2015)
I. coffeana	CML 4011	MN658676	MN689581	MN689589	Guimarães et al. (2021)
I. coffeana	CML 4014	MN658683	MN746440	MN746443	Guimarães et al. (2021)
I. coffeana	CML 4016	MN658684	MN746441	MN746444	Guimarães et al. (2021)
I. coffeana	CML 4019	MN658679	MN689586	MN689594	Guimarães et al. (2021)
I. coffeana	N-L-7	MG309792	N/A	N/A	Mao et al. (2018)
I. coffeana	Y-L-43	MG309793	N/A	N/A	Mao et al. (2018)
I.coffeana	W-S-35	MG309794	N/A	N/A	Mao et al. (2018)
I.nduratia crispans	MONT 2347T	EU195297	N/A	N/A	Mitchell et al. (2008)
I. darjeelingensis	NFCCI 3095T	JQ409997	N/A	N/A	Saxena et al. (2014)
I. equiseti	JCM 18233T	JX089322	N/A	N/A	Suwannarach et al. (2013)
I. fengyangensis	CGMCC 2862T	HM034856	HM034849	HM034843	Zhang et al. (2010)
I.ghoomensis	NFCCI 3234T	KF537625	N/A	N/A	Meshram et al. (2015)
I. heveae	RTM5–IV3T	KF850712	N/A	N/A	Siri-Udom et al. (2015)
I. indica	NFCCI 3235T	KF537626	N/A	N/A	Meshram et al. (2015)
I. kashayum	NFCCI 2947T	KC481680	N/A	N/A	Meshram et al. (2013)
I. musae	JCM 18230T	JX089323	N/A	N/A	Suwannarach et al. (2013)
I. oryzae	JCM 18231T	JX089321	N/A	N/A	Suwannarach et al. (2013)
I. rosea	MONT 2098T	AH010859	N/A	N/A	Worapong et al. (2002)
Induratia sp.	CML 4013	MN658681	MN689583	MN689591	Guimarães et al. (2021)
Induratia sp.	CML 4015	MN658682	MN689584	MN689592	Guimarães et al. (2021)
I. strobelii	NFCCI 2907T	JQ409999	N/A	N/A	Meshram et al. (2014)
I. suthepensis	JCM 18232T	JN558830	N/A	N/A	Suwannarach et al. (2013)
I. suturae	MSUB 2380T	JF938595	N/A	N/A	Kudalkar et al. (2012)

I. thailandica	MFLUCC 17-2669T	MK762707	MK791283	MK776960	Samarakoon et al. (2020)
I. tigerensis	NFCCI 3172T	JQ409998	N/A	N/A	Saxena et al. (2015)
I. vitigena	MONT P-15T	AY100022	N/A	N/A	Daisy et al. (2002)
I. yucatanensis	MEXU 25511T	FJ917287	N/A	N/A	González et al. (2009)
I. yunnanensis	CGMCC 3.18908T	MG866046	MG866059	MG866066	Chen et al. (2019)
I. ziziphi	MFLUCC 17-2662T	MK762705	MK791281	MK776958	Samarakoon et al. (2020)
Emarcea castanopsidicola	CBS 117105T	AY603496	MK791285	MK776962	Duong et al. (2004) Samarakoon et al. (2020)
Emarcea eucalyptigena	CBS 139908T	KR476733	MK791286	MK776963	Duong et al. (2004) Samarakoon et al. (2020)

[a]Abbreviations: *BCC* BIOTEC Culture Collection, Thailand, *BISH* Herbarium Pacificum (BISH), Bishop Museum, Hawaii, *CBS* Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, *CGMCC* China General Microbiological Culture Collection Center, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China, *CML* Coleção Micológica de Lavras, Departamento de Fitopatologia, Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil, *COAD* Culture Collection of the Universidade Federal de Viçosa, Brazil, *CBS* CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands, *JCM* Japan Collection of Microorganisms, Japan, *JDR* Herbarium of Jack D. Rogers, *LGMF* LabGeM Culture Collection, Federal University of Parana (UFPR), Curitiba, Brazil, *MEXU* Herbario Nacional, *MFLUCC* Mae Fah Luang University Culture Collection, Chiang Rai, Thailand, *MONT* Montana State University Herbarium, Plant Sciences and Plant Pathology, Montana State University, Bozeman, Montana, USA. Types strains are indicated with [T].

[b] Sequences unavailable in GenBank are indicated by "N/A".

Table 3. Overview of the regions used for phylogenetic analyses (including outgroup) and best nucleotide substitution models.

Parameters of matrices used to generate phylogenies												
ITS				***rpb2***				***Tub2***				**Combined**
Length bp	**Variable sites**	**Informative sites**	**Substitution model**	**Length bp**	**Variable sites**	**Informative sites**	**Substitution model**	**Length bp**	**Variable sites**	**Informative sites**	**Substitution model**	**Substitution model**
539	203	88	K2+G	893	361	286	T92+G	1034	386	268	K2+G	TN93+G

Abbreviation nucleotide substitution models: K2 = Kimura-2-paramters; T92=Tamura 3-parameter; TN93=Tamura-Nei. Rate variation among sites: G=Gamma distributed rate variation among sites.

Antimicrobial activity

To evaluate the antagonistic activity of all *Induratia* strains, six plant pathogens were used. Mycelial growth of the pathogens was determined in Petri plates with two compartments in the following manner: both compartments were filled with MEA to test *R. solani, B. cinerea, C. lindemuthianum, S. sclerotiorum* and *F. verticillioides*. For *Phytophthora* sp. one of the compartments was filled with carrot sucrose agar (CSA). A mycelial plug of 5 mm diameter from the colony margin of an actively growing *Induratia* culture was placed on MEA in one compartment of the Petri dish that was wrapped with five layers of plastic film and incubated at 25 °C. After two days of incubation, a mycelial plug of each of the pathogens was placed at the other compartment. Controls were the pathogens grown without *Induratia* strains. The diameter of the colonies was measured when the mycelium in the control plates reached the edge of the Petri dish. To assess the viability of the pathogens, the plugs used in the treatments were transferred to fresh MEA plates and incubated at 25 °C for 15 days. These assays were installed in a completely random design with three replicates.

Biocontrol assays with *Induratia* strain FTB

All experiments described below were done with strain FTB, which was selected in the assays described above. To select the best substrate for growth and VOCs production, the isolate was grown on the following media: 1) malt extract agar (MEA) composed of 20 g of malt extract (Himedia) and 20 g of agar per liter; 2) potato dextrose agar (PDA) (Himedia) at 39 g per liter; 3) rice; 4) maize; 5) millet medium; the last three composed of 166.7 g of grinded grain and 20 g of agar per liter. A 5-mm mycelial disc of isolate FTB was transferred to one compartment of plate containing each of the media described above and three days later a mycelial disc of *B. cinerea* was placed on the other section of the Petri plate filled with PDA. The growth of *B. cinerea* was evaluated five days after transference and incubation at 25 °C with 12 h photoperiod. Millet was chosen as the growth substrate for strain FTB and its chemical composition is shown in Table 4. Millet was used in two ways: 1) millet medium prepared with 166.7 g of grinded millet, 20 g of agar, and distilled water to one liter; 2) 100 g of whole millet grains and 100 mL of distilled water were placed in transparent plastic bags measuring 30 x 40 cm. Both millet medium and whole millet grains were autoclaved for 20 min

at 120 °C. The bags containing whole millet grains were seeded with 15 mycelial discs of strain FTB and incubated for 21 days at 25 °C. Whole grains colonized by strain FTB were used to perform assays to study mycelial growth and germination of *B. cinerea* conidia. Mycelial growth and conidia germination of *B. cinerea* was determined on Petri plated divided into two sections in the following manner: one of the sections was filled with 10 mL of millet medium or 8 g of whole millet grains colonized by strain FTB for 21 days and the other section of the Petri plate was filled with 10 mL of PDA. Mycelial discs of strain FTB were only transferred to the millet medium and mycelial discs of *B. cinerea* were only transferred to the PDA medium. Plates with millet medium received mycelial discs of strain FTB and were incubated for 0, 2, 4 and 6 days at 5, 10, 15, 20, 25 and 30 ºC. Following the incubation, mycelial discs of *B. cinerea* were placed on the PDA section of the Petri plates and incubated for three days in the different temperatures mentioned above and 12-h photoperiod. The assay was done in a factorial scheme with four incubation times, six temperatures and three replicates. In another assay, Petri plates were filled with whole grains colonized with strain FTB for 21 days in one section and the PDA section received mycelial discs of *B. cinerea* on the same day and were incubated at 5, 10, 15, 20, 25 and 30 °C. A similar assay was conducted to evaluate conidia germination, but instead of PDA, water-agar 2% was used in the other section that received 100 µL of a suspension containing $2x10^5$ *B. cinerea* conidia/mL. Petri plates were incubated at 5, 10, 15, 20, 25 and 30 ºC as described above and conidia germination was evaluated 48 h after spreading the suspension. For all experiments described above the controls consisted of plates containing *B. cinerea* only. These assays were installed in a completely random design with three replicates.

Antimicrobial activity of strain FTB in millet grains

To test the inhibition of *R. solani, Rhizopus stolonifera, B. cinerea, C. lindemuthianum, S. sclerotiorum, F. verticillioides, Phytophthora* sp. and *P. carotovorum,* Petri dishes with two compartments were used. In one compartment of the Petri dish, 4g of millet colonized with *Induratia* strain FTB (21 days of growth) and the other compartment contained 10 mL of the appropriate culture medium (PDA – for most pathogens and CSA for *Phytophthora* sp.). The control was the pathogen at one compartment and millet grains without *Induratia* FTB at the other. The chemical composition of millet grains used in this study is shown in Table 4. Mycelial growth of the pathogens was evaluated when the controls reached the edge of the plates. The mycelial disks were transferred to separate plates containing fresh culture medium and observed for up to 30 days to verify their viability.

Table 4. Chemical composition of millet grains used in this study.

Macronutrientes (g.Kg^{-1})					
N	**P**	**K**	**CA**	**Mg**	**S**
30.33±0.81	6.20±0.95	4.93±0.81	0.77±0.15	1.93±0.15	0.93±0.06
Micronutrientes (mg.Kg^{-1})					
Zn	**Cu**	**Fe**	**Mn**	**B**	
53.30±1.11	6.47±0.70	110.40±5.43	15.10±0.61	6.83±1.59	
Total proteins (g.Kg^{-1})					
191.58±6.78					

Control of *Botrytis cinerea* in closed strawberries boxes

Strain FTB was chosen to assess the control of *B. cinerea* in closed strawberry boxes. Sachets measuring 7cm x 4 cm, with one face with plastic and the other with paper were filled with 40 g of millet grains colonized for 21 days at 25 ºC by strain FTB. The sachets were placed with the plastic face upwards on the bottom of standard commercial plastic trays containing four strawberries. Treatments were: T1 (treatment 1: millet grains colonized by FTB); C1 (control 1: millet without FTB); C2 (control 2: without millet nor FTB). The trays were incubated at 10, 15, 20 and 25 ºC. To determine the first day of evaluation, the fruits were visually inspected for any contamination or deterioration signals (Valenzuela et al., 2015). Assays were installed in a completely random design with four replicates containing four fruits per replicate.

To check the maximum time that *Induratia* FTB was capable of producing inhibitory volatiles, an *in vitro* test was carried out. Every 7 days, a sample containing 4g of millet colonized by *Induratia* FTB was placed in one half of the split plate and in the other half, a mycelium disk of *B. cinerea* was placed on PDA medium. Controls were millet grains without *Induratia* FTB at one side and *B. cinerea* at the other.

Identification of volatile organic compound

Volatiles produced by *Induratia* strains grown for 3 days at 25 ºC on MEA medium and millet placed inside “Supelco” tubes were extracted through solid phase microextraction

(SPME) and analyzed by gas chromatography coupled to a mass spectrometer (GC-MS Shimadzu QP 2010 Plus, Japan). Volatiles extracted from MEA medium and millet without strains served as controls. SPME extraction was operated in the headspace mode for VOCs extraction with the following parameters: fiber DVB/CAR/PDMS (Divinylbenzene, Carboxen, and Polydimethylsiloxane), extraction temperature of 55 °C, extraction time of 35 min and desorption time in the GC injector of 1 min. A GC-MS QP 2010 Ultra (Shimadzu, Japan) gas chromatograph coupled to a mass spectrometer equipped with a HP-5 column (5% phenyl-95% dimethylsiloxane) with dimensions 30 m × 0.25 mm × 0.25 μm was used for separation and identification of the volatiles produced. The injector temperature was 250 °C, the interface temperature 240 °C and the ion source temperature 200 °C. The injector was operated in splitless mode. The carrier gas was He 5.0 at 1.0 mL min. The temperature program of the GC oven was as follows: an increase from 40 °C to 160 °C at 3 min and then an increase to 240 °C at 10 °C min. Initial identification of the unknown volatiles produced by *Induratia* species was done as follow: the mass spectra of each peak in the chromatogram was extracted using the Automated Mass Spectral Deconvolution and Identification System (AMDIS) v.2.63 software. The identification of VOCs was performed by comparing the peak mass spectra in samples with spectra in the NIST (National Institute of Standards and Technology) Mass Spectral Library Search Program v. 1.7 (NIST, Washington, DC, USA) and by comparing the retention indices obtained experimentally (RI Exp.) with the retention rates in the literature (RI Lit.) (Nist 2013; Adams 2007). For comparing mass spectra, we considered only peaks with a spectra similarity greater than 80%. The experimental retention indices were obtained by the injection of a homologous series of alkanes. Peaks were identified in the chromatograms only when mass spectra data agreed with the retention indices.

3 RESULTS

Strain characteristics

The best growth temperature for the *Induratia* strains tested was 25 °C, but all of them grew in a range from 15 to 25 °C. The strains P3 1T and P2 2T could still grow at 30 °C, while the other four C3 5T, FTB 01, P2 1T and F1 5T did not, but none of them grew at 35 °C. Spores were never produced (Table 5). All strains were white and smooth after 14 days of growth at 25 ºC on MEA (Fig. 1).

Table 5. Colony diameter of *Induratia* strains at different temperatures.

Isolate	Colony diameter (cm)						
	15°C	20°C	23°C	25°C	28°C	30°C	35°C
P3 1T	1,4	2,6	2,6	3,9	2,5	1,6	0
C3 5T	1,5	2,4	3,3	3,6	1,8	0	0
FTB 01	2,4	3,2	4	4,5	0,8	0	0
P2 2T	1,7	2,4	2,4	3,6	2,1	0,1	0
P2 1T	2,3	2,4	2,4	3,4	2,1	0	0
F1 5T	1,9	3	3,3	3,4	1,7	0	0

Phylogenetic analyses

Phylogenetic analysis based on ITS showed that the six strains grouped into three clades. One of these clades corresponded to the species *I. coffeana* (C35T and FTB). The second clade, which is still unresolved, contained *I. coffeana* and the type strain of *I. yucatanensis* together with three strains from this study (P21T, P22T and P15T). The strain P31T grouped in a third clade, that contained the type strains of *Induratia equiseti*, *Induratia suturae, I. thailandica, I. vitigena, I. ziziphin*, and two *Induratia* sp. (Fig. 2). In the *rpb2* phylogeny, strains P21T, P22T and P15T were in the *I. coffeana* clade, and strain P31T was grouped with two undescribed *Induratia* species. (Fig. 23). In the *Tub2* phylogeny (Fig. 4) and in the combined tree (Fig. 5) strains P21T, P22T and P15T were close to *I. coffeana* strains. However, the there is no data for the type strain of *I. coffeana* and the subclades indicate possible new undescribed species in this clade. These two phylogenies indicate that strain P31T corresponds to an undescribed species (Figs. 3-5).

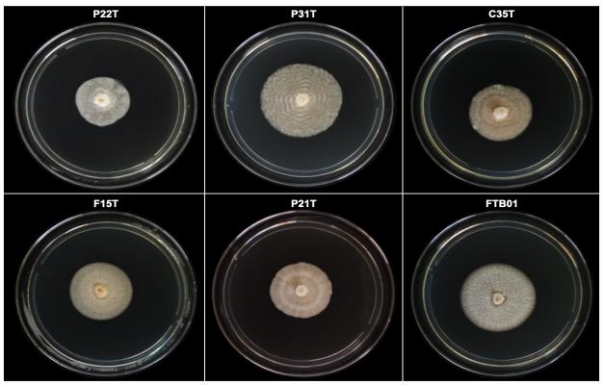

Fig. 1. Colony of the *Induratia* strains used in this study incubated at 23 °C on malt extract agar for 14 days.

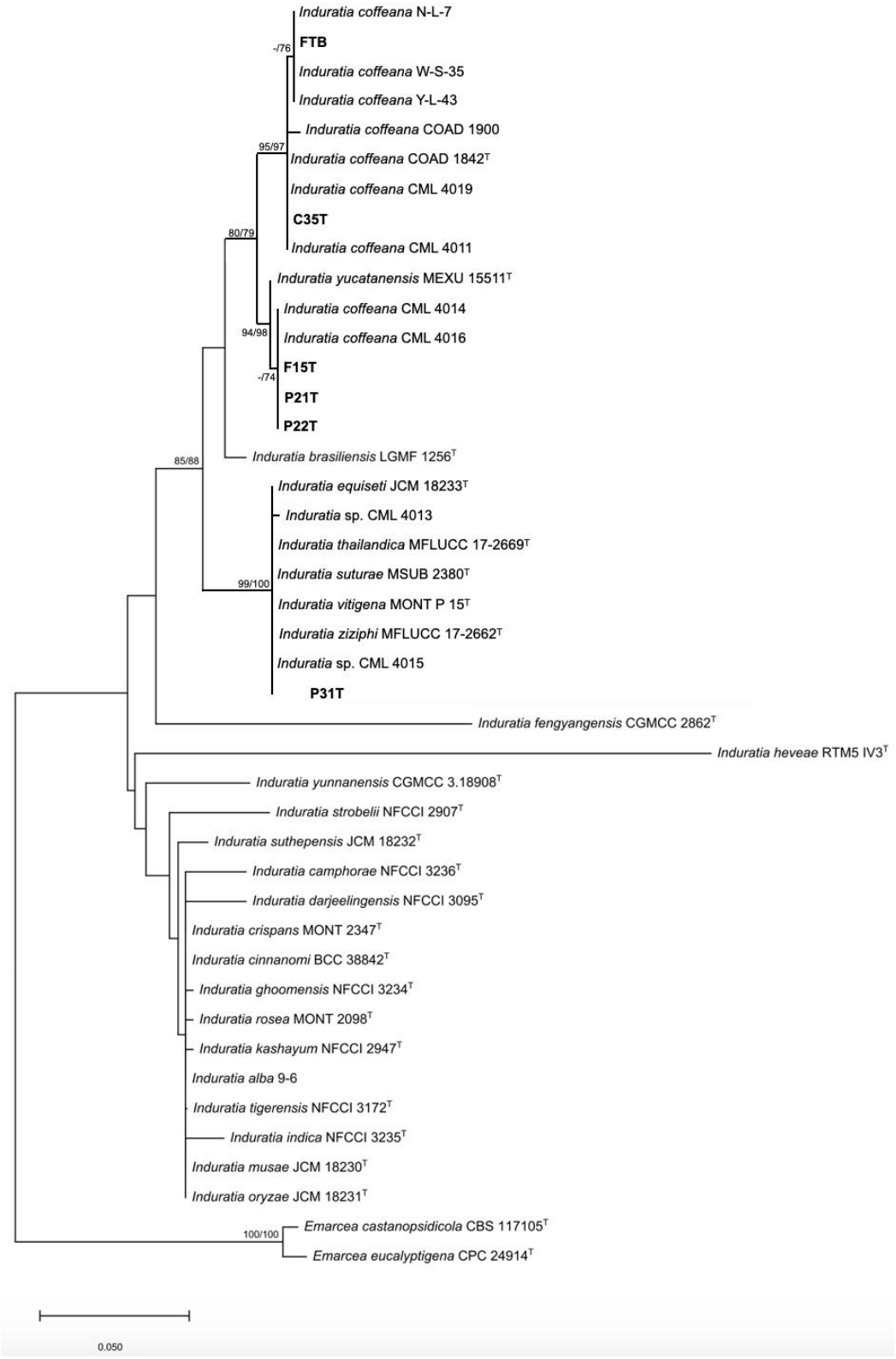

Fig. 2. Maximum Likelihood phylogenetic tree based on ITS showing relationships among *Induratia* species. Strains from this study are highlighted in bold. Bootstrap values ≥ 70% (ML/MP) are shown at the internodes. A minus sign (–) refers to support values lower than 70%. Symbol T refer to Type strains.

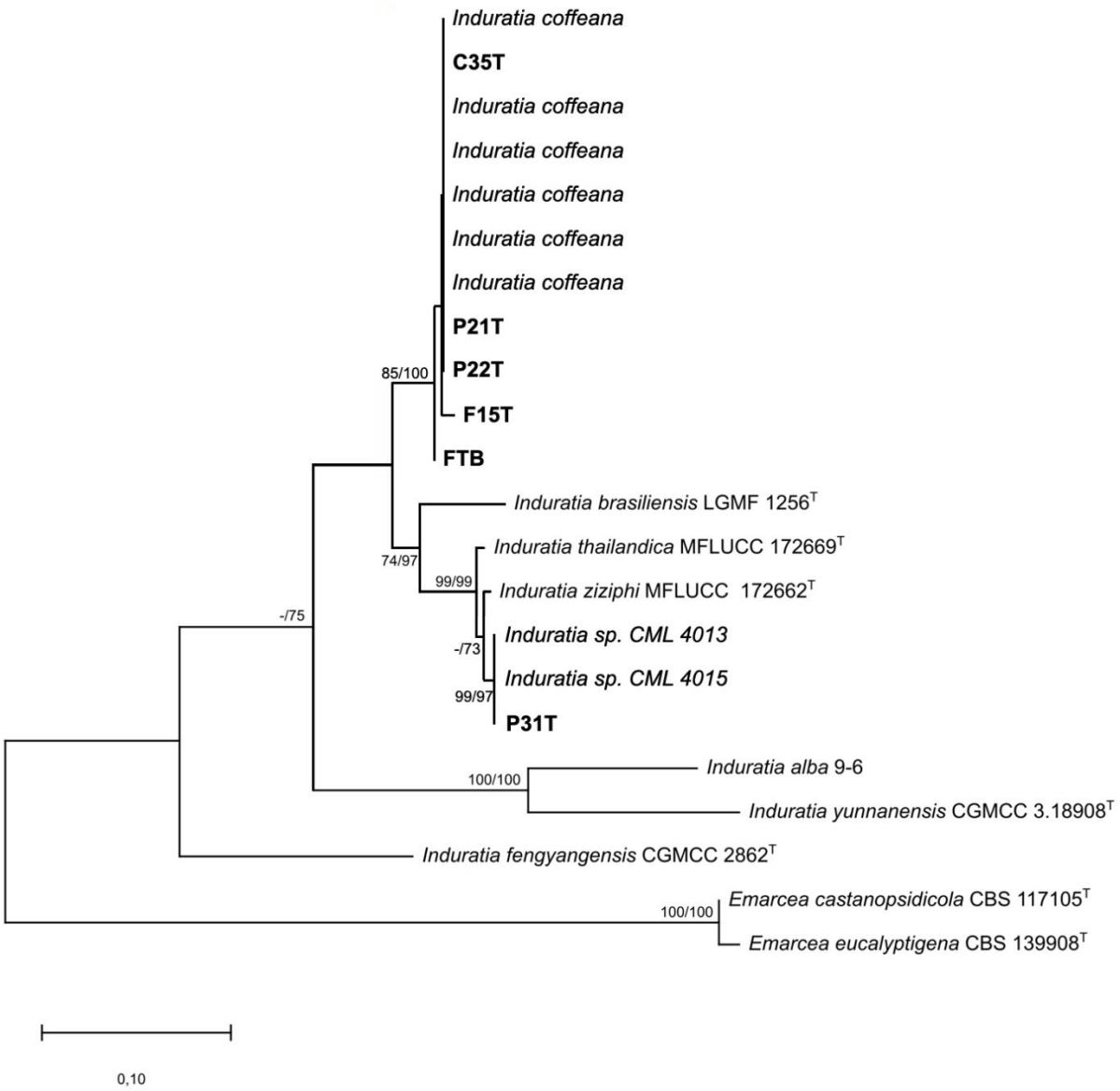

Fig. 3. Maximum Likelihood phylogenetic tree based on *rpb2* showing relationships among *Induratia* species. Strains from this study are highlighted in bold. Bootstrap values ≥ 70% (ML/MP) are shown at the internodes. A minus sign (–) refers to support values lower than 70%. Symbol T refer to Type strains.

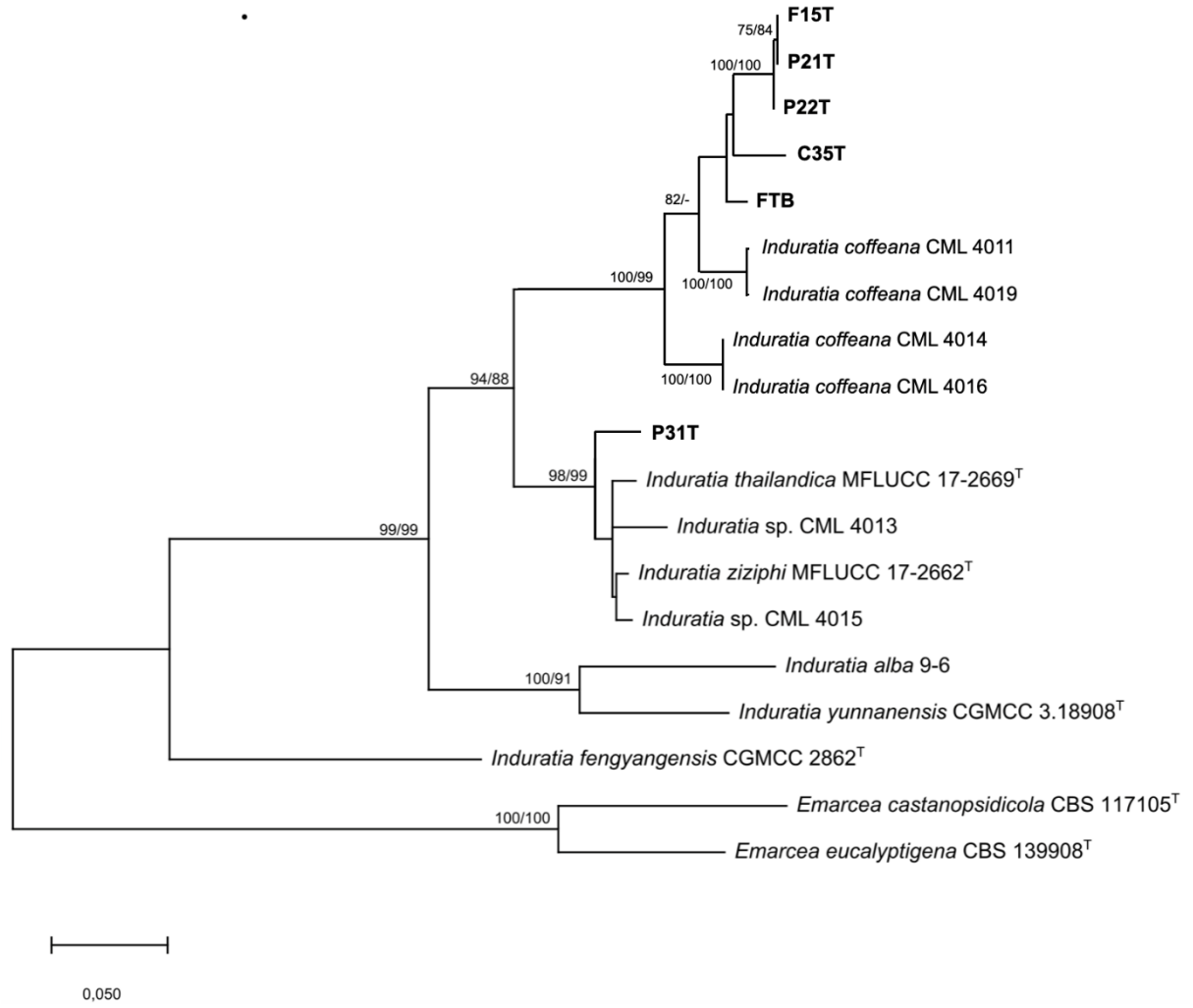

Fig. 4. Maximum Likelihood phylogenetic tree based on *Tub2* showing relationships among *Induratia* species. Strains from this study are highlighted in bold. Bootstrap values ≥ 70% (ML/MP) are shown at the internodes. A minus sign (–) refers to support values lower than 70%. Symbol T refer to Type strains.

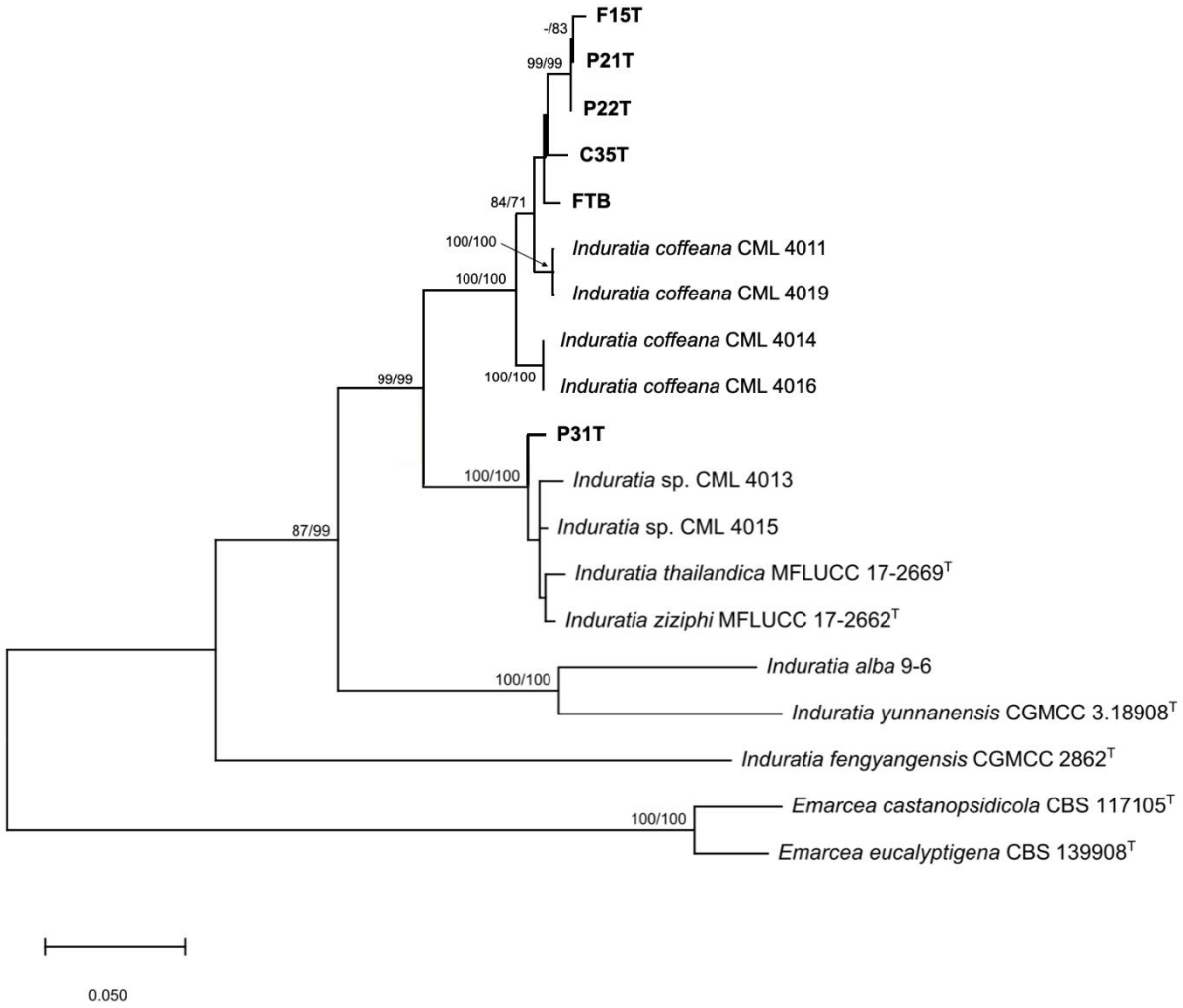

Fig. 5. Maximum Likelihood phylogenetic tree based on concatenated ITS-*rpb2*-*Tub2* sequences showing relationships among *Induratia* species. Strains from this study are highlighted in bold. Bootstrap values ≥ 70% (ML/MP) are shown at the internodes. A minus sign (–) refers to support values lower than 70%. Symbol T refer to Type strains.

Antimicrobial activity

The strains *I. coffeana* FTB and *Induratia* P31T were able to completely inhibit the mycelial growth and kill *S. sclerotiorum*. The volatile metabolites produced by FTB were also able to inhibit the mycelium growth of *R. solani* and *B. cinerea*, but were not able to kill these pathogens in the tested conditions (Table 6). The VOCs of the strain *I. coffeana* P21T showed the weakest antimicrobial activity, slightly inhibiting *C. lindemuthianum*, *S. sclerotiorum*, *Phytophthora* sp. and showing no inhibition activity on *R. solani*, *B. cinerea* and *F. verticillioides* (Table 6).

Among the pathogens tested, *F. verticillioides* was the least sensitive when exposed to VOCs with the highest inhibition produced by strain FTB (32.4%), whereas strains C3 5T, P2 1T and F1 5T were not able to inhibit it. *Sclerotinia sclerotiorum* was the most sensitive to VOCs and its growth was reduced by all strains. Strain FTB showed the best results *in vitro* for most phytopathogens and was chosen for further experiments employing strawberries in closed boxes.

The VOCs produced by *I. coffeana* strain FTB in millet grains *in vitro* inhibited and killed *R. solani*, *B. cinerea*, *Phytophthora* sp., *C. lindemuthianum*, *S. sclerotiorum* after exposure. However, *F. verticillioides* and *Pectobacterium carotovorum* were inhibited but not killed (Table 7).

Table 6. Antimicrobial activity of the VOCs produced by *Induratia* strains.

Strain	*Rhizoctonia solani*		*Botrytis cinerea*		*Phytophthora* sp.		*Colletotrichum lindemunthianum*		*Sclerotinia sclerotiorum*		*Fusarium verticillioides*	
	Inib.[a]	**Viab.[b]**	**Inib.[a]**	**Viab.[b]**	**Inib.[a]**	**Viab.[b]**	**Inib.[a]**	**Viab.[b]**	**Inib.[a]**	**Viab.[b]**	**Inib.[a]**	**Viab.[b]**
P31T	42.86	Alive	33.33	Alive	70.48	Alive	24.76	Alive	100.00	Alive	12.38	Alive
C3 5T	0.00	Alive	23.81	Alive	19.05	Alive	9.52	Alive	33.33	Alive	0.00	Alive
FTB	70.48	Alive	98.10	Alive	40.00	Alive		Alive	100.00	Alive	32.38	Alive
P2 2T	50.48	Alive	0.00	Alive	0.00	Alive	43.81	Alive	95.24	Alive	19.05	Alive
P2 1T	0.00	Alive	0.00	Alive	47.62	Alive	9.52	Alive	14.29	Alive	0.00	Alive
F1 5T	0.00	Alive	30.48	Alive	51.43	Alive	17.14	Alive	57.14	Alive	0.00	Alive

[a] Inibitory rate (%); [b] Viability.

Table 7. Antimicrobial activity of volatiles produced by *I. coffeana* FTB grown for 21 days in millet to verify the growth (cm) and viability of several pathogens exposed to volatiles

Pathogen	Growth and viability	
	Negative Control	***I. coffeana* FTB**
Rhizoctonia solani	4 cm and alive	inhibit and kill
Botrytis cinerea	4 cm and alive	inhibit and kill
Phytophthora sp.	4 cm and alive	inhibit and kill
Rhizopus stolonifer	4 cm and alive	inhibit and kill
Colletotrichum lindemuthianum	4 cm and alive	inhibit and kill
Sclerotinia sclerotiorum	4 cm and alive	inhibit and kill
Fusarium verticillioides	4 cm and alive	0.8 cm and alive
Pectobacterium carotovorum	growth and alive	alive

Control of *Botrytis cinerea* in closed strawberries boxes

Most fruits in the control showed symptoms of *B. cinerea* at the end of the experiment in different temperatures, whereas fruits treated with *I. coffeana* FTB did not show *B. cinerea* incidence (Figs. 6 and 7). Strain FTB grown in millet grains increased the shelf-life at 10, 15, 20 and 25 ºC in 6, 3, 3 and 5 days, respectively (Table 8). The sachet containing *I. coffeana* FTB has shown to be effective until before 63 days (Fig. 8). This test confirms that during the 7 days of the experiment with the fruits, the carrier formulation of *I. coffeana* FTB produced volatile compounds with the ability to inhibit *B. cinerea*.

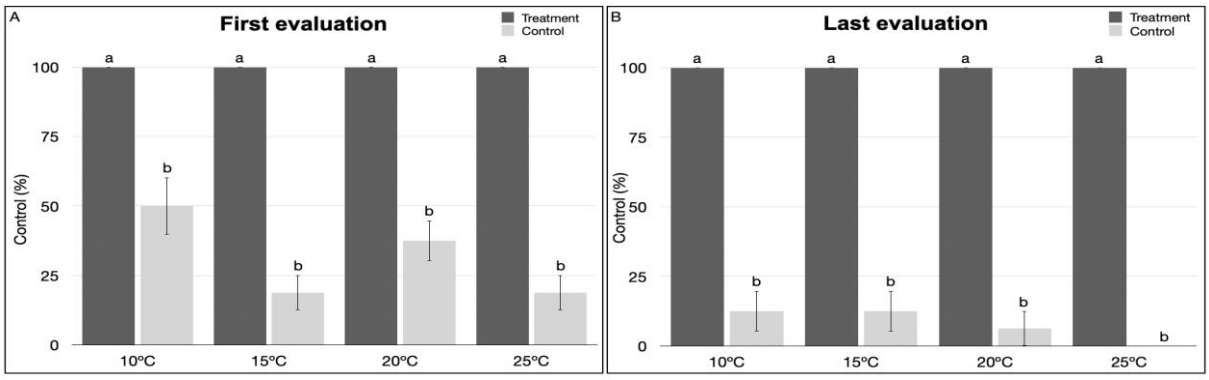

Fig. 6. Percentage of control of *Botrytis cinerea* in strawberry fruits with *Induratia coffeana* FTB at the end of the experiment at different temperatures. Fruits were treated with a sachet containing whole millet grains colonized by *Induratia coffeana* FTB (Treatment) or without Control). The values are means of 4 replicates containing 4 fruits per replicate. A- First evaluation. B - Last evaluation

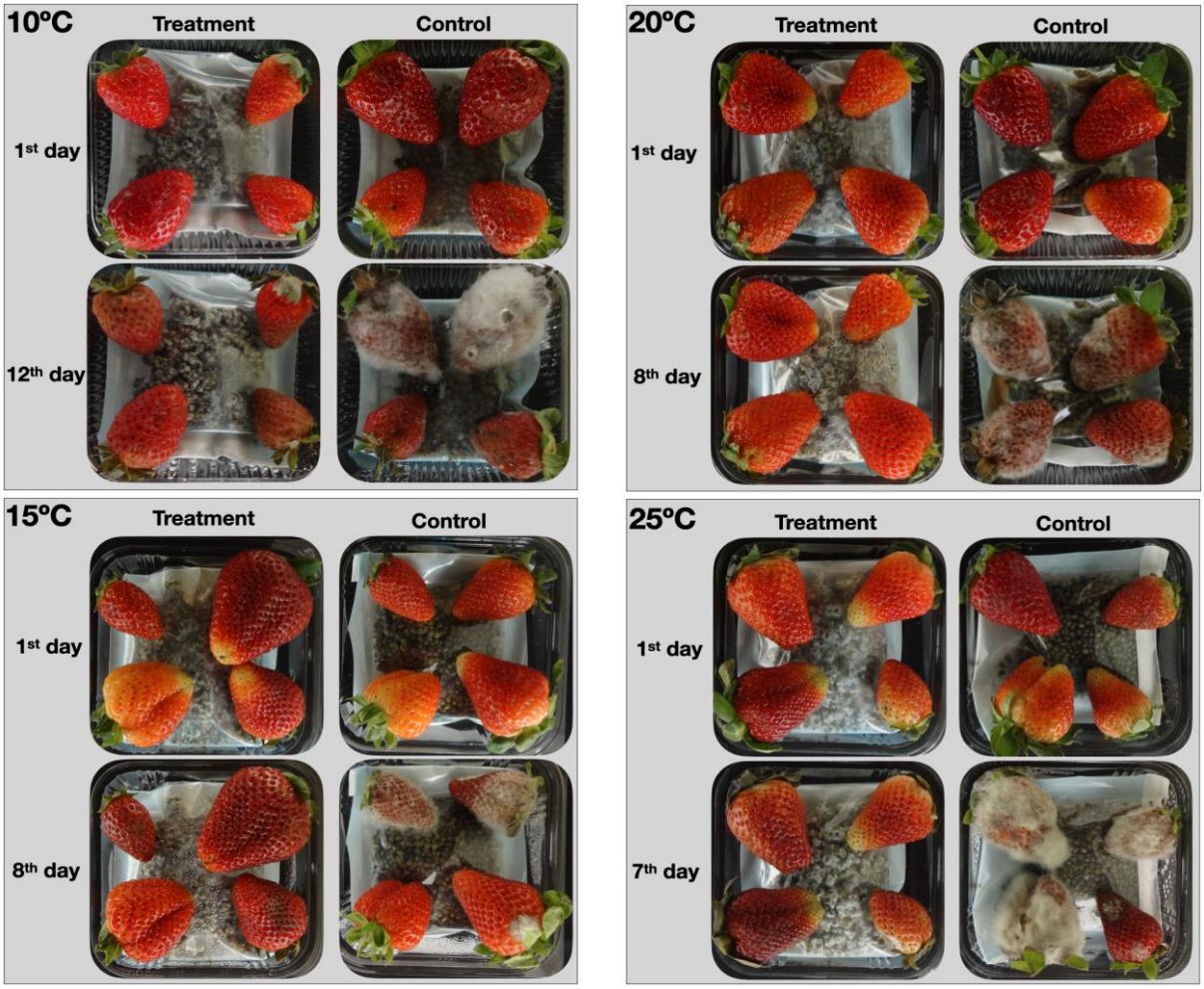

Fig. 7. Aspect of the strawberries treated with sachets containing whole millet grains colonized by *Induratia coffeana* FTB or with whole millet grains without *Induratia* sp. (Control 1) or without both (Control 2) at different temperatures. The upper panels at each temperature show the fruits at the beginning of the experiment (1st day) and the lower panels show the fruits at the end of experiment (12th day, for 10ºC; 8th day, for 15ºC; 8th day, for 20ºC; 7th day, for 10ºC).

Table 8. Percentage of control of *B. cinerea* at the first and last day of each temperature incubation.

	***B. cinerea* control (%)**							
	10ºC		**15ºC**		**20ºC**		**25ºC**	
	Day 6	**Day 12**	**Day 5**	**Day 8**	**Day 3**	**Day 6**	**Day 2**	**Day 7**
Treatment	100	100	100	100	100	100	100	100
Control	50	18.75	25	0	43.75	12.5	3.25	0

Identification of volatile organic compounds

A total of 17 compounds were determined by comparison with the GC-MS library (Table 9). These VOCs were not detected in the non-inoculated controls. All strains produced phenylethyl alcohol. For C3 5T, F1 5T, FTB 01, P2 1T, P2 2T and P3 1T a total of 6, 12, 6, 8, 9 and 12 VOCs, respectively were identified. It was not possible to determine the relative concentration of each compound in these analyses.

Day	*B. cinerea* inhibition
0	Yes
7	Yes
14	Yes
21	Yes
28	Yes
35	Yes
42	Yes
49	Yes
56	Yes
63	No
70	No

Fig. 8. Time in days that *I. coffeana* FTB was able to produce volatiles that completely inhibited *B. cinerea.* Every 7 days a sample was taken from the same plastic bag and placed in a split Petri dish in one half, and in the other half, a mycelium disk of *B. cinerea*. At day 0 was already possible to observe the complete inhibition of *B. cinerea*. At day 63 it there was no complete inhibition of the pathogen.

Table 9. VOCs produced by *Induratia* strains on MEA medium 25 °C after 3 days by SPME GC-MS analysis. The symbol * means *Induratia coffeana* FTB cultivated in millet after 21 days.

RI Exp.	RI Lit.	Possible compound	C3 5T	F1 5T	P2 1T	P2 2T	P3 1T	FTB 01	FTB 01*
684	680.18	2-methyl-propanoate	x	x		x	x	x	
733	731.16	3-methyl-1-butanol		x					
737	734.88	2-methyl-1-butanol		x					x
766	766.98	2-methylpropyl acetate		x					x
790	788.84	Isobutanoic acid	x	x		x	x	x	
804	799.53	Hexanal	x	x	x	x	x		
851	854.23	2-furanmethanol		x	x	x	x		
870	868.51	Hexanol			x	x	x		
875	874.93	3-Methylbutyl acetate		x			x		
878	877.26	2-Methylbutyl acetate		x			x		
911	904.31	2-nonene	x						
1091	1084.45	3-nonanone	x						
1119	1112.63	Phenylethyl alcohol	x	x	x	x	x	x	x
1257	1252.89	Phenylethyl acetate			x	x	x	x	
1392	1391.35	b-element		x	x		x		
1437	1435.13	a-guaien		x	x	x	x	x	
1492	1490.32	d-guaien			x	x	x	x	
1523	1532.00	Epiglobulol							x
1456	1456.00	Dehydroaromandendrene							x
753	756.00	Isobutyrate<ethyl>							x
846	848.00	Butanoic acid, 2-methyl, Ethyl ester							x
1000	1000.00	3-octanol							x
1523	1532.00	Epiglobulol							x
		Ethanol							x
		Acetic acid ethyl ester							x

4 DISCUSSION

In this prospective study of endophytic fungi from tissues of the hemiparasite plant *S. flexicaulis*, six strains of genus *Induratia* were isolated and their potential to control *Botrytis cinerea* in strawberry was assessed. *Induratia* species are the most studied among the volatile-producing fungi (Silva *et al.* 2019). They have a great relevance in agriculture, medicine and industry (Strobel and Daisy 2003; Tan and Zou 2001). In Brazil, there are few reports on *Induratia* species (Guimaraes et al., 2021).

Generally, fungal identification is based on morphological features and phylogenetic analysis (Mao *et al*. 2018). The assexual stage of reproduction is commonly analyzed in the identification of fungi, however, the genus *Induratia* does not routinely produce conidia in culture media. In the same fashion, sexual spores are also not commonly formed by these fungi and were only found 20 years after the original description of this genus (Samarakoon *et al.* 2020). Thus, it is indispensable to use multi-locus molecular phylogenetic analysis as a tool to support the correct identification. Samarakoon et al. (2020) in agreement with other studies on Sordariomycetes and other Ascomycota (Hongsanan *et al.* 2018; Chen *et al.* 2019) affirmed that

ITS sequences are not enough for species delimitation and additional DNA loci must be sequenced. Thus, the authors suggest the identification of *Induratia* species by phylogenetic analyses of the loci ITS, LSU, SSU, *RPB2, TUB2*, specially *RPB*2 and *TUB2*.

The six *Induratia* strains used in this study were identified by phylogenetic analysis of ITS, *RPB2* and *TUB2* sequences. Unfortunately, sequences of *RPB2* and *TUB2* are not available for most type strains of the described *Induratia* species. This limited our identification and therefore, sequences for *RPB*2 and *TUB2* of species described without these genes must be obtained. Future descriptions of new species must contain the complete set of genes described above.

Each strain obtained in this study produced a different group of VOCs under the same conditions. The VOCs produced by *Induratia* sp. are well known by their capacity to inhibit and kill bacteria, fungi, oomycetes, insects and nematodes (Daisy *et al.* 2002; Grimme *et al.* 2007; Strobel and Daisy 2003; Worapong *et al.* 2001; Lacey and Neven 2006), but these VOCs can change depending on substrate, temperature, days of incubation and other conditions (Ezra *et al.* 2003; Gabler *et al.* 2006; Suwannarach *et al.* 2017). A total of 17 VOCs were identified and just phenylethyl alcohol was common for all strains. Curiously, strains P2 2T, P2 1T, F1 5T, C35T and FTB were all identified as *I. coffeana* and they produced 10 different compounds. This demonstrates that the VOCS are not correlated with the phylogenetic status, which is in accordance with other studies (Mao *et al.* 2018; Samarakoon *et al.* 2020). The six strains studied here produced some compounds that are already known for their properties, such as phenylethyl alcohol, 2-furanmethanol, 3-methyl butanol, 2-methyl butanol with antimicrobial activity (Fialho *et al.* 2011; Lingappa *et al*. 1969). The potential to control plant pathogens was evident in the six strains isolated in this study and it was shown by other authors (Yang *et al.* 1994).

Strain *I. coffeana* FTB showed the best results for most pathogens and was chosen for *in vivo* experiments with strawberries in closed boxes. Strawberries have a short shelf-life as fresh fruits because of the occurrence of *B. cinerea, R. stolonifer* and other post-harvest pathogens (Labuza and Breene 1989; Bautista-Baños *et al.* 2003). Attempts to control these pathogens with fungicides make strawberry one of the fruits with the highest amounts of pesticide residues (Anvisa 2017). The activity of *Induratia* sp. against *B. cinerea* was already demonstrated by other authors (Strobel 2006; Mercier *et al.* 2007; Mercier and Jimenez 2009; Banguela-Castillo *et al.* 2015), but products registered to control this pathogen in post-harvest are lacking in Brazil. In our study, *I. coffeana* FTB grown on whole millet grains was able to control *B. cinerea* on strawberries. The fact that the VOCs produced by *Induratia* strains do not

leave toxic residues in the treated products and the lack of pathogenicity of these fungi to humans and other animals are great advantages of this biocontrol agents. The sachet containing millet grains colonized by *I. coffeana* FTB could increase the shelf-life of strawberries in air tight closed plastic boxes in 6, 3, 3 and 5 days, respectively, at 10, 15, 20 and 25 ºC. Therefore, a patent on the formulation and its use as a biofumigant was deposited (See next section).

In conclusion, this study reports the isolation, selection and identification of *Induratia* strains with potential to inhibit partially or completely a variety of plant pathogens. One new species of *Induratia* will be further described in future studies. The strain *I. coffeana* FTB was able to completely inhibit *B. cinerea in vitro* and *in vivo*, increasing strawberries shelf-life to up to 6 days, dependent on the temperature. This strain has the potential to be developed into a commercial product to control post-harvest decays in strawberries.

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MANUSCRIPT 2

Diversity and multifunctional potential for plant growth promotion in bacteria from soil and rhizosphere

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Abstract

Beneficial microorganisms play essential roles in soil fertility, plant nutrition and health. In this study, we examined the potential of a collection of 138 bacterial strains to promote plant growth. The strains were isolated from the rhizosphere of two monocotyledonous and two dicotyledonous plant species and from bare fallow soil, all from the same site. The most common trait was phosphorus (P) solubilisation from aluminium phosphate (in 66.7% of the strains), whereas solubilisation of P from phytic acid (6.5%) and from iron phosphate (5.8%) were the least common and were only detected in bacterial strains from faba bean and oilseed rape. All bacterial strains inhibited the growth of *F. graminearum* (from 5.4% to 87.2%). In total, 10 genera were identified among the strains by 16S rRNA sequencing and *Pseudomonas* was the most common in monocotyledonous plants and in bulk soil, while *Stenotrophomonas* was dominant in the rhizosphere of the dicotyledonous. Combinations of bacterial strains improved the spectrum of *in vitro* activity in most cases, however, wheat growth was generally lower. These strains have potential to be used as biofertilizers and/or biocontrol agents and further studies should be pursued to develop them into practical solutions for a more sustainable agricultural production.

Keywords: *Fusarium*; macronutrients; siderophores; *Triticum*.

1. Introduction

Plants release exudates that can alter the diversity and abundance of microorganisms in the rhizosphere, which in turn may affect plant development [1]. Some microorganisms can increase the availability of soil nutrients for plants as well as inhibit or kill phytopathogenic microbes thereby increasing plant growth [2,3]. Beneficial microbial populations are essential components of the soil and rhizobacteria are determinants of soil fertility and plant health, enabling plants to overcome both abiotic and biotic stresses [4].

Nitrogen (N), phosphorus (P) and potassium (K) are the primary macronutrients for plants with P being the second most applied nutrient in many cropping systems. It is estimated that half of the P applied as fertiliser is not assimilated by plants but rather remains unavailable in recalcitrant forms in soil [5]. Phosphorus is involved in many metabolic processes in plants, and it is also an essential element of several cellular components [6]. Phosphorus is abundant in soil in both organic and inorganic forms and cations such as Ca^{2+}, Mg^{2+}, Fe^{3+} and Al^{3+} can bind phosphate anions and precipitate them, leading to immobilization [5,7]. The fixed P form in alkaline soils is $Ca_3(PO_4)_2$, while aluminium (Al^{3+}) and iron (Fe^{3+}) produce insoluble complexes with the phosphate ion (PO_4^{3-}) in acidic soils, where phosphate mineralization by plants is low [5,8]. This is mainly due to the chemical formula of P that is commonly fixed to aluminium (Al) or iron (Fe), which prevent P uptake by plants [9,10]. Organic phosphorus forms such as phytic acid may contribute to more than 50% of total P in soil. However, phosphate-mineralizing rhizobacteria can convert organic P in the soil into an inorganic P form that plants can use through for example the activity of phytase enzymes [5,11,12]. Furthermore, phosphate solubilising root associated bacteria are capable of making insoluble inorganic forms of P available for plant uptake through the secretion of organic acids [5]. As such, bacterial components of the root microbiome can contribute to P cycling in agricultural systems.

Potassium is the third most important nutrient for plant growth, after N and P. It is directly involved in cell elongation and although it is an abundant element in soil, less than 2% of soil K can be directly absorbed by plants as most of it is fixed in silicate minerals [13]. A diverse group of microorganisms can release K, solubilising fixed forms and making them available to plants. Microorganisms solubilise K by secretion of organic acids, acidolysis, chelation, decomposition of organic matter and crop residues, exchange reactions and complexation [14–17]. In addition to macronutrients, iron is also very abundant in soils, but its availability to plants is low due to the form it occurs [18], and in response, plants release substances into the rhizosphere that promote iron solubility [19,20]. However, under adverse conditions, rhizobacteria benefit plants through siderophore secretion and Fe chelation, which is ultimately made available to the plant upon bacterial cell death [18].

Rhizobacteria can enhance plant growth directly by solubilising and increasing the uptake of nutrients such as N, P and K and indirectly by suppressing phytopathogens through multiple mechanisms. In one of these mechanisms, the bacterial ability to produce siderophores that chelate Fe makes this micronutrient unavailable to plant pathogens. Other mechanisms are the synthesis of antifungal metabolites such as antibiotics and cell wall degrading enzymes, production of volatile organic compounds capable of inhibiting fungal growth, competition with pathogens for nutrients and the capacity to induce plant systemic resistance systems [4,21].

Wheat is one of the most important cereals on a global scale and fusarium head blight caused by *Fusarium graminearum* is the most severe floral disease of wheat worldwide. This pathogen reduces yield and contaminates the grains with mycotoxins, mainly nivalenol and deoxynivalenol, which also threaten the health of humans and domestic animals that consume infected crops. Epidemics develop around the fourth or fifth years of wheat cultivation in producing areas such as Europe, United States, Africa and Brazil [22]. Several bacteria can

inhibit *F. graminearum* and their deployment in agriculture could help to promote plant health and soil fertility.

Most of the fertilizers used in agriculture are obtained from the exploitation of non-renewable mineral sources, frequently at high environmental costs. On the other hand, a diverse group of microorganisms are known to enhance nutrient availability to plants, mainly P, N, K, sulphur (S), calcium (Ca) and zinc (Zn) [23–25]. Nowadays, many of these microorganisms are commercialised as biofertilizers and biofungicides. However, there is a need to increase the availability of products containing these beneficial microbes in the market.

In this study, we aimed at exploring the functional diversity and potential of rhizosphere and bulk soil derived bacteria from different crop hosts in a single soil type to solubilise and mineralise nutrients and antagonise plant pathogens to promote plant growth. The end goal of this study is to employ these bacteria alone or in combinations to develop bioproducts to improve plant growth and contribute to increase the sustainability of agriculture.

2. Materials and Methods

2.1. Microorganisms used in the study

The bacterial strains were obtained from the rhizosphere of healthy oat (*Avena sativa*), wheat (*Triticum aestivum*), oilseed rape (*Brassica napus*), faba bean (*Vicia faba*) as well as from bare fallow soil. All samples were collected from soil or plants at flowering growth stage grown in the same site, at Furzefield, Rothamsted experimental field, Harpenden, United Kingdom (UK). A 1-g subsample of the recovered rhizosphere soil or bulk soil was diluted in water in 10-fold steps and plated onto 10% tryptic soy broth (TSB) supplemented with 15g/L of BD Bacto agar™ dehydrated agar (Fisher). Individual colonies were obtained and stored in 25% glycerol at -80°C until required for further studies. A total of 138 bacterial strains were obtained and deposited in the collection of microorganisms maintained at the Molecular

Microbial Ecology Group at Rothamsted Research, UK. *Fusarium graminearum* strain 602.10 was obtained from Dr Kevin M. King (Rothamsted Research, UK), grown on potato dextrose agar (PDA) and was preserved at -80°C in 15% glycerol.

2.2. DNA extraction, PCR and 16S rRNA gene sequencing

The bacterial strains were subjected to 16S rRNA gene sequencing for taxonomic identification. Bacterial strains were cultured overnight in 1/10 TSB at 28°C and the genomic DNA was extracted using an extraction buffer containing SDS [26]. The 16S rRNA gene PCR was carried out with bacterial DNA extracts using the primers FD1 (5'-AGAGTTTGATCCTGGCTCAG-3') and RD1 (5'- AAGGAGGTGATCCAGCC-3'). PCR reactions were carried out using the DreamTaqTM Green PCR 2x Master Mix (ThermoScientific). Once prepared, the samples were placed in a thermocycler for PCR and subjected to the following conditions: 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 60°C for 30 s, 72°C for 1 min and a final extension step of 72°C for 5 min. A single PCR amplicon was generated for each strain with no apparent unspecific amplification in the gel. The purification and sequencing of 16S rRNA gene PCR products with the Sanger method was carried out by Eurofins MWG/Operon (Germany) using a PCR product concentration of 10 ng/µl with the FD1 primer. MegaBlast was used in the searches of the curated rRNA gene database with only type strains of the bacterial species included as subjects in all sequence comparisons.

2.3. Phosphate solubilisation assays (Ca, Al and Fe, NaIHP)

Four different P sources were used and named P1, P2, P3 and P4 as tricalcium, aluminium and iron phosphate and soluble phytate (NaIHP), respectively. The bacterial strains were grown overnight in TSB 1/10 and 1 µl of each bacterial strain was spotted on agar plates

containing the different P sources. A total of 138 bacterial strains were tested and 16 different strains were spotted per Petri dish (90 mm) containing the different media and the assays were performed in triplicates.

2.3.1. *Tricalcium phosphate (P1)*

To detect bacteria that utilise tricalcium phosphate $Ca_3(PO_4)_2$ as a P source, Pikovskaya agar medium [27] containing per litre, 10 g dextrose, 5 g $Ca_3(PO_4)_2$, 0.5 g $(NH_4)_2SO_4$; 0.2 g NaCl, 0.1 g $MgSO_4.7H_2O$, 0.2 g KCl, 0.5 g yeast extract, 0.002 g $MnSO_4.H_2O$, 0.002 g $FeSO_4.7H_2O$ and 15 g agar was used. The plates were incubated at 26°C for 5 days and the strains able to solubilise tricalcium phosphate produced a clear halo around the colony. The solubilisation indices were calculated as ((colony diameter + halo diameter) / colony diameter), where 0 indicated no bacterial growth and no solubilisation, 1 indicated bacterial growth but no solubilisation, indices higher than 1 indicated bacterial growth and solubilisation.

2.3.2. *Aluminium phosphate (P2) and iron phosphate (P3)*

To detect bacteria that can utilize aluminium phosphate ($AlPO_4$) or iron phosphate ($FePO_4$) as P sources, a modified basal medium was prepared according to [9], which contained per litre, 10 g sucrose, 0.1 g NaCl, 0.5 g $MgS0_4.7H_20$, 0.2 g yeast extract; 0.5 g NH_4Cl, 0.1 g $MnSO_4.H_2O$, 2 g $FePO_4$ or 5 g $AlPO_4$, 20 g agar and 0.025g bromocresol green. The strains were transferred as described above and plates were incubated at 26°C for 24 h for $AlPO_4$ and for 5 days for $FePO_4$. The ability to solubilise $FePO_4$ was scored as positive or negative, whereas solubilisation of $AlPO_4$ was scored as a solubilisation index as described above for $Ca_3(PO_4)_2$.

2.3.3. *Soluble phytate (P4)*

To detect bacteria that utilize phytic acid or inositol hexaphosphoric acid dodecasodium salt (Na-IHP (Na)) (Sigma Aldrich) as the unique source of P, the solid phytic acid-specific medium was used. This medium was prepared according to Unno et al. [11], based on modified phytic acid specific broth, which contained per litre, 15 g agar, 10 g Na-IHP, 1.0 g $(NH_4)_2SO_4$, 0.1 g $MgSO_4 \cdot 7H_2O$, 7.0 g KCl, 0.1 g $CaCl_2 \cdot 2H_2O$, 1.0 ml 0.1 M FeNa-EDTA, 1.0 ml of a trace element solution (per litre, 15.0g $Na_2EDTA \cdot 2H_2O$, 0.43 g $ZnSO_4 \cdot 7H_2O$, 0.24 g $CoCl_2 \cdot 6H_2O$, 0.99 g $MnCl_2 \cdot 4H_2O$, 0.22 g $Na_2MoO_4 \cdot 2H_2O$, 0.19 g $NiCl_2 \cdot 6H_2O$, 0.08 g $Na_2SeO_3 \cdot 6H_2O$, 0.15 g H_3BO_3) and 0.01 g bromocresol green as a pH indicator. Bacterial strains were distributed on plates in grid format and incubated as described above for tricalcium phosphate. The ability to mineralise phytic acid was scored as negative or positive by the coloration around the colonies, from light to dark green of bromocresol green caused by a change in pH. Negative strains did not show any colour change around the colonies.

2.4 Casein Hydrolyzation (CH)

Microorganisms with hydrolytic activity can break the peptide bonds that link amino acids in proteins. These bonds and nitrogen are released in the process of protein hydrolysis [28]. The casein agar medium is used to detect hydrolysing microorganisms [29] and agar supplemented with skimmed milk was used as a casein source. The medium contained per litre, 50.0 g skimmed milk, 5.0 g pancreatic digest of casein, 2.5 g yeast extract, 1.0 g glucose and 12.5 g agar (Hardy Diagnostics). The growth of bacteria, transfer to plates and halo scoring for nitrate (NO_3) index calculation were done as described above for $Ca_3(PO_4)_2$. The plates were incubated at 26°C for 24 h for evaluation.

2.5 Potassium solubilisation assay (K)

The 138 strains were tested using the modified Aleksandrow medium to detect bacteria that utilise potassium aluminium silicate as a unique K source. This medium was prepared as described by Zhang and Kong [13], using potash feldspar (Bath Potters, UK) as an insoluble potassium source. Aleksandrow's medium contained per litre, 5 g glucose, 0.5 g $MgSO_4.7H_2O$, 0.1 g $CaCO_3$, 0.005 g $FeCl_3.6H_2O$, 2 g $Ca_3(PO_4)_2$, 2g washed potash feldspar, 0.5 g $(NH_4)_2SO_4$; 0.2 g NaCl, 0.2 g KCl, 0.5 g yeast extract, 0.002 g $MnSO_4.H_2O$ and 15 g agar. Growth of the inoculum, transfer to plates, incubation at 26°C for 5 days and halo scoring for solubilisation index determination were done as described above for $Ca_3(PO_4)_2$.

2.6 Siderophore production assay (Sid)

The ability of the bacterial strains to produce siderophores was detected on chrome azurol sulphonate (CAS) agar plates as described by [30,31]. Growth of the inoculum, transfer to plates and halo scoring for siderophore index calculation were done as described above for $Ca_3(PO_4)_2$. The CAS plates were incubated at 26°C for 24 h before evaluation.

2.7 Antifungal activity

Antagonistic activity of the bacterial strains against *F. graminearum*, abbreviated here as Fg, was verified by using the dual culture technique with three replicates. The bacterial strains were cultured overnight in TSB at 26°C at 180 rpm and 10 µl of each strain was streaked 15 mm from the edge of the 90-mm Petri dish containing a mixture of 25% PDA + 10% TSA and 15 g of agar per litre. After 2 days, a 5-mm mycelial plug of *F. graminearum* was placed in the centre of the Petri dish. Fungal mycelial growth was recorded after 4 days at 26°C and a plug of *F. graminearum* alone in a plate was the control to determine the inhibition percentage.

2.8 Compatibility among strains and wheat growth promotion

Four strains were selected for the growth promotion studies based on positive outcomes in the multifunctional assays described above. Strain P19(7)a was among the best at solubilising P from all sources (Ca, Al, Fe and NaIHP); strain P3(3)a was among the best for siderophore production; P14(9)a was among the best for NO_3 hydrolysation and K solubilisation; and P4(20) was among the best for NO_3 hydrolysation. These strains were tested *in vitro* for their growth compatibility with two-by-two combinations and four replicates were prepared according to Barbosa et al. [32]. Compatibility was tested by streaking the strains vertically and horizontally, crossing each other on 10% TSA medium and incubating at 26°C for 48 h. Growth inhibition indicated incompatibility, whereas overgrowth indicated compatibility.

Strains P19(7)a, P14(9)a, P3(3)a and P4(20) were tested individually and in all possible combinations of two strains and also all four strains were combined for their capacity to solubilise or mineralise P from different sources, siderophore production, K solubilisation and NO_3 hydrolysation by using the assays described above with four replicates per treatment. For the plate transfers, the OD_{600} for each strain was adjusted to 0.1 and 1 µL of the suspension was used for individual strains. In combinations of two and four strains, 0.5 µL and 0.25 µL of each strain were used, respectively, to ensure biomass parity regardless of strain mixture complexity.

For plant growth promotion assays, wheat seeds cv. Cadenza were surface-sterilised with 70% ethanol for 10 min, 1.5% sodium hypochlorite for 1 h and rinsed five times with autoclaved distilled water. The bacterial strains were grown overnight, the concentration of the cell suspension was adjusted to OD_{600}=0.1 and the seeds were soaked in individual or combined suspensions and shaken overnight at 150 rpm. For each treatment (individual or combined bacterial strains), 25 seeds in 4 replicates were incubated 7 days at 20±2˚C in germination paper size 25 × 38 cm (Anchor Paper) and were watered with distilled water once at the first day. Total root length was determined with the WinRhizo^{TM} system, a root-measuring device with a unique overlap correction method [33].

2.9 Data analysis

All data were evaluated for normality and homogeneity of variance using Shapiro-Wilk's and Bartlett's tests, respectively. The index data were subjected to the analysis of variance followed by mean comparison with Scott-Knott's test ($P<0.05$). Data were transformed when needed and subsequent analysis of variance were carried out. Statistical analyses were conducted using R [34] and the statistical package agricolae [35]. A Pearson's correlation analysis was performed between all functional bioassays using the software PAST 4.03 [36].

3. Results

3.1 The diversity of culturable bacteria varies according to the isolation source

The electropherograms from the 16S rRNA gene sequences showed two outcomes: 109 strains with clear, single-peak electropherograms with high-quality sequences, whereas 29 strains showed mixed electropherograms, indicative of low-quality sequences. The 109 strains with high quality sequences were identified at the genus level and the 29 strains with low quality sequences could not be identified (Supplementary Fig. S1 and Table S1).

The most abundant genera were *Pseudomonas* (44.2%) and *Stenotrophomonas* (23.2%), which were found in all isolation sources. Eight other genera were also identified (Fig. 1). *Pseudomonas* was the most representative genus in strains from the rhizosphere of the grasses (wheat and oat) and bare fallow soil, whereas *Stenotrophomonas* was the most common genus in strains from the rhizosphere of the dicotyledonous species, faba bean and oilseed rape (Fig. 1). However, some bacterial genera appeared to be more specific, for example, *Arthrobacter* was only found in the rhizosphere of wheat and oat, *Comamonas* only in the rhizosphere of faba bean, *Lelliottia* only in the rhizosphere of oilseed rape and *Paenisporosarcina* was exclusively found in bulk soil (Fig. 1).

3.2 The number of functional groups was higher among strains from dicotyledonous plants

All seven functional groups occurred among strains from faba bean and oilseed rape, whereas only five groups occurred among strains from oat, wheat and bare fallow soil (Fig. 2). The functional group profile in bulk soil most resembled the monocotyledonous grasses (oat and wheat) as opposed to the dicotyledonous plants (faba bean and oilseed rape) (Fig. 2). The ability to solubilise P from phytic acid and from iron phosphate occurred only in strains from the dicotyledonous plants (Fig. 2).

The most widespread trait was the ability to solubilise P from aluminium phosphate, which was present in 66.7% of the strains studied, followed by K solubilisation (61.5%) and P solubilisation from tricalcium phosphate (52.2%), while the least common abilities were solubilisation of P from phytic acid (6.5%) and from iron phosphate (5.8%) (Fig. 2). The capacity to hydrolyse casein and release nitrogen was more common in strains from faba bean (70%) and oilseed rape (78%) than in strains from wheat (8.7%), oat (40%) and soil (45.5%). On the other hand, the ability to synthesise siderophores and to solubilise K was more common in strains from the monocotyledonous plants (70 and 78%) and soil than in strains from the dicotyledonous plants (40 and 48%) (Fig. 2).

In addition to the presence or absence of functional ability, bacterial traits evaluated in this study displayed a great quantitative variability among strains. For example, the siderophore production index, which indicates the amount of siderophores secreted, ranged from 0 to 4.58 cm, the aluminium phosphate solubilisation index from 0 to 4.38 cm, while nitrogen hydrolysation index ranged from 0 to 2.38 cm (Fig. 3; Supplementary Table S2). Although the ability to solubilise P from aluminium phosphate was more common in strains from monocotyledonous plants (77-88%) against (53-65%) in dicotyledonous plants, the highest indices of aluminium phosphate solubilisation were found among strains from faba bean and

oilseed rape (Figs. 2 and 3; Supplementary Table S2). Similarly, K solubilisation was more common in oat and wheat (82-85% of the strains) than in faba bean and oilseed rape (39-49%), but the highest indices for K solubilisation were shown by strains from faba bean (Fig. 3; Supplementary Table S2). Siderophore synthesis was more widespread among strains from monocotyledonous plants, notably, strains from oat exhibited the highest siderophore indices (Fig. 3; Supplementary Table S2).

All bacterial strains had the ability to inhibit the growth of *F. graminearum*, which ranged from 5.4% to 87.2% of inhibition compared to control plates with no inoculant added. However, higher inhibitory activities (>76%) were found only among strains from faba bean and oilseed rape (Fig. 3; Supplementary Table S2). In general, strains from bare fallow soil and wheat had low plant growth promoting potential compared to the other strains in the collection (Fig. 3; Supplementary Table S2).

3.3 The majority of the strains possess multiple potential growth-promoting traits

A total of 23 unique profiles or functional group profiles were found among the 138 strains studied. These profiles are the individual or combined capabilities shown by the bacterial strains. The only trait common to all bacterial strains was inhibition of *F. graminearum* growth. Most bacterial strains, 125 out of 138, demonstrated more than one of the potential growth-promoting traits evaluated and only two strains possessed all eight traits (Table 1).

The most numerous genera, *Pseudomonas* and *Stenotrophomonas* had at least one representative strain in nine and 13 profiles, respectively (Table 1) and some of the traits were correlated with each other. For example, the majority of the *Stenotrophomonas* strains (22 out of 32) were antagonistic to *F. graminearum* and were also capable of hydrolysing casein, whereas most *Pseudomonas* strains (45 out of 61) shared at least five traits: ability to solubilise P from aluminium and tricalcium phosphate, synthesis of siderophores, K solubilisation, and

antagonism against *F. graminearum* (Table 1). Strains of *Pseudomonas* were on average more antagonistic against *F. graminearum* than strains of *Stenotrophomonas* (Supplementary Table S2). Furthermore, solubilisation of K and P from tricalcium phosphate were significantly correlated (R=0.7; *P*=0.0001) as these two traits occurred together in 67 strains (Table 1; Supplementary Fig. S2). The least common traits were solubilisation of P from phytic acid and from iron phosphate, which occurred in 8 and 9 strains, respectively. These two traits were highly correlated (R=0.95; *P*=0.0001) and present in strains representative of the following genera: *Pseudomonas* (1), *Serratia* (1), *Lelliottia* (2), *Stenotrophomonas* (1) and four strains that were not identified (Fig. 1; Table 1). Production of siderophores was positively correlated with solubilisation of K (R=0.63; *P*=0.0001), where 66 strains had these traits together and 25 strains had either one or the other; it was also correlated with solubilisation of P from aluminium phosphate (R=0.64; *P*=0.0001), with 70 strains sharing this trait and 23 presenting them separately. Similarly, solubilisation of P from aluminium phosphate and solubilisation of K were positively correlated (R=0.54; *P*=0.0001), with 83 strains showing the traits together and only 12 possessed one but not the other (Table 1; Supplementary Table S2 and Fig. S2).

3.4 Single strains generally promoted more wheat growth than combinations

Four combinations were compatible while two were incompatible among the four strains selected for this part of the study. Compatibility and incompatibility were verified by the absence or presence, respectively, of inhibition zones when two strains grow together (Supplementary Table S3). Strain P19(7)a was the only one compatible with the other three strains. Strain P3(3)a was compatible with P19(7)a and P4(20), but not with P14(9)a. Strain P14(9)a was incompatible with P3(3)a and P4(20). These strains were inoculated on wheat seeds individually or in combinations of two strains as well as one combination of all four strains to test their potential for plant growth. Additionally, the strains were evaluated in *in vitro*

bioassays to verify the interference of the combinations on their ability to solubilise nutrients from different sources and to produce siderophores. It should be noted that P4(20), P14(9)a and P19(7)a were all isolated from the bean rhizosphere, whereas P3(3)a was isolated from oat rhizosphere.

In general, the combination of strains increased their spectrum of activity in the *in vitro* bioassays. For five out of six tested, the presence of the trait in one of the strains led to its addition in the combination. However, the trait solubilisation of P from Ca_3PO_4 was suppressed in four of the six combinations with at least one of the strains with this property. Only in one of the combinations (P14(9)a + P19(7)a) an increase in the values of most indices was observed (Fig. 4A).

Root length was significantly longer for wheat plants treated with any bacterial strain applied individually or in combinations when compared to the untreated control. However, in two combinations out of seven, the root length was significantly shorter than in plants treated with individual strains and the other combinations, but it was still longer than the untreated control. Irrespective of the compatibility between strains, combinations in general yielded plants with smaller shoot lengths. The shoot length was significantly shorter in six out of the seven combinations tested when compared with plants treated with individual strains (Fig. 4B).

4. Discussion

The rhizosphere of plants and soils have been shown by many authors to be rich sources for the isolation of culturable plant growth promoting bacteria [37]. Due to the fact that these beneficial bacteria enhance the fitness of their host upon inoculation, they have been intensively exploited in recent times to develop inoculants for agricultural uses [38]. Among the benefits brought about by these bacteria, increased growth and defence against pathogens are in the forefront. Growth promotion is mostly induced by beneficial bacteria through the production

of phytohormones such as IAA and by the solubilization of recalcitrant nutrients that are otherwise unavailable to plants [38-40]. It has recently been shown by multivariate analyses of numerous studies that plants favour associations with hormone-producing bacteria in nutrient-rich soils and with nutrient solubilisers when growing in poor soils [25, 41].

A total of 138 culturable bacterial strains associated with the rhizosphere of healthy faba bean, oilseed rape, oat, wheat plants and bare fallow soil were investigated for their potential to promote plant growth with a variety of bioassays *in vitro*. These bioassays included the ability to solubilise four different types of P: tricalcium phosphate (P1), aluminium phosphate (P2), iron phosphate (P3), phytic acid (P4); casein hydrolyzation (CH), potassium (K) solubilization, siderophore (Sid) production and inhibition of *F. graminearum* growth (Fg). Solubilisation of nutrients in poor soils has the potential to contribute to modern agriculture by decreasing the use of synthetic and environmentally expensive chemical fertilisers [37].

Plants growing in soils with high organic matter contents are less responsive to biofertilizers, i.e., the microbes that assist plants in nutrient acquisition. The best-known effects of biofertilizers are responses from plants growing in nutrient-poor soils [25]. Several mechanisms are known to be involved in biofertilization by bacteria, including solubilisation of nutrients, production of siderophores and biological fixation of N [37]. The most exploited microbes in agriculture are the N-fixing bacteria [42]. However, our focus in this study was on nutrient solubilisation because after N, P and K are the next most limiting nutrients for plant growth. Furthermore, the root colonisation potential of associative nitrogen fixing bacteria is limited when compared to mutualistic associations of rhizobia with legumes. In addition, bacteria that solubilise P and K secrete organic acids that can solubilise recalcitrant forms of these nutrients, that are common in most soils, into forms labile to plants.

Phosphorus is present in soils in two forms: inorganic P (Pi) and organic P (Po), and because only 0.1% of the total P in soils is readily available to plants, chemical fertilizers are applied to

cope with this deficiency [43]. However, the phosphate anions in chemical fertilizers are highly reactive and are easily complexed with Ca^{2+}, Fe^{3+} and Al^{3+}, forming insoluble inorganic salts. Furthermore, organic P is mostly immobilised in organic matter, in the form of salts of phytic acid also known as soil phytate [8]. Most of the phosphate fertilizers that are not immobilised leach to groundwater and are responsible for water eutrophication that led to poisonous cyanobacterial and algal bloom formation [44]. It is estimated that the amount of P accumulated in agricultural soils would be sufficient to sustain maximum crop production for 100 years if it were available [45,46]. Therefore, microorganisms are an eco-friendly and cost-effective approach to solubilise Pi and mineralise Po in soil for the sustainable development of agricultural crops [38]. Several mechanisms are employed by bacteria to increase the availability of P to plants: 1) secretion of mineral-dissolving compounds such as organic acids or H^+ ions, protons, hydroxyl anions and CO_2 or metal chelating agents such as siderophores and HCN; 2) production of extracellular enzymes such as phytase to solubilise Po (biochemical mineralisation); and 3) release of P during substrate degradation (biological P mineralisation) [8,38]. In this study we considered the first two mechanisms and found that P-solubilising bacteria are present in soil and in the rhizosphere of different plant species in a manner that depends on the source of P being utilised. While tricalcium phosphate (P1) and aluminium phosphate (P2) solubilisers were present in relatively high numbers in all samples, iron phosphate (P3) and phytate (P4) mineralisers were only found less commonly in the strains derived from the rhizosphere of dicotyledonous plants. Interestingly, there was a strong positive correlation between the ability of the bacterial strains to solubilise P from iron phosphate and phytate. From the nine strains that possessed these traits, only one was not able to solubilise iron phosphate (Fig. 3). These low densities and the co-occurrence of the two traits have been observed previously by other authors [47].

Regarding the source of insoluble phosphate used in our screenings, tricalcium phosphate (P1) was used as proxy for inorganic P source in alkaline soils, whereas iron and aluminium phosphate was a proxy for inorganic P in acidic soils and phytate for soils rich in Po [48]. Our initial tests for growth promotion were not done in soil and therefore no conclusion can be drawn on the ability of these bacteria to promote growth in soil as several traits besides nutrient solubilisation may be involved in the final outcome, such as the capacity to colonise the rhizosphere of a given plant species [38]. As such, the ability to solubilise P is not always correlated with plant growth promotion [49].

Potassium is essential for cell turgor and elongation [50]. Only 1-2% of the soil K can be directly utilised by plants, whereas 90-98% is fixed in silicate minerals [13]. Bacteria and fungi are able to solubilise K by several mechanisms, including secretion of organic acids, chelation and decomposition of organic matter [14,17]. In this study, we found that K solubilisation was found in more than 60% of the strains, the second most common trait, evidencing the potential for K solubilization in this collection.

The 138 strains were identified by 16S rRNA gene sequencing were representative of 10 genera, which varied according to their origin of isolation. The most common genus in dicotyledonous plant rhizospheres was *Stenotrophomonas*, while *Pseudomonas* was the most common in monocotyledonous plants and in soil. The fact that some bacterial genera were more numerous in the rhizosphere of monocotyledonous as opposed to dicotyledonous and vice-versa probably reflects the exudates of these plants, that select specific communities with capacity to utilise them more efficiently [51]. These findings could be important for the design of host specific microbial inoculants. Plants are the key determinants of the composition of microbial communities in the rhizosphere. They determine the diversity, activity and densities of microbes directly through the secretion of root exudates, which vary according to plant species and genotypes and the nutrient status of the soil [52,53]. Additionally, plants influence the microbial

communities by changing the chemical properties of the soil [54]. Previous work has revealed that under Fe deficiency monocots increase their root exudation of phytosiderophores whereas dicots release protons into the soil environment [55]. In future studies it will be interesting to discover the differential root exudate chemistry between mono and dicotyledonous plants and how this affects the selection of root associated microbiota.

The number of genera recovered in our study was only 10 because the approach used a single isolation medium (1/10TSA) for subsequent screening for solubilisation of nutrients. However, it is well known from NGS and metagenomics data that the total number of bacterial genera in soil and in the rhizosphere is much higher than the number of genera we can cultivate in artificial media [56]. Besides plants, bacteria can also auto-regulate their composition, activity and diversity by producing quorum sensing molecules that allow them to respond in a coordinated way to external stimuli [57]. Some bacterial groups have a higher degree of hierarchy in soil or in the rhizosphere and are called core species as they regulate broader activities in the bacterial communities [58].

It is relatively well established in the scientific literature that dicotyledonous plants, e.g. legumes, exert a stronger effect on the abundance and diversity of microbial communities in the rhizosphere than monocotyledonous plants, e.g. grasses [53, 54, 59, 60]. In our study, this influence was noticed in a relatively small collection of culturable bacteria. The range of functional groups in culturable bacteria in the rhizosphere of the dicotyledonous plants (faba bean and oilseed rape) was greater than in the rhizosphere of monocotyledonous plants (oat and wheat) and in soil. Monocotyledonous plants are known to produce root exudates with a higher C:N ratio than dicotyledonous plants, making these exudates more difficult to utilise. On the other hand, dicotyledonous plants secrete more amino acids, sugars and flavonoids in their root exudates than monocotyledonous plants [61,62]. These differences could account for the diversity and the number of functional groups we observed in this study. Interestingly, the

functional groups observed among strains from soil were more similar to the ones observed in monocotyledonous plants, which supports the previous observation that dicotyledonous plants exert a stronger influence over their rhizosphere microbiome than monocotyledonous plants, whose community members are more similar to the bulk soil in their PGPR activity profile.

The identification of 29 strains was not possible due to mixed chromatograms that resulted from direct PCR amplifications and sequencing by the Sanger method. These mixed chromatograms probably resulted from the natural heterogeneity in the 16S rRNA gene in genomes of diverse bacterial groups [63]. Although all bacterial cultures were purified from single colonies and the PCR amplifications have always shown only one visible band in the gels, mixtures of strains cannot be completely ruled out. Variations as high as 9.7% were reported among copies of the 16S rRNA gene in the same bacterial genome [64,65]. This variation is especially high in strains of *Bacillus* [66], but also occurs in many other bacterial genera [63]. It was surprising to us that *Bacillus* spp. were not identified among the strains used in this study. It is possible that at least part of the non-identified strains are *Bacillus* species that could not be identified due to the heterogeneity in the 16S rRNA gene. This explanation is plausible when we consider that *Bacillus* strains can house over 10 copies of the 16S rRNA gene [67]. Indeed, it has been previously shown that 49% of the culturable bacterial strains from cocoa seeds had heterogeneity in their 16S rRNA gene, which greatly complicated their identification [63]. In our study, approximately 21% of the total number of strains could not be identified at this time, but they may still be identified in the future by methods such as cloning the 16S rRNA gene and sequencing from the vector, or by whole genome sequencing.

The combination of bacterial strains has been shown to increase their effectiveness in agricultural applications [68–71]. Successful combinations of bacterial strains depend on their compatibility, which may be determined in Petri dish assays [32,72]. The results of this study showed that most combinations, independent of their compatibility, resulted in an increased

spectrum of *in vitro* activity. However, wheat growth was not improved by most combinations. We must view this result with caution though, as the negative treatment was a no inoculant control. As such, it could be the case that the bacterial biomass inoculation could amount to a fertilisation effect regardless of PGPR activity. Furthermore, our experiments were done only for the initial phase of wheat growth in soil-free germination paper, without the addition of recalcitrant nutrient substrates. Future studies should be carried out to verify if the strains and their combinations will improve plant growth in the presence of the relevant recalcitrant nutrients or in soil, where the influence of these strains to solubilise and release of nutrients can be assessed in the context of strain persistence and plant biomass response. In this context, it will also be interesting to test these strains in systems with high and low nutrient status. It should also be noted that we selected the most promising strains for wheat inoculation regardless of the strain source. All selected strains were from either oat or bean rhizospheres, and future wheat *in planta* studies using this collection should also include wheat strains or screen the promising oat and bean strains on the plant species that they were initially isolated from to test the importance of host specificity of inoculants.

To conclude, in this study, we performed the initial stages of a broad characterisation of 138 strains with multiple traits that are potentially useful in the development of bacterial-based agricultural products to improve plant growth, with special emphasis on nutrient solubilisation.

Declaration of competing interests

The authors declare no conflict of interest.

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Figure legends:

Fig. 1. Identity at the genus level of the 138 bacterial strains used in this study according to the isolation source. (A) Percentage of each bacterial genus among the 138 studied strains. (B) Distribution of the bacterial genera according to the origin. The numbers between parenthesis represent the number of strains per isolation source. The bacterial strains were identified by sequencing a fragment of the 16S rRNA gene. The identification was done by comparing by Blast searches the sequences obtained in this study with the ones deposited in a curated database containing only type strains of described species. Strains that yielded low quality sequences are labelled as not identified.

Fig. 2. Distribution of functional groups of bacterial strains from soil and rhizosphere. The percentages indicate the number of strains that present each respective functional trait. The percentages for each trait do not add up to 100% because most strains possess multiple traits. The numbers between parenthesis represent the total number of strains according to their isolation origin. The functional traits were determined in plate bioassays and scored either as positive or negative for P3 - iron phosphate ($FePO_4$) and P4 - phytic acid (Na-IHP) and as an index for P1 - tricalcium phosphate ($Ca_3(PO_4)_2$), P2 - aluminium phosphate ($AlPO_4$), CH - casein hydrolyzation, K - potassium solubilisation and Sid - siderophore production. An asterisk indicates that not all strains were tested.

Fig. 3. Heatmap showing the relative indices for eight different traits in the bacterial strains studied. The shades of grey demarcate the isolation source. The identification of the strains at the genus level was done by 16S sequence analysis. The colours indicate the presence of a respective trait and the strength of the colour indicates the value of the index, whereas the white colour indicates the complete absence of the trait in consideration. The symbol X means that

this particular strain not tested. All traits shown were determined in plate assays and scored as positive or negative for P3 (iron phosphate) and P4 (phytic acid) and as an index for P1 (tricalcium phosphate), P2 (aluminium phosphate), CH (casein hydrolyzation), K (potassium) solubilisation and Sid (siderophores production). The strains highlighted in blue were selected for the combination studies.

Fig. 4. *In vitro* potential grow-promoting traits and wheat growth promotion by four selected bacterial strains and their combinations. (A) Four strains were tested alone or in six two-by-two combinations and one combination containing all four strains for their ability to solubilise P from three sources: P1 - tricalcium phosphate ($Ca_3(PO_4)_2$), P2 - aluminium phosphate ($AlPO_4$), and P4- phytic acid Na-IHP (phytic acid); CH - Casein hydrolysation, K - potassium solubilisation, Sid - siderophores production. All bioassays were done in plates and scored as numerical indices according to the size of the halo around the colonies. Solubilisation of P from $FePO_4$ was not tested. The signal "+" indicates compatibility, "-" indicates incompatibility and "+/-" indicates the presence of both compatible and incompatible interactions in the combination. (B) Shoot and root length of wheat seedlings seven days after the treatment. Means were calculated from four replicates of 25 seeds for each treatment. Black circles in the legend indicate that the bacterial strain in consideration was present and grey circles indicate the absence of the strain. The negative control was not treated with any bacterial strain. Columns with the same same letters are not significantly different according Scott-Knott's test at 5% probability.

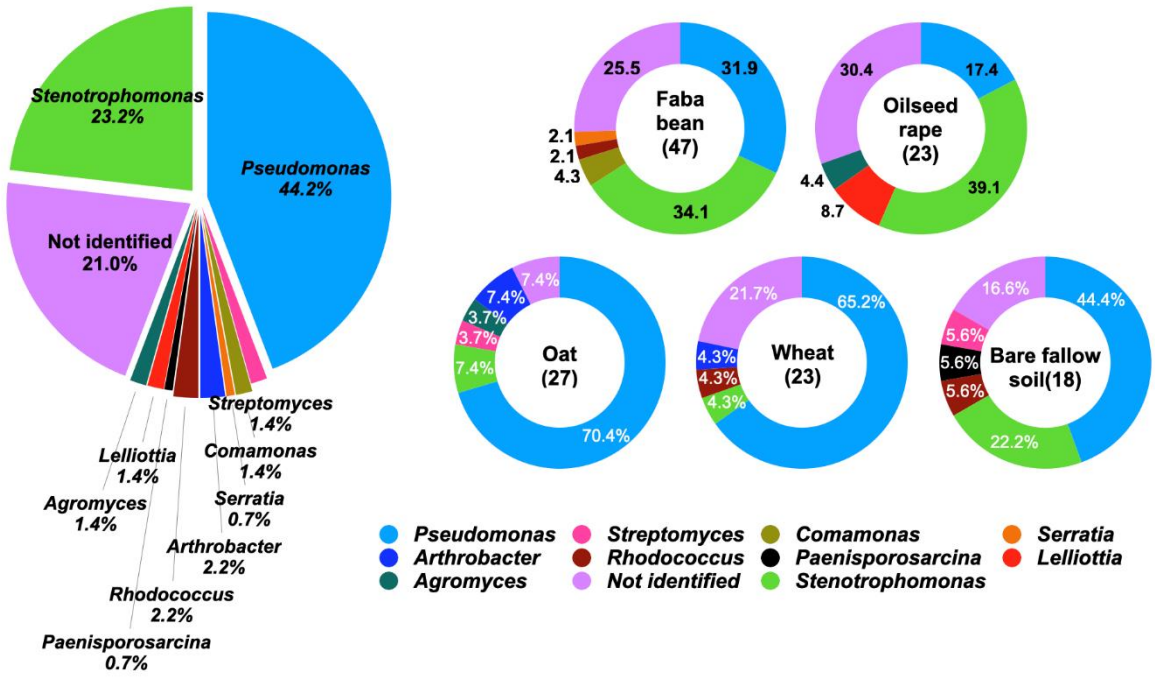

Figure 1.

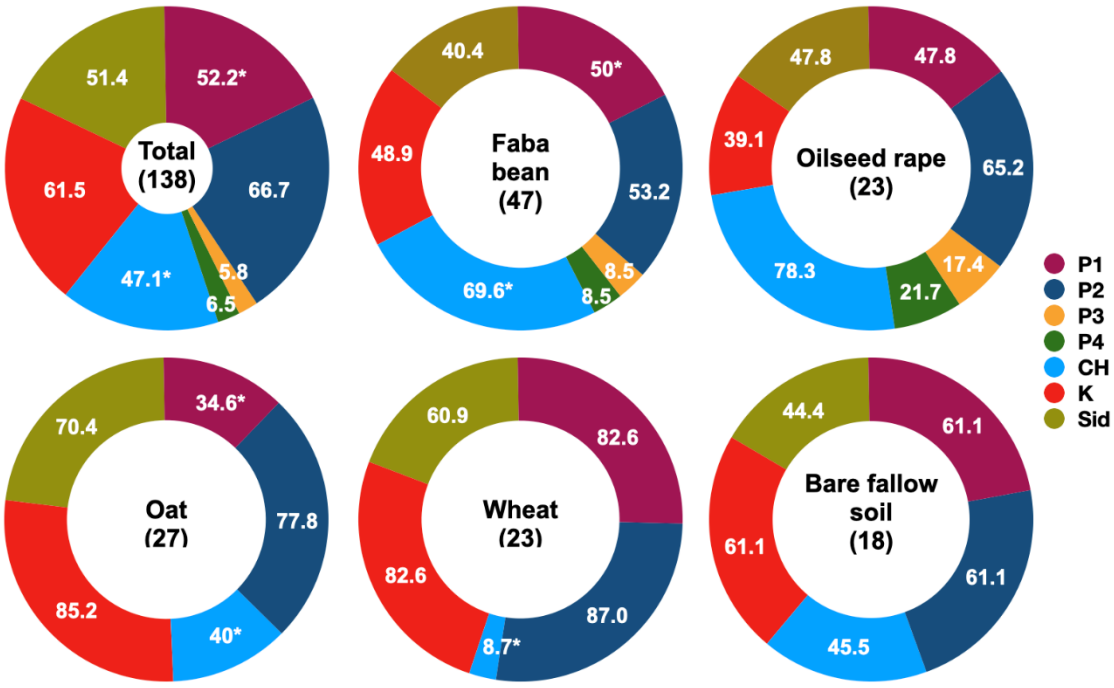

Figure 2.

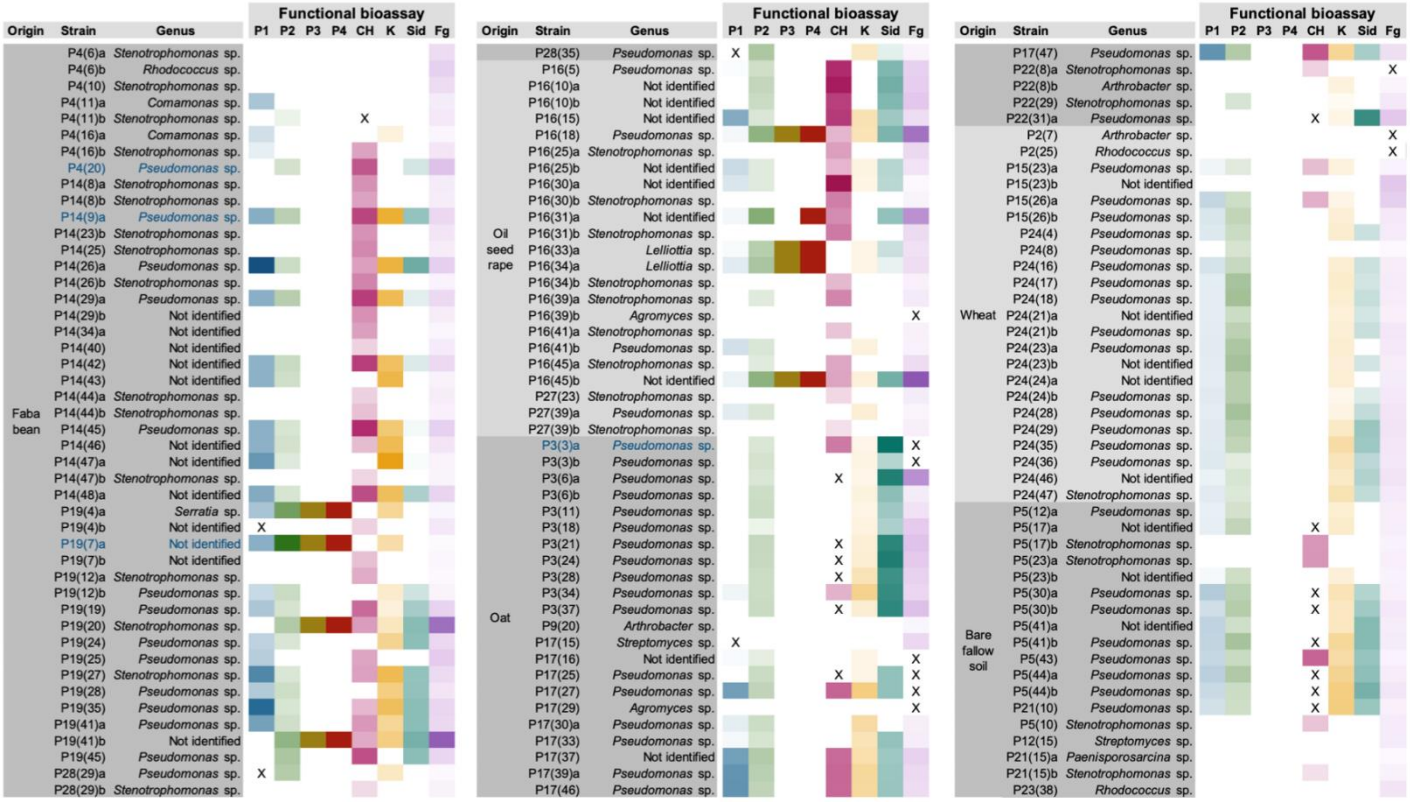
Functional bioassay
Origin Strain Genus P1 P2 P3 P4 CH K Sid Fg
Faba bean
P4(6)a Stenotrophomonas sp.
P4(6)b Rhodococcus sp.
P4(10) Stenotrophomonas sp.
P4(11)a Comamonas sp.
P4(11)b Stenotrophomonas sp.
P4(16)a Comamonas sp.
P4(16)b Stenotrophomonas sp.
P4(20) Pseudomonas sp.
P14(8)a Stenotrophomonas sp.
P14(8)b Stenotrophomonas sp.
P14(9)a Pseudomonas sp.
P14(23)b Stenotrophomonas sp.
P14(25) Stenotrophomonas sp.
P14(26)a Pseudomonas sp.
P14(26)b Stenotrophomonas sp.
P14(29)a Pseudomonas sp.
P14(29)b Not identified
P14(34)a Not identified
P14(40) Not identified
P14(42) Not identified
P14(43) Not identified
P14(44)a Stenotrophomonas sp.
P14(44)b Stenotrophomonas sp.
P14(45) Pseudomonas sp.
P14(46) Not identified
P14(47)a Not identified
P14(47)b Stenotrophomonas sp.
P14(48)a Not identified
P19(4)a Serratia sp.
P19(4)b Not identified
P19(7)a Not identified
P19(7)b Not identified
P19(12)a Stenotrophomonas sp.
P19(12)b Pseudomonas sp.
P19(19) Pseudomonas sp.
P19(20) Stenotrophomonas sp.
P19(24) Pseudomonas sp.
P19(25) Pseudomonas sp.
P19(27) Stenotrophomonas sp.
P19(28) Pseudomonas sp.
P19(35) Pseudomonas sp.
P19(41)a Pseudomonas sp.
P19(41)b Not identified
P19(45) Pseudomonas sp.
P28(29)a Pseudomonas sp.
P28(29)b Stenotrophomonas sp.
Functional bioassay
Origin Strain Genus P1 P2 P3 P4 CH K Sid Fg
Oil seed rape
Oat
P28(35) Pseudomonas sp.
P16(5) Pseudomonas sp.
P16(10)a Not identified
P16(10)b Not identified
P16(15) Not identified
P16(18) Pseudomonas sp.
P16(25)a Stenotrophomonas sp.
P16(25)b Not identified
P16(30)a Not identified
P16(30)b Stenotrophomonas sp.
P16(31)a Not identified
P16(31)b Stenotrophomonas sp.
P16(33)a Lelliottia sp.
P16(34)a Lelliottia sp.
P16(34)b Stenotrophomonas sp.
P16(39)a Stenotrophomonas sp.
P16(39)b Agromyces sp.
P16(41)a Stenotrophomonas sp.
P16(41)b Pseudomonas sp.
P16(45)a Stenotrophomonas sp.
P16(45)b Not identified
P27(23) Stenotrophomonas sp.
P27(39)a Pseudomonas sp.
P27(39)b Stenotrophomonas sp.
P3(3)a Pseudomonas sp.
P3(3)b Pseudomonas sp.
P3(6)a Pseudomonas sp.
P3(6)b Pseudomonas sp.
P3(11) Pseudomonas sp.
P3(18) Pseudomonas sp.
P3(21) Pseudomonas sp.
P3(24) Pseudomonas sp.
P3(28) Pseudomonas sp.
P3(34) Pseudomonas sp.
P3(37) Pseudomonas sp.
P9(20) Arthrobacter sp.
P17(15) Streptomyces sp.
P17(16) Not identified
P17(25) Pseudomonas sp.
P17(27) Pseudomonas sp.
P17(29) Agromyces sp.
P17(30)a Pseudomonas sp.
P17(33) Pseudomonas sp.
P17(37) Not identified
P17(39)a Pseudomonas sp.
P17(46) Pseudomonas sp.
Functional bioassay
Origin Strain Genus P1 P2 P3 P4 CH K Sid Fg
Wheat
Bare fallow soil
P17(47) Pseudomonas sp.
P22(8)a Stenotrophomonas sp.
P22(8)b Arthrobacter sp.
P22(29) Stenotrophomonas sp.
P22(31)a Pseudomonas sp.
P2(7) Arthrobacter sp.
P2(25) Rhodococcus sp.
P15(23)a Pseudomonas sp.
P15(23)b Not identified
P15(26)a Pseudomonas sp.
P15(26)b Pseudomonas sp.
P24(4) Pseudomonas sp.
P24(8) Pseudomonas sp.
P24(16) Pseudomonas sp.
P24(17) Pseudomonas sp.
P24(18) Pseudomonas sp.
P24(21)a Not identified
P24(21)b Pseudomonas sp.
P24(23)a Pseudomonas sp.
P24(23)b Not identified
P24(24)a Not identified
P24(24)b Pseudomonas sp.
P24(28) Pseudomonas sp.
P24(29) Pseudomonas sp.
P24(35) Pseudomonas sp.
P24(36) Pseudomonas sp.
P24(46) Not identified
P24(47) Stenotrophomonas sp.
P5(12)a Pseudomonas sp.
P5(17)a Not identified
P5(17)b Stenotrophomonas sp.
P5(23)a Stenotrophomonas sp.
P5(23)b Not identified
P5(30)a Pseudomonas sp.
P5(30)b Pseudomonas sp.
P5(41)a Not identified
P5(41)b Pseudomonas sp.
P5(43) Pseudomonas sp.
P5(44)a Pseudomonas sp.
P5(44)b Pseudomonas sp.
P21(10) Pseudomonas sp.
P5(10) Stenotrophomonas sp.
P12(15) Streptomyces sp.
P21(15)a Paenisporosarcina sp.
P21(15)b Stenotrophomonas sp.
P23(38) Rhodococcus sp.

Figure 3.

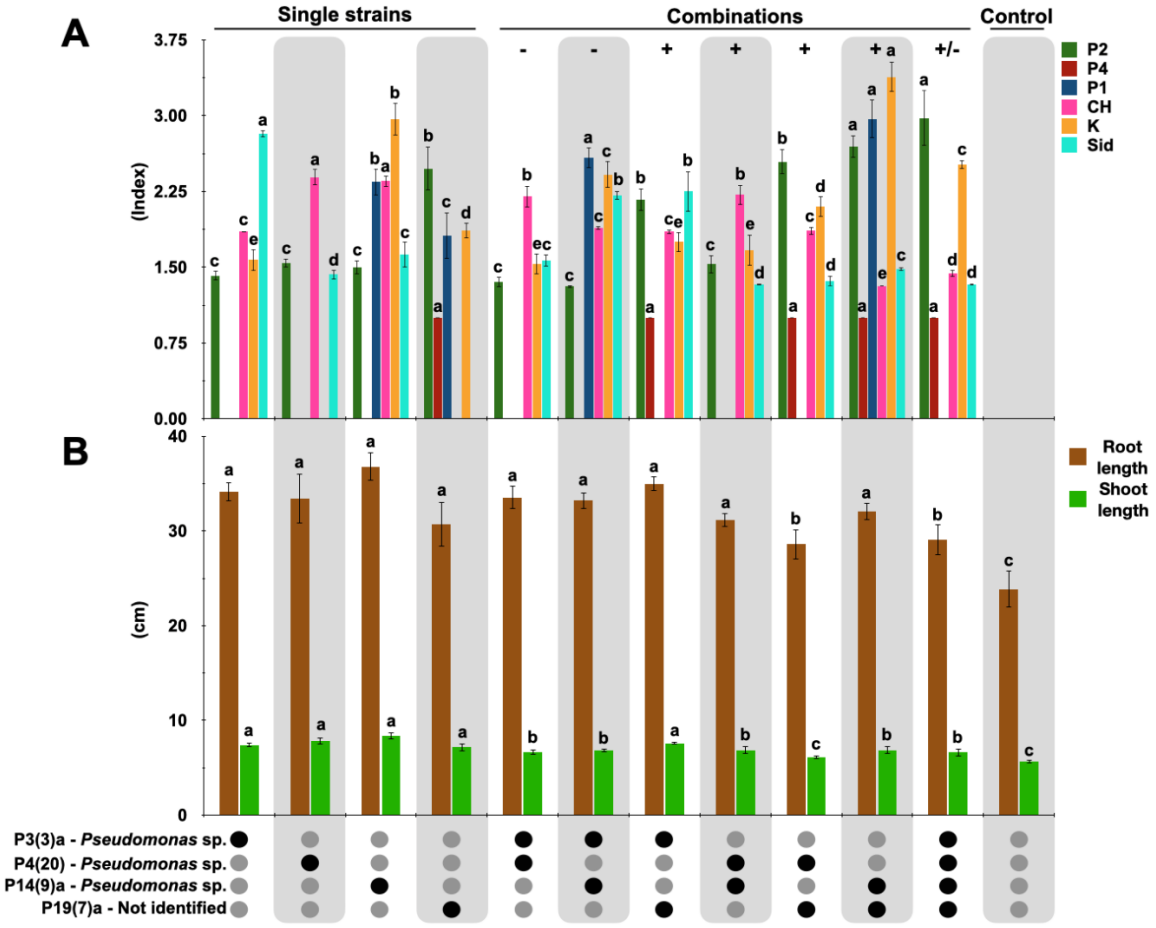
A
Single strains
Combinations
Control
P2
P4
P1
CH
K
Sid
(Index)
B
Root length
Shoot length
(cm)
P3(3)a - Pseudomonas sp.
P4(20) - Pseudomonas sp.
P14(9)a - Pseudomonas sp.
P19(7)a - Not identified

Figure 4.

Table 1. Distribution of bacterial strains in unique profiles defined by the eight functional traits evaluated in this study. The bacterial strains were identified by sequencing a fragment of the 16S rRNA gene and the bioassays were done on Petri plates and scored either as positive or negative or as indices. The numbers between parenthesis (Num.) represent the total number of strains that possess the trait under consideration and the number of strains in each genus is shown between parenthesis in each profiles. The traits evaluated were: P1 (tricalcium phosphate), P2 (aluminium phosphate), P3 (iron phosphate), P4 (phytic acid), CH (casein hydrolyzation), K (potassium) and Sid (siderophore production).

P1[a]	P2	P3	P4	CH[a]	K	Sid	Fg[b]	Num.	Genus
○	○	○	○	○	○	○	●	(13)	*Arthrobacter* (2); *Rhodococcus* (2); *Stenotrophomonas* (2); *Rhodococcus* (1); *Paenisporosarcina* (1); *Streptomyces* (2); *Agromyces* (2); Not identified (1)
○	●	○	○	○	○	○	●	(2)	*Pseudomonas* (1); *Stenotrophomonas* (1)
●	○	○	○	○	○	○	●	(1)	*Comamonas* (1)
○	○	○	○	○	●	○	●	(1)	*Arthrobacter* (1)
○	○	○	○	●	○	○	●	(27)	*Stenotrophomonas* (22); Not identified (5)
○	●	○	○	●	○	○	●	(2)	*Stenotrophomonas* (2)
●	○	○	○	●	○	○	●	(2)	*Stenotrophomonas* (1); *Pseudomonas* (1)
○	●	○	○	○	●	○	●	(2)	*Stenotrophomonas* (1); *Pseudomonas* (1)
●	○	○	○	○	●	○	●	(1)	*Comamonas* (1)
○	○	○	○	○	●	●	●	(1)	*Pseudomonas* (1)
○	●	○	○	●	○	●	●	(4)	*Pseudomonas* (2); Not identified (2)
●	●	○	○	○	●	○	●	(11)	*Pseudomonas* (6); Not identified (5)
○	●	○	○	○	●	●	●	(11)	*Pseudomonas* (11)
●	●	○	○	●	○	●	●	(1)	*Pseudomonas* (1)
●	●	○	○	●	●	○	●	(3)	*Pseudomonas* (2); Not identified (1)
○	●	○	○	●	●	●	●	(1)	*Pseudomonas* (1)
●	●	○	○	○	●	●	●	(26)	*Stenotrophomonas* (1); *Pseudomonas* (20); Not identified (5)

●	●	○	●	●	○	●	●	(1)	Not identified (1)
●	●	●	●	○	●	○	●	(2)	*Serratia* (1); Not identified (1)
●	●	○	○	●	●	●	●	(20)	*Pseudomonas* (13); *Stenotrophomonas* (1); Not identified (6)
○	●	●	●	●	●	●	●	(2)	*Lelliotia* (2)
●	●	●	●	○	●	●	●	(2)	*Stenotrophomonas* (1); Not identified (1)
●	●	●	●	●	●	●	●	(2)	*Pseudomonas* (1); Not identified (1)

[a] For the definition of these profiles, strains not tested for CH and P1 were considered as negative for these traits.
[b] Strains not tested for *F. graminearum* inhibition were considered as positive for this trait because all tested strains showed some level of inhibition.

Supplementary material:

Diversity and multifunctional potential for plant growth promotion in bacteria from soil and rhizosphere

Larissa M. de Oliveira, Vanessa N. Kavamura, Ian M. Clark, Tim H. Mauchline and Jorge T. De Souza

Table S1. Identification of the strains used in this study with partial sequences of the 16S RNA gene. Sequences were compared with the ones deposited in public databases and the identity was determined with Megablast only with type strains of the species included in the search.

Strain code	Identification in this study	Crop/Isolation source	Accession number [a]	Fragment size (bp) [b]	Closest match in databases/ accession number	Identity (%)
P16(39)b	*Agromyces* sp.	Oilseed rape	OQ457212	970	*Agromyces cerinus* subsp. *nitratus* DSM 8596 (NR_115030.1)	99.79
P17(29)	*Agromyces* sp.	Oat	OQ457213	965	*Agromyces terreus* DS-10 (NR_044184.1)	98.86
P2(7)	*Arthrobacter* sp.	Wheat	OQ457214	915	*Arthrobacter ginsengisoli* DCY81 (NR_178602.1)	99.23
P22(8)b	*Arthrobacter* sp.	Oat	OQ457215	967	*Arthrobacter oryzae* KV-651 (NR_041545.1)	99.07
P9(20)	*Arthrobacter* sp.	Oat	OQ457216	927	*Arthrobacter oryzae* KV-651 (NR_041545.1)	99.78
P4(11)a P4(16)a	*Comamonas* sp.	Faba bean	OQ457217	885	*Comamonas piscis* CN1 16S (NR_148635.1)	97.29
P16(33)a	*Lelliottia* sp.	Oilseed rape	OQ457218	966	*Lelliottia amnigena* JCM1237 (NR_024642.1)	99.38
P16(34)a	*Lelliottia* sp.	Oilseed rape	OQ457219	978	*Lelliottia amnigena* JCM1237 (NR_024642.1)	99.69
P21(15)a	*Paenisporosarcina* sp.	Bare fallow soil	OQ457256	979	*Paenisporosarcina macmurdoensis* CMS 21w (NR_025573.1)	99.59
P17(30)a	*Pseudomonas* sp.	Oat	OQ457220	906	*Pseudomonas baltica* MBT-2 (NR_181571.1)	99.56
P24(29) P24(21)b P24(36) P24(24)b	*Pseudomonas* sp.	Wheat	OQ457221	906	*Pseudomonas laurylsulfativorans* AP3_22 (NR_179728.1)	99.34

P24(35) P24(16) P15(26)a P24(23)a P24(18) P24(28) P24(17)						
P19(12)b	*Pseudomonas* sp.	Faba bean	OQ457222	900	*Pseudomonas baetica* a390 (NR_116899.1)/ *P. helmanticensis* OHA11 (NR_126220.1)	99.33
P14(26)a P19(35) P14(9)a	*Pseudomonas* sp.	Faba bean	OQ457223	1191	*Pseudomonas trivialis* P 513/19 (NR_028987.1)/ *P. poae* P 527/13 (NR_028986.1)/ *P. kairouanensis* KC12 (NR_179450.1)/ *P. allii* MAFF 301514 (NR_179337.1)	99.92
P17(47) P17(27)	*Pseudomonas* sp.	Oat	OQ457224	1188	*Pseudomonas trivialis* P 513/19 (NR_028987.1)/ *P. poae* P 527/13 (NR_028986.1)/ *P. kairouanensis* KC12 (NR_179450.1)/ *P. allii* MAFF 301514 (NR_179337.1)	99.92
P19(28) P19(41)a P19(25) P19(24) P19(19)	*Pseudomonas* sp.	Faba bean	OQ457225	881	*Pseudomonas marginalis* LMG 2210 (NR_027230.1)/ *P. rhodesiae* CIP 104664 (NR_024911.1)	99.66
P14(29)a P14(45)	*Pseudomonas* sp.	Faba bean	OQ457226	631	*Pseudomonas trivialis* P 513/19 (NR_028987.1)/ *P. poae* P 527/13 (NR_028986.1)	99.68
P24(8)	*Pseudomonas* sp.	Wheat	OQ457227	683	*Pseudomonas marginalis* ICMP 3553 (NR_117821.1)/ *P. petroselini* MAFF 311094 (NR_179384.1)/ *P. extremaustralis* 14-3 (NR_114911.1)	100
P27(39)a	*Pseudomonas* sp.	Oilseed rape	OQ457228	625	*Pseudomonas kielensis* MBT-1 (NR_181570.1)	99.52
P17(33)	*Pseudomonas* sp.	Oat	OQ457229	881	*Pseudomonas umsongensis* Ps 3-10 (NR_025227.1)	100

P19(45)	*Pseudomonas* sp.	Faba bean	OQ457230	355	*Pseudomonas antarctica* CMS 35 (NR_025586.1)/ *P. allii* MAFF 301514(NR_179337.1)/ *P, simiae* OLi (NR_042392.1)/ *P. poae* P 527/13 (NR_028986.1)	99.44
P4(20) P28(35)	*Pseudomonas* sp.	Faba bean	OQ457231	881	*Pseudomonas lactis* DSM 29167 (NR_156986.1)/ *P. azotoformans* NBRC 12693 (NR_113600.1)/ *P. paralactis* DSM 29164 (NR_156987.1)	99.89
P16(5)	*Pseudomonas* sp.	Oilseed rape	OQ457232	881	*Pseudomonas lactis* DSM 29167 (NR_156986.1)/ *P. azotoformans* NBRC 12693 (NR_113600.1)/ *P. paralactis* DSM 29164 (NR_156987.1)	99.89
P21(10) P5(30)a	*Pseudomonas* sp.	Bare fallow soil	OQ457233	1197	*Pseudomonas soyae* JL117 (NR_181891.1)	99.5
P5(30)b	*Pseudomonas* sp.	Bare fallow soil	OQ457234	626	*Pseudomonas soyae* JL117 (NR_181891.1)	99.52
P5(41)b P5(43) P5(44)a P5(44)b	*Pseudomonas* sp.	Bare fallow soil	OQ457235	624	*Pseudomonas soyae* JL117 (NR_181891.1)	99.52
P16(18)	*Pseudomonas* sp.	Oilseed rape	OQ457236	624	*Pseudomonas soyae* JL117 (NR_181891.1)	99.52
P15(23)a	*Pseudomonas* sp.	Wheat	OQ457237	319	*Pseudomonas kielensis* MBT-1 (NR_181570.1)	99.69
P3(28)	*Pseudomonas* sp.	Oat	OQ457238	620	*Pseudomonas helmanticensis* OHA11 (NR_126220.1)	99.68
P17(25)	*Pseudomonas* sp.	Oat	OQ457239	634	*Pseudomonas baetica* a390 (NR_116899.1)	99.84
P28(29)a	*Pseudomonas* sp.	Faba bean	OQ457240	501	*Pseudomonas corrugata* CFBP:2431 (NR_117826.1)	99.20
P24(4) P15(26)b	*Pseudomonas* sp.	Wheat	OQ457241	505	*Pseudomonas corrugata* CFBP:2431 (NR_117826.1)	99.80
P5(12)a	*Pseudomonas* sp.	Bare fallow soil	OQ457242	716	*Pseudomonas helmanticensis* OHA11 (NR_126220.1)/ *P. baetica* a390 (NR_116899.1)	99.44
P17(39)a	*Pseudomonas* sp.	Oat	OQ457243	282	*Pseudomonas extremorientalis* KMM 3447 (NR_025174.1)/ *P. marginalis* ICMP 3553 (NR_117821.1)/ *P. simiae* OLi (NR_042392.1)/ *P. trivialis* P 513/19 (NR_028987.1)	97.16
P3(3)b P3(6)b	*Pseudomonas* sp.	Oat	OQ457244	906	*Pseudomonas crudilactis* UCMA 17988 (NR_179985.1)	99.89

P3(11) P3(18)						
P16(41)b	*Pseudomonas* sp.	Oilseed rape	OQ457245	906	*Pseudomonas crudilactis* UCMA 17988 (NR_179985.1)	99.89
P3(24) P3(21) P3(3)a P22(31)a P3(37) P3(34) P17(46) P3(6)a	*Pseudomonas* sp.	Oat	OQ457246	1201	*Pseudomonas helmanticensis* OHA11 (NR_126220.1)	99.92
P2(25)	*Rhodococcus* sp.	Wheat	OQ457247	927	*Rhodococcus corynebacterioides* DSM 20151(NR_041873.1)	99.89
P4(6)b	*Rhodococcus* sp.	Faba bean	OQ457248	904	*Rhodococcus qingshengii* JCM 15477 djl-6 (NR_043535.1)	99.56
P23(38)	*Rhodococcus* sp.	Bare fallow soil	OQ457249	877	*Rhodococcus globerulus* DSM 43954 (NR_118617.1)	100
P19(4)a	*Serratia* sp.	Faba bean	OQ457250	980	*Serratia proteamaculans* DSM 4543 (NR_025341.1)	99.18
P16(34)b P27(39)b P27(23) P16(41)a P16(30)b P16(25)a P16(31)b	*Stenotrophomonas* sp.	Oilseed rape	OQ457253	1219	*Stenotrophomonas rhizophila* e-p10 (NR_121739.1)	100
P14(47)b P14(8)a P28(29)b P4(6)a	*Stenotrophomonas* sp.	Faba bean	OQ457251	1219	*Stenotrophomonas rhizophila* e-p10 (NR_121739.1)	100

P4(11)b P19(12)a P14(23)b P19(27) P4(16)b P4(10) P19(20) P14(25)						
P5(10) P21(15)b	*Stenotrophomonas* sp.	Bare fallow soil	OQ457252	1219	*Stenotrophomonas rhizophila* e-p10 (NR_121739.1)	100
P22(29)	*Stenotrophomonas* sp.	Oat	OQ457254	1219	*Stenotrophomonas rhizophila* e-p10 (NR_121739.1)	100
P24(47)	*Stenotrophomonas* sp.	Wheat	OQ457255	1219	*Stenotrophomonas rhizophila* e-p10 (NR_121739.1)	100
P22(8)a	*Stenotrophomonas* sp.	Oat	OQ457257	858	*Stenotrophomonas rhizophila* e-p10 (NR_121739.1)	99.88
P14(26)b P14(44)b P14(44)a	*Stenotrophomonas* sp.	Faba bean	OQ457258	858	*Stenotrophomonas rhizophila* e-p10 (NR_121739.1)	99.88
P5(17)b P5(23)a	*Stenotrophomonas* sp.	Bare fallow soil	OQ457259	858	*Stenotrophomonas rhizophila* e-p10 (NR_121739.1)	99.88
P16(39)a P16(45)a	*Stenotrophomonas* sp.	Oilseed rape	OQ457260	429	*Stenotrophomonas rhizophila* e-p10 (NR_121739.1)	100
P14(8)b	*Stenotrophomonas* sp.	Faba bean	OQ457261	1013	*Stenotrophomonas rhizophila* e-p10 (NR_121739.1)	99.80
P12(15)	*Streptomyces* sp.	Bare fallow soil	OQ457262	993	*Streptomyces globisporus* KCTC 9026 (NR_118107.1)/ *S. mediolani* NBRC 15427 (NR_112465.1)/ *S. albovinaceus* CSSP418 (NR_115373.1)/ *S. griseinus* NBRC 12869 (NR_112311.1)/ *S. globisporus* NBRC 12867 (NR_112309.1)/ *S. pluricolorescens* NBRC 12808 (NR_112284.1)/ *S. tanashiensis* CSSP723 (NR_043369.1)/ *S. rubiginosohelvolus* NBRC 12912 (NR_041093.1)	100

P17(15)	*Streptomyces* sp.	Oat	OQ457263	988	*Streptomyces clavifer* NRRL B-2557 (NR_043507.1)/ *S. mutomycini* NBRC 100999 (NR_041421.1)	100
P14(48)a	Not identified	Faba bean				
P19(7)b	Not identified	Faba bean				
P14(42)	Not identified	Faba bean				
P19(7)a	Not identified	Faba bean				
P14(47)a	Not identified	Faba bean				
P14(43)	Not identified	Faba bean				
P14(29)b	Not identified	Faba bean				
P14(34)a	Not identified	Faba bean				
P14(40)	Not identified	Faba bean				
P14(46)	Not identified	Faba bean				
P19(4)b	Not identified	Faba bean				
P19(41)b	Not identified	Faba bean				
P16(10)a	Not identified	Oilseed rape				
P16(10)b	Not identified	Oilseed rape				
P16(15)	Not identified	Oilseed rape				
P16(25)b	Not identified	Oilseed rape				
P16(31)a	Not identified	Oilseed rape				
P16(45)b	Not identified	Oilseed rape				
P16(30)a	Not identified	Oilseed rape				
P15(23)b	Not identified	Wheat				
P24(46)	Not identified	Wheat				
P24(24)a	Not identified	Wheat				
P24(23)b	Not identified	Wheat				
P24(21)a	Not identified	Wheat				
P17(37)	Not identified	Oat				

P17(16)	Not identified	Oat
P5(41)a	Not identified	Bare fallow soil
P5(17)a	Not identified	Bare fallow soil
P5(23)b	Not identified	Bare fallow soil

[a] Accession numbers obtained for the strains used in the study. Strains with identical sequences had only one representative sequence deposited. Some strains did not yield useful sequences and could not be identified.
[b] Size of the fragments obtained.
Strains printed in blue were chosen for the combination experiments.

Table S2. Response of each individual strain to the eight functional traits performed in this study. The following bioassays were performed in Pedtri dishes with specific media to detect and quantify the activity: P1 - tricalcium phosphate ($Ca_3(PO_4)_2$), P2 - aluminium phosphate ($AlPO_4$), iron phosphate ($FePO_4$), and P4- phytic acid (Na-IHP); CH - Casein hydrolysation, K - potassium solubilisation, Sid - siderophores production, and *Fusarium graminearum* inhibition (Fg).

Genus	Strain	Isolation source	$Ca_3(PO_4)_2$ (P1)	$AlPO_4$ index (P2)	$FePO_4$ (P3)	Na-IHP (P4)	Casein index (CH)	(K) index	(Sid) index	(Fg) inhibition (%)
Agromyces sp.	P16(39)b	Oilseed rape	1	1	0	0	1	1	0	Nd[a]
Agromyces sp.	P17(29)	Oat	1	0	0	0	1	1	0	Nd
Arthrobacter sp.	P2(7)	Wheat	1	1	0	0	1	1	0	Nd
Arthrobacter sp.	P22(8)b	Oat	0	1	0	0	1	1.44	0	10.47
Arthrobacter sp.	P9(20)	Oat	1	1	0	0	1	1	0	9.3
Comamonas sp.	P4(11)a	Faba bean	1.93	1	0	0	1	0	0	18.6
Comamonas sp.	P4(16)a	Faba bean	1.52	1	0	0	1	1.44	0	8.91
Lelliottia sp.	P16(33)a	Oilseed rape	1.09	2.16	1	1	1	1.35	1.79	28.68
Lelliottia sp.	P16(34)a	Oilseed rape	1.34	2.38	1	1	1	1.35	1.37	33.33
Paenisporosarcina sp.	P21(15)a	Bare fallow soil	0	0	0	0	1	1	0	6.98
Pseudomonas sp.	P17(30)a	Oat	1.3	1.83	0	0	1	2.24	1	16.67
Pseudomonas sp.	P24(29)	Wheat	1.36	2.15	0	0	1	1.75	2.48	27.13
Pseudomonas sp.	P24(21)b	Wheat	1.32	2.08	0	0	1	1.57	1.82	23.26
Pseudomonas sp.	P24(36)	Wheat	1.38	1.48	0	0	1	1.92	2.01	22.87
Pseudomonas sp.	P24(24)b	Wheat	1.35	2.17	0	0	1	1.27	1.52	21.32
Pseudomonas sp.	P24(35)	Wheat	1.36	2.02	0	0	1	2.2	2.44	24.03
Pseudomonas sp.	P24(16)	Wheat	1.46	1.92	0	0	1	1.61	1.77	17.83
Pseudomonas sp.	P15(26)a	Wheat	1.66	1.8	0	0	1.43	1.36	1	46.12
Pseudomonas sp.	P24(23)a	Wheat	1.34	2.33	0	0	1	1.63	1	21.32

Pseudomonas sp.	P24(18)	Wheat	1.31	2.51	0	0	1	1.61	1.84	17.05
Pseudomonas sp.	P24(28)	Wheat	1.31	2.46	0	0	1	1.37	1.91	25.58
Pseudomonas sp.	P24(17)	Wheat	1.32	2.42	0	0	1	1.7	1.69	18.22
Pseudomonas sp.	P19(12)b	Faba bean	1.6	1.88	0	0	1	1.68	1.73	11.63
Pseudomonas sp.	P14(26)a	Faba bean	3.77	1.84	0	0	1.52	3.56	3.13	46.51
Pseudomonas sp.	P19(35)	Faba bean	3.35	1.66	0	0	1.39	3.16	2.7	40.31
Pseudomonas sp.	P14(9)a	Faba bean	2.29	2.13	0	0	2	3.68	2.56	37.98
Pseudomonas sp.	P17(47)	Oat	2.71	2.25	0	0	1.9	2.38	2.28	31.78
Pseudomonas sp.	P17(27)	Oat	2.52	2.03	0	0	1.84	2.67	2.61	Nd
Pseudomonas sp.	P19(28)	Faba bean	1.84	1.81	0	0	1	2.67	2.61	24.81
Pseudomonas sp.	P19(41)a	Faba bean	2.49	1.79	0	0	1.56	2.37	2.71	37.6
Pseudomonas sp.	P19(25)	Faba bean	1.79	1	0	0	1.49	1	0	49.61
Pseudomonas sp.	P19(24)	Faba bean	1.72	1.62	0	0	1	2.44	2.68	37.98
Pseudomonas sp.	P19(19)	Faba bean	1.9	1.71	0	0	1.8	1.48	2.32	42.64
Pseudomonas sp.	P14(29)a	Faba bean	2.28	2.17	0	0	2.05	3.28	1.44	36.82
Pseudomonas sp.	P14(45)	Faba bean	2.26	1.66	0	0	2.13	2.84	1.18	44.57
Pseudomonas sp.	P24(8)	Wheat	1	1.68	0	0	1	1	0	6.98
Pseudomonas sp.	P27(39)a	Oilseed rape	1.33	1.63	0	0	1	1.58	1	10.47
Pseudomonas sp.	P17(33)	Oat	1.11	1.69	0	0	1	1.96	2.58	17.05
Pseudomonas sp.	P19(45)	Faba bean	1	2.38	0	0	1.95	1	2.69	40.31
Pseudomonas sp.	P4(20)	Faba bean	1	1.74	0	0	1.91	1.6	1.66	52.71
Pseudomonas sp.	P28(35)	Faba bean	Nd	2.33	0	0	1	1.8	1.42	11.63
Pseudomonas sp.	P16(5)	Oilseed rape	1.06	1.8	0	0	2.14	1	2.86	44.57
Pseudomonas sp.	P21(10)	Bare fallow soil	1.5	1.81	0	0	Nd	2.69	2.5	14.34
Pseudomonas sp.	P5(30)a	Bare fallow soil	1.83	2.09	0	0	Nd	1.97	2.11	14.73
Pseudomonas sp.	P5(30)b	Bare fallow soil	1.54	2.21	0	0	Nd	1.81	2.38	20.93

Pseudomonas sp.	P5(41)b	Bare fallow soil	1.69	2.43	0	0	Nd	2.33	2.78	18.6
Pseudomonas sp.	P5(43)	Bare fallow soil	1.65	1.88	0	0	1.84	2.18	2.54	12.79
Pseudomonas sp.	P5(44)a	Bare fallow soil	1.75	2.3	0	0	Nd	2.45	2.67	17.05
Pseudomonas sp.	P5(44)b	Bare fallow soil	1.6	1.83	0	0	Nd	2.64	3.01	24.42
Pseudomonas sp.	P16(18)	Oilseed rape	1.13	2.83	1	1	1.4	1.97	2.92	78.29
Pseudomonas sp.	P15(23)a	Wheat	1.18	1.52	0	0	1.36	1.46	1	12.4
Pseudomonas sp.	P3(28)	Oat	1	1.85	0	0	Nd	1.96	3.83	44.19
Pseudomonas sp.	P17(25)	Oat	1	1.88	0	0	Nd	1.35	2.6	Nd
Pseudomonas sp.	P28(29)a	Faba bean	Nd	2.22	0	0	1	1.79	1	6.98
Pseudomonas sp.	P24(4)	Wheat	1.38	1.86	0	0	1	1.65	1.77	18.22
Pseudomonas sp.	P15(26)b	Wheat	1.41	2.01	0	0	1	1.55	1	20.93
Pseudomonas sp.	P5(12)a	Bare fallow soil	1.26	1.74	0	0	1	1.6	0	16.28
Pseudomonas sp.	P17(39)a	Oat	2.7	2.13	0	0	1.83	2.32	2.5	36.82
Pseudomonas sp.	P3(3)b	Oat	1	1.73	0	0	1	1.39	2.09	Nd
Pseudomonas sp.	P3(6)b	Oat	1	1.83	0	0	1	1.43	2.67	18.6
Pseudomonas sp.	P3(11)	Oat	1	1.88	0	0	1	1.36	2.43	28.29
Pseudomonas sp.	P3(18)	Oat	1	1.31	0	0	1	1.42	2.21	49.61
Pseudomonas sp.	P16(41)b	Oilseed rape	1.5	1.62	0	0	1	1.46	1	13.95
Pseudomonas sp.	P3(24)	Oat	1	1.92	0	0	Nd	1.61	4.33	50
Pseudomonas sp.	P3(21)	Oat	1	1.93	0	0	Nd	1.57	4.12	52.33
Pseudomonas sp.	P3(3)a	Oat	1.11	1.61	0	0	Nd	1.33	4.58	Nd
Pseudomonas sp.	P22(31)a	Oat	1	1	0	0	Nd	1.34	3.92	50.78
Pseudomonas sp.	P3(37)	Oat	1	1.89	0	0	Nd	1.6	4.06	53.1
Pseudomonas sp.	P3(34)	Oat	1.18	1.83	0	0	1.42	2.51	3.94	38.95
Pseudomonas sp.	P17(46)	Oat	2.71	2.03	0	0	1.82	2.3	2.51	34.11
Pseudomonas sp.	P3(6)a	Oat	1	1.61	0	0	Nd	1.32	4.28	68.99

Rhodococcus sp.	P2(25)	Wheat	1	1	0	0	1	1	0	Nd
Rhodococcus sp.	P4(6)b	Faba bean	1	1	0	0	1	1	0	44.57
Rhodococcus sp.	P23(38)	Bare fallow soil	1	1	0	0	1	1	0	26.36
Serratia sp.	P19(4)a	Faba bean	1.93	3.3	1	1	1	2.36	1	9.3
Stenotrophomonas sp.	P16(34)b	Oilseed rape	1	1	0	0	1.38	1	0	14.34
Stenotrophomonas sp.	P27(39)b	Oilseed rape	1	0	0	0	1.18	0	0	15.89
Stenotrophomonas sp.	P27(23)	Oilseed rape	1	0	0	0	1.21	0	0	18.6
Stenotrophomonas sp.	P16(41)a	Oilseed rape	1	1	0	0	1.32	0	0	14.73
Stenotrophomonas sp.	P16(30)b	Oilseed rape	1	1	0	0	1.7	1	0	11.24
Stenotrophomonas sp.	P16(25)a	Oilseed rape	1	1	0	0	1.52	1	0	37.98
Stenotrophomonas sp.	P16(31)b	Oilseed rape	1	1	0	0	1.72	1	0	35.27
Stenotrophomonas sp.	P14(47)b	Faba bean	1	1	0	0	1.23	0	0	11.24
Stenotrophomonas sp.	P14(8)a	Faba bean	1	1	0	0	1.59	1	0	18.6
Stenotrophomonas sp.	P28(29)b	Faba bean	1	0	0	0	1.2	0	0	13.18
Stenotrophomonas sp.	P4(6)a	Faba bean	1	1	0	0	1	0	0	34.11
Stenotrophomonas sp.	P4(11)b	Faba bean	1	1.32	0	0	Nd	0	0	21.71
Stenotrophomonas sp.	P19(12)a	Faba bean	1	1	0	0	1.45	0	0	6.98
Stenotrophomonas sp.	P14(23)b	Faba bean	1	1	0	0	1.59	1	0	25.58
Stenotrophomonas sp.	P19(27)	Faba bean	2.9	1.6	0	0	1.51	3.29	2.58	35.66
Stenotrophomonas sp.	P4(16)b	Faba bean	1.28	1	0	0	1.5	0	0	24.03
Stenotrophomonas sp.	P4(10)	Faba bean	1	1	0	0	1	0	0	34.5
Stenotrophomonas sp.	P19(20)	Faba bean	1	2.26	1	1	1.53	1.85	2.76	79.84
Stenotrophomonas sp.	P14(25)	Faba bean	1	1	0	0	1.64	1	0	17.83
Stenotrophomonas sp.	P5(10)	Bare fallow soil	1	1	0	0	1.32	0	0	19.77
Stenotrophomonas sp.	P21(15)b	Bare fallow soil	1	1	0	0	1.18	0	0	13.95
Stenotrophomonas sp.	P22(29)	Oat	1	1.65	0	0	1	1.16	0	21.32

Stenotrophomonas sp.	P24(47)	Wheat	1.35	1.58	0	0	1	2	1.92	20.54
Stenotrophomonas sp.	P22(8)a	Oat	1	1	0	0	1.24	0	0	Nd
Stenotrophomonas sp.	P14(26)b	Faba bean	1	1	0	0	1.66	1	0	12.4
Stenotrophomonas sp.	P14(44)b	Faba bean	1	1	0	0	1.34	0	0	14.34
Stenotrophomonas sp.	P14(44)a	Faba bean	1	1	0	0	1.25	0	0	17.83
Stenotrophomonas sp.	P5(17)b	Bare fallow soil	1	1	0	0	1.57	0	0	13.95
Stenotrophomonas sp.	P5(23)a	Bare fallow soil	1	1	0	0	1.58	0	0	15.5
Stenotrophomonas sp.	P16(39)a	Oilseed rape	1	1.52	0	0	1.66	1	0	20.16
Stenotrophomonas sp.	P16(45)a	Oilseed rape	1	1.51	0	0	1.48	0	0	34.11
Stenotrophomonas sp.	P14(8)b	Faba bean	1	1	0	0	1.5	1	0	17.05
Streptomyces sp.	P12(15)	Bare fallow soil	1	1	0	0	1	1	0	19.38
Streptomyces sp.	P17(15)	Oat	Nd	0	0	0	0	1	0	38.37
Not identified	P14(48)a	Faba bean	2.34	1.66	0	0	1.96	3.16	2.21	40.7
Not identified	P19(7)b	Faba bean	1	1	0	0	1.34	0	0	8.53
Not identified	P14(42)	Faba bean	2.23	1.89	0	0	2.04	3.11	1.53	36.43
Not identified	P19(7)a	Faba bean	2.28	4.38	1	1	1	2.16	1	5.43
Not identified	P14(47)a	Faba bean	2.66	1.55	0	0	1	4.21	1.12	12.02
Not identified	P14(43)	Faba bean	2.24	1.79	0	0	1	3.42	1	13.18
Not identified	P14(29)b	Faba bean	1	1	0	0	1.6	0	0	17.44
Not identified	P14(34)a	Faba bean	1	1	0	0	1.52	0	0	8.53
Not identified	P14(40)	Faba bean	1	1	0	0	1.25	0	0	18.99
Not identified	P14(46)	Faba bean	2.24	1.74	0	0	1.37	3.3	1	25.19
Not identified	P19(4)b	Faba bean	Nd	1	0	0	1.24	0	0	20.93
Not identified	P19(41)b	Faba bean	1	2.78	1	1	1.46	2.65	3	85.27
Not identified	P16(10)a	Oilseed rape	1	1.98	0	0	2.27	1	3.08	47.29
Not identified	P16(10)b	Oilseed rape	1	1.88	0	0	2.03	1	2.84	51.16

Not identified	P16(15)	Oilseed rape	2.33	1.61	0	0	2.06	2.03	2.42	32.56
Not identified	P16(25)b	Oilseed rape	1.58	1.52	0	0	1.4	1.42	2.07	25.97
Not identified	P16(31)a	Oilseed rape	1.14	2.97	0	1	1.63	1	2.6	68.22
Not identified	P16(45)b	Oilseed rape	1.22	2.84	1	1	1.49	1.49	3.04	87.21
Not identified	P16(30)a	Oilseed rape	1.42	1.51	0	0	2.38	1.37	2.04	6.98
Not identified	P15(23)b	Wheat	1	1	0	0	1	1	0	50.78
Not identified	P24(46)	Wheat	1.23	1.66	0	0	1	1.75	2.16	27.13
Not identified	P24(24)a	Wheat	1.31	2.17	0	0	1	1.59	1	18.6
Not identified	P24(23)b	Wheat	1.32	2.45	0	0	1	1.55	1.68	17.44
Not identified	P24(21)a	Wheat	1.3	1.85	0	0	1	1.5	1.66	15.5
Not identified	P17(37)	Oat	2.52	2.25	0	0	1.8	2.1	2.44	43.41
Not identified	P17(16)	Oat	1.08	1.44	0	0	1	1.34	1	Nd
Not identified	P5(41)a	Bare fallow soil	1.7	1.79	0	0	1	1.81	2.78	17.83
Not identified	P5(17)a	Bare fallow soil	1.27	2.01	0	0	Nd	1.7	1	16.28
Not identified	P5(23)b	Bare fallow soil	1.13	1.81	0	0	1	1.51	1	13.95

[a] Nd - Not determined.

Strains printed in blue were chosen for the combination experiments.

Table S3. Compatibility in two by two combinations of four selected strains. A positive sign indicates compatibility and a negative indicates incompatibility in the specific combination. Compatibility was determined by plating two strains in a perpendicular streak and overgrowth indicated compatibility, whereas a break at the meeting point indicated incompatibility.

	P3(3)a	P4(20)	P14(9)a	P19(7)a
P3(3)a	+	+	−	+
P4(20)	+	+	−	+
P14(9)a	−	−	+	+
P19(7)a	+	+	+	+

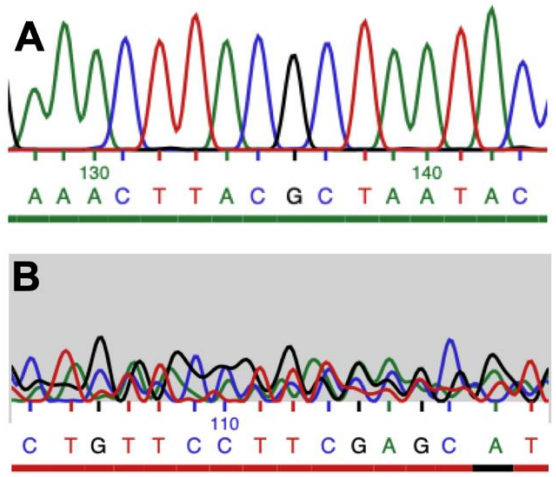

Fig. S1. Representative electropherograms obtained from 16S RNA fragments amplified by PCR and sequenced with the Sanger method. All strains were pure colonies and all PCR products yielded one single band. (A) Clear peaks indicating that the reaction contained only one PCR product. (B) Mixed peaks indicating a mixture of PCR products resulting from the natural heterogeneity in 16S RNA sequences or mixtures of strains.

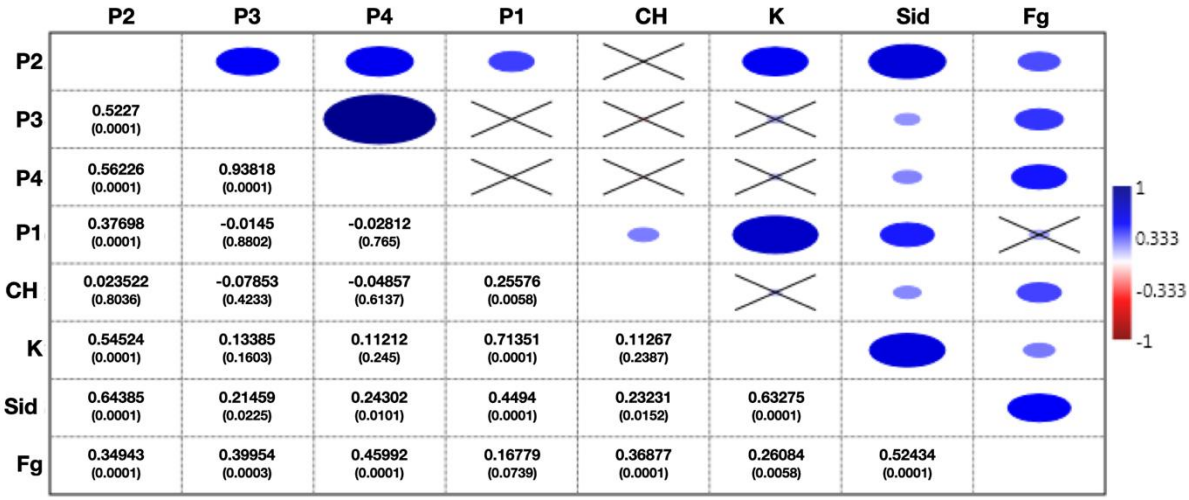

Fig. S2. Correlations between traits assayed in this study. Pearson's correlation coefficient was calculated and *P* values > 0.05 are not significant (between parenthesis at the lower left part of the figure) and are shown with a x at the upper right part of the figure. The scale shows positive correlations (closer to 1) as shades of blue and negative correlations in shades of red. Bioassays were performed for the following traits: P1 - tricalcium phosphate solubilisation, P2 - aluminium phosphate solubilisation, P3 - iron phosphate solubilisation, P4 - phytate mineralisation, CH - hydrolisation of casein, K-potassium solubilisation, Sid - siderophore production, Fg - *Fusarium graminearum* inhibition.

PATENT 1

Manuscript prepared according to the standard of Instituto Nacional da Propriedade Industrial

Biofungicida a base do fungo *Muscodor* sp. para o controle de podridões em frutos embalados

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Natureza Patente: 10 - Patente de Invenção (PI)

Título da Invenção ou Modelo de Utilidade (54): Biofungicida a base do fungo Muscodor sp. para o controle depodridões em frutos embalados

Resumo: A presente invenção provê um conjunto de compostos orgânicos voláteis com ação fungicida que é produzido naturalmente pelo isolado Muscodor sp. FTB01. Mais particularmente, a invenção refere-se a uma formulação de Muscodor e método para preparar eusar esta formulação. Esta invenção inclui um processo e produto para controle do fungo fitopatogênico Botrytis cinerea em morangosembalados. No entanto, a técnica pode ser expandida para o controle de outros fungos fitopatogênicos e o procedimento tambémpode ser aplicado em outros frutos e vegetais em pós-colheita. Estainvenção é considerada sustentável, pois é de origem biológica e não interfere no meio ambiente.

Figura a publicar: Fig 1

Dados do Inventor (72)

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CEP:

País: BRASIL

Telefone:

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Email:

Documentos anexados

Tipo Anexo	Nome
Contrato Social	1. Estatuto da UFLA.pdf
Portaria	2. Portaria de nomeação de reitor - JOÃO CHRYSOSTOMO.pdf
Comprovante de pagamento de GRU 200	3. Comprovante de pagamento.pdf
Documento de Cessão	4. Declaracao de inventor - Larissa.pdf
Documento de Cessão	5. Declaração de inventor Jorge.pdf
Documento de Cessão	6. Declaração de inventor - Fernando.pdf
Documento de Cessão	7. Declaração de inventor - Allan.pdf
Documento de Cessão	8. Declaração de inventor - Márcio.pdf
Contrato de trabalho	9. Jorge - declaracaoFuncional.pdf
Procuração	10. PROCURAÇÃO.pdf
Resumo	11. Resumo.pdf
Desenho	12. Figuras.pdf
Relatório Descritivo	13. Relatorio descritivo.pdf
Reivindicação	14. Reinvindicacoes.pdf

Acesso ao Patrimônio Genético

☑ Declaração Negativa de Acesso - Declaro que o objeto do presente pedido de patente de invenção não foi obtido em decorrência de acesso à amostra de componente do Patrimônio Genético
Brasileiro, o acesso foi realizado antes de 30 de junho de 2000, ou não se aplica.

Declaração de veracidade

☑ Declaro, sob as penas da lei, que todas as informações acima prestadas são completas e verdadeiras.

ESTATUTO

TÍTULO I
DA UNIVERSIDADE

Art. 1º A Universidade Federal de Lavras - UFLA, instituição federal de ensino superior, com sede e foro na cidade de Lavras, Estado de Minas Gerais, criada pela Lei nº 8.956 de 15 de dezembro de 1994, por transformação da Escola Superior de Agricultura de Lavras, fundada em 1908, federalizada pela Lei nº
4.307 de 23 de dezembro de 1963 e transformada em autarquia de regime especial pelo Decreto nº 70.686 de 07 de junho de 1972, é pessoa jurídica de direito público, regendo-se pela legislação federal vigente, por este Estatuto, pelo Regimento Geral e pelas resoluções e normas emanadas dos Conselhos Universitário e de Ensino, Pesquisa e Extensão.

Art. 2º A Universidade gozará de autonomia didático-científica, disciplinar, administrativa e de gestão financeira e patrimonial, nos termos da legislação vigente, e reger-se-á pelos seguintes princípios:

I. liberdade de ensino, pesquisa e extensão, bem como de divulgação do pensamento, da arte e do saber;
II. pluralismo de idéias e de concepções pedagógicas;
III. gestão democrática e participativa;
IV. valorização dos seus recursos humanos;
V. indissociabilidade entre ensino, pesquisa e extensão;
VI. respeito à pessoa e a seus direitos fundamentais;
VII. intercâmbio permanente com instituições nacionais, estrangeiras e internacionais;
VIII. compromisso com a paz, com a defesa dos direitos humanos e com a preservação do meio ambiente;
IX. compromisso com a cultura, a ética, a liberdade e a democracia;
X. compromisso com a formação de cidadãos altamente qualificados para o exercício profissional;
XI. compromisso com o desenvolvimento econômico, o bem estar social e a melhoria da qualidade de vida da população brasileira.

CAPÍTULO I
DOS OBJETIVOS

Art. 3º A Universidade Federal de Lavras tem por objetivos:

I. estimular a criação cultural e o desenvolvimento do espírito científico e do pensamento reflexivo;
II. formar diplomados nas diferentes áreas de conhecimento, aptos para a inserção em setores profissionais e para a participação no desenvolvimento da sociedade brasileira, e colaborar na sua formação contínua;
III. incentivar o trabalho de pesquisa e investigação científica, visando ao desenvolvimento da ciência e da tecnologia e da criação e difusão da

cultura, e, desse modo, desenvolver o entendimento do ser humano e domeio em que vive;

IV. promover a divulgação de conhecimentos culturais, científicos e técnicosque constituem patrimônio da humanidade e comunicar o saber através do ensino, de publicações ou de outras formas de comunicação;

V. suscitar o desejo permanente de aperfeiçoamento cultural e profissional e possibilitar a correspondente concretização, integrando os conhecimentos que vão sendo adquiridos numa estrutura intelectualsistematizadora do conhecimento de cada geração;

VI. estimular o conhecimento dos problemas do mundo presente, em particular os nacionais e regionais, prestar serviços especializados à comunidade e estabelecer com esta uma relação de reciprocidade;

VII. promover a extensão, junto à população, visando à difusão das conquistas e benefícios resultantes da criação cultural e da pesquisa científica e tecnológica geradas na instituição;

VIII. desenvolver as ciências, as letras, as artes, o esporte e a saúde, visando à preservação e à melhoria da qualidade de vida.

CAPÍTULO II
DA
ORGANIZAÇÃO

Art. 4º A Universidade se organizará com base nos seguintes princípios:

I. unidade de patrimônio e administração;

II. estrutura orgânica com base em departamentos;

III. unidade das funções de ensino, pesquisa e extensão, vedada aduplicação de meios para fins idênticos ou equivalentes;

IV. plena utilização dos recursos humanos, materiais e de qualquer ordem;

V. universalidade do conhecimento;

VI. flexibilidade de métodos e critérios, com vista às peculiaridades regionais e às possibilidades de combinação dos conhecimentos, para novos cursos e programas de pesquisa e de extensão.

TÍTULO II
DA ADMINISTRAÇÃO UNIVERSITÁRIA

Art. 5º São órgãos da administração superior da Universidade:

I. Conselho Universitário;

II. Conselho de Ensino, Pesquisa e Extensão;

III. Conselho de Curadores;

IV. Reitoria.

CAPÍTULO I
DO CONSELHO UNIVERSITÁRIO

SEÇÃO I
DA ORGANIZAÇÃO

Art. 6º Os órgãos colegiados e comissões terão, em qualquer caso, no mínimo,setenta por cento de docentes em sua composição.

Art. 7º O Conselho Universitário é o órgão superior de deliberação coletiva da Universidade, em matéria de administração financeira e política universitária, e se compõe:

I. do Reitor - como seu presidente;
II. do Vice-Reitor - como seu vice-presidente;
III. de um representante do Conselho de Ensino, Pesquisa e Extensão, eleito por seus pares;
IV. dos chefes dos Departamentos Didático-Científicos;
V. de representantes das classes pertencentes à Carreira do Magistério Superior previstas na legislação vigente, eleitos por seus pares, de acordo com a proporcionalidade definida pelo Conselho Universitário no seu Regimento Interno;
VI. de dois representantes da comunidade lavrense sem vínculo empregatício com a Universidade, escolhidos pelos membros do Conselho Universitário, entre indicações de clubes de serviço, associações ou outras entidades representativas da sociedade civil;
VII. de cinco representantes dos servidores técnico-administrativos, eleitos por seus pares;
VIII. de quatro representantes do corpo discente, sendo dois dos cursos de graduação e dois dos cursos de pós-graduação, eleitos por seus pares.

§ 1º O mandato dos representantes do Conselho de Ensino, Pesquisa e Extensão, das classes docentes, da comunidade e dos servidores técnico- administrativos, será de dois anos, permitida uma recondução.

§ 2º O mandato dos representantes do corpo discente será de um ano, permitida uma recondução.

§ 3º Juntamente com os membros representantes, serão eleitos suplentes que completarão o mandato, em caso de impedimento definitivo do titular.

SEÇÃO II
DO FUNCIONAMENTO

Art. 8º O funcionamento do Conselho Universitário será definido no seu Regimento.

SEÇÃO III
DA COMPETÊNCIA

Art. 9º Compete ao Conselho Universitário:

I. formular a política global da Universidade;
II. aprovar normas para a avaliação do desempenho institucional;
III. aprovar o Estatuto e o Regimento Geral da Universidade, submetendo-os à autoridade competente para a aprovação final;

IV. aprovar alterações e emendas a este Estatuto e ao Regimento Geral, por, pelo menos, dois terços da totalidade de seus membros, em sessão especialmente convocada, submetendo-as à autoridade competente para homologação;
V. aprovar o seu regimento interno e os dos órgãos de administração, ensino, pesquisa e extensão e dos órgãos suplementares;
VI. aprovar as vinculações orgânicas dos órgãos Suplementares;
VII. aprovar a criação, agregação, desmembramento, incorporação ou fusãoe extinção de órgãos ou unidades;
VIII. aprovar a criação, a organização e a extinção, em sua sede, de cursos eprogramas, por proposta do Conselho de Ensino, Pesquisa e Extensão;
IX. aprovar a alienação de bens imóveis;
X. aprovar os símbolos da Universidade;
XI. dispor sobre a elaboração e execução do orçamento da Universidade;
XII. aprovar o relatório anual de atividades e a prestação de contas doReitor;
XIII. organizar, em reunião conjunta com o Conselho de Ensino, Pesquisa e Extensão e Conselho de Curadores, as listas de nomes para a escolha enomeação do Reitor e do Vice-Reitor da Universidade, de acordo com a legislação vigente;
XIV. eleger dois de seus membros docentes, para representá-lo no Conselho de Curadores;
XV. apreciar recursos contra atos do Reitor e do Conselho de Ensino, Pesquisa e Extensão;
XVI. outorgar os títulos de Mérito Universitário, Doutor "Honoris causa", Professor "Honoris causa" e de "Professor Emérito";
XVII. participar da outorga dos graus e diplomas, em sessão solene;
XVIII. criar câmaras e comissões permanentes ou temporárias, para estudo deassuntos específicos;
XIX. deliberar sobre outras matérias atribuídas à sua competência, neste estatuto, no regimento geral e nos regimentos internos, bem como sobreas questões omissas.

CAPÍTULO II
DO CONSELHO DE ENSINO, PESQUISA E EXTENSÃO – CEPE

SEÇÃO I
DA ORGANIZAÇÃO

Art. 10. O Conselho de Ensino, Pesquisa e Extensão - CEPE, órgão superiorde deliberação coletiva, autônomo em sua competência, responsável pela coordenação de todas as atividades de ensino, pesquisa e extensão da Universidade, será integrado pelos seguintes membros:

I. Reitor, como seu presidente;
II. Vice-Reitor, como seu vice-presidente;
III. Pró-Reitores;
IV. 07 (sete) Coordenadores dos Cursos de Graduação, eleitos por seuspares, com mandato de 02 (dois) anos, permitida uma recondução;

V. 04 (quatro) coordenadores dos Cursos de Pós-Graduação, eleitos por seus pares, com mandato de 02 (dois) anos, permitida uma recondução;
VI. 04 (quatro) representantes do corpo discente, eleitos por seus pares, com mandato de 01 (um) ano, permitida uma recondução;
VII. 02 (dois) representantes dos servidores técnico-administrativos, eleitos por seus pares, com mandato de 02 (dois) anos, permitida uma recondução;
VIII. 02 (dois) representantes da comunidade lavrense, sem vínculo empregatício com a Universidade, escolhidos pelos membros do Conselho de Ensino, Pesquisa e Extensão, entre indicações de clubesde serviço, associações ou outras entidades representativas da sociedade civil.

Parágrafo único. Juntamente com os membros representantes, serão eleitos suplentes, que completarão o mandato, em caso de impedimento definitivo do titular.

SEÇÃO II
DO FUNCIONAMENTO

Art. 11. O funcionamento do Conselho de Ensino, Pesquisa e Extensão será definido no seu Regimento.

SEÇÃO III
DA COMPETÊNCIA

Art. 12. Compete ao Conselho de Ensino, Pesquisa e Extensão:

I. estabelecer as diretrizes dos órgãos de ensino, pesquisa e extensão, de modo a coordenar as programações, impedindo a duplicação de meios para fins idênticos ou equivalentes;
II. exercer, como órgão deliberativo e consultivo, a jurisdição universitária nos campos do ensino, da pesquisa e da extensão;
III. aprovar o seu Regimento Interno e as respectivas modificações, por doisterços de seus membros, submetendo-o ao Conselho Universitário;
IV. fixar normas complementares às do Regimento Geral sobre processo seletivo, currículos, matrículas, transferências, verificação do rendimento escolar, revalidação de diplomas de estrangeiros, aproveitamento de estudos, além de outras matérias de sua competência;
V. propor normas para o processo seletivo e fixar o número inicial de vagaspara cada curso;
VI. opinar ou propor sobre a criação, agregação, desmembramento, incorporação ou fusão e extinção de órgãos ou unidades;
VII. opinar e propor sobre a criação e extinção de cursos e programas;
VIII. aprovar alterações curriculares, criar ou extinguir disciplinas ou modificara sua distribuição e denominação, mediante proposta dos Colegiados deCurso;
IX. aprovar ou modificar o calendário escolar;
X. deliberar e propor sobre a criação, desmembramento ou extinção de departamento didático-científico;

XI. deliberar e propor sobre a criação e distribuição de cargos de magistério;
XII. opinar ou propor sobre a celebração de contratos e convênios,referentes ao ensino, pesquisa e extensão;
XIII. propor normas para provimento de cargos de magistério;
XIV. propor a contratação ou rescisão de contrato de pessoal docente;
XV. aprovar critérios para contratação de professores visitantes e substitutos;
XVI. deliberar sobre o afastamento de pessoal docente e técnico- administrativo;
XVII. eleger um de seus membros para representá-lo no Conselho Universitário;
XVIII. eleger dois de seus membros docentes para representá-lo no Conselho de Curadores;
XIX. organizar, em reunião conjunta com o Conselho Universitário e Conselho de Curadores, as listas de nomes para a escolha e nomeação do Reitor e do Vice-Reitor da Universidade, de acordo com a legislação vigente;
XX. aprovar projetos institucionais de pesquisa, planos de cursos e planosde atividades de extensão;
XXI. deliberar sobre taxas, contribuições e emolumentos;
XXII. criar câmaras e comissões permanentes ou temporárias, para estudo deassuntos específicos;
XXIII. julgar os recursos sobre matéria de sua competência;
XXIV. deliberar originalmente ou em grau de recurso, sobre qualquer outra matéria de sua esfera de competência, não prevista neste Estatuto, no Regimento Geral e nos demais Regimentos Internos.

Parágrafo único. Não se aplica o disposto no inciso XII do *caput* aos convênios de estágios curriculares.

Art. 13. Das decisões do Conselho de Ensino, Pesquisa e Extensão caberá recurso ao Conselho Universitário, em face de razões de legalidade e demérito.

CAPÍTULO III
DO CONSELHO DE CURADORES

SEÇÃO I
DA ORGANIZAÇÃO

Art. 14. O Conselho de Curadores, órgão de fiscalização econômico-financeira da Universidade, compõe-se:

I. de 06 (seis) representantes do Conselho Universitário, escolhidos por seus pares, dentre os docentes, com mandato de dois anos, permitida recondução;

II. de 06 (seis) representantes do Conselho de Ensino, Pesquisa e Extensão, escolhidos por seus pares, dentre os docentes, com mandato de dois anos, permitida uma recondução;

III. de um representante do Ministério da Educação, com mandato de dois anos;

IV. de um representante do corpo discente, eleito por seus pares, com mandato de um ano, permitida uma recondução;

V. de dois representantes da comunidade externa, escolhido pelo Conselho Universitário, dentre os cidadãos que residam há mais de cinco anos emLavras e que estejam em exercício de profissão definida, com mandato de dois anos, vedada a recondução;

VI. de um representante dos servidores técnico-administrativos, eleitos por seus pares, com mandato de dois anos, permitida uma recondução.

SEÇÃO II
DO FUNCIONAMENTO

Art. 15. O Conselho de Curadores reunir-se-á em sessão ordinária, no início decada ano ou quando convocado por seu Presidente ou por um terço de seus membros.

Parágrafo único. Em sua primeira reunião, os membros do Conselho deCuradores elegerão o seu presidente.

SEÇÃO III
DA COMPETÊNCIA

Art. 16. Ao Conselho de Curadores, compete:

I. fiscalizar os atos inerentes à execução orçamentária, examinando ou mandando examinar, a qualquer tempo, a contabilidade e documentaçãorespectiva;
II. analisar a prestação de contas anual do Reitor e emitir parecer conclusivo, para encaminhamento ao Conselho Universitário;
III. organizar, em reunião conjunta com o Conselho Universitário e Conselho de Ensino, Pesquisa e Extensão, as listas de nomes para a escolha e nomeação do Reitor e do Vice-Reitor da Universidade, de acordo com a legislação vigente.

CAPÍTULO IV
Da Reitoria

Art. 17. A Reitoria, órgão executivo que superintende todas as atividadesuniversitárias, é exercida pelo Reitor e compreende:

I. Pró-Reitorias;
II. Órgãos de Apoio e Assessoramento;
III. Órgãos da Administração Geral;
IV. Órgãos Suplementares.

Parágrafo único. A constituição, atribuições e competências das unidades a que se refere este artigo serão definidas no Regimento Geral e nos Regimentos Internos.

Art. 18. Ao Reitor, compete representar a Universidade, coordenar e superintender todas as atividades universitárias.

Art. 19. O Reitor será substituído, em suas faltas e impedimentos, pelo Vice- Reitor, e este em caráter transitório, pelo Pró-Reitor previamente designado pelo Reitor.

Art. 20. O Reitor e o Vice-Reitor serão eleitos e nomeados em conformidade com o disposto na legislação vigente.

Parágrafo único. O mandato do Reitor, salvo disposição legal em contrário, será de quatro anos.

TÍTULO III
DA ADMINISTRAÇÃO DO ENSINO, DA PESQUISA E DA EXTENSÃO

CAPÍTULO I
Dos Departamentos Didático-Científicos

Art. 21. O Departamento é a menor fração da estrutura universitária para todosos efeitos de organização administrativa, didático-científica e de distribuição de pessoal e compreenderá disciplinas afins.

Art. 22. A administração de cada departamento será exercida:

I. pelo Chefe do Departamento;
II. pela Assembléia Departamental.

Art. 23. O Chefe e o Subchefe do Departamento serão eleitos pela Assembléia Departamental, dentre os docentes do quadro permanente, e terão mandatos coincidentes com o do Reitor.

§ 1º Nas faltas e impedimentos do Chefe, a chefia será exercida pelo Subchefe.

§ 2º Nas faltas e impedimentos do Chefe e do Subchefe, exercerá a chefia o docente mais antigo, no exercício do magistério, no Departamento.

Art. 24. Integram a Assembléia Departamental os docentes em exercício e as representações discente e técnico-administrativa.

§ 1º A representação discente será de até 15% dos integrantes da Assembléia Departamental, com mandato de um ano, permitida uma recondução.

§ 2º A representação dos servidores técnico-administrativos será de até 15% dos integrantes da Assembléia Departamental, eleita pelos servidores lotados no Departamento, com mandato de um ano, permitida uma recondução.

Art. 25. O Departamento, como elemento fundamental da estrutura universitária, é aberto a toda a Universidade, em decorrência do princípio que veda a duplicidade de meios para fins idênticos ou equivalentes.

Art. 26. As atribuições e funcionamento do Departamento serão estabelecidas no Regimento Geral e no Regimento Interno de cada departamento, aprovado pelo Conselho Universitário.

CAPÍTULO II
Dos Colegiados de Curso

Art. 27. O Colegiado de Curso, responsável pelo gerenciamento acadêmico-administrativo dos cursos, será composto de sete membros, sendo:

I. um coordenador indicado pelo Pró-Reitor respectivo;
II. quatro representantes dos docentes envolvidos no curso, escolhidos pelo coordenador e homologados pelo Pró-Reitor respectivo;
III. um representante discente, de graduação ou de pós-graduação, do curso em questão, eleito pelos seus pares, com mandato de um ano, permitida uma recondução;
IV. um representante dos servidores técnico-administrativos, envolvidos com as atividades do respectivo curso, com mandato de dois anos, permitida a recondução.

Parágrafo único. As atribuições e funcionamento do Colegiado de Curso serão estabelecidas no Regimento Geral e no Regimento Interno de cada Colegiado, aprovados pelo Conselho Universitário.

CAPÍTULO III
Do Ensino

Art. 28. O ensino, atividade básica da Universidade Federal de Lavras, abrangerá os seguintes cursos e programas:

I. cursos seqüenciais por campo de saber, de diferentes níveis de abrangência, abertos a candidatos que atendam aos requisitos estabelecidos pelos órgãos competentes;
II. de graduação, abertos a candidatos que tenham concluído o ensino médio ou equivalente e tenham sido classificados em processo seletivo;
III. de pós-graduação, compreendendo programas de mestrado e doutorado, cursos de especialização, aperfeiçoamento e outros, abertos a candidatos diplomados em cursos de graduação e que atendam às exigências estabelecidas pelos órgãos competentes;

IV. de extensão, abertos a candidatos que atendam aos requisitos estabelecidos em cada caso pelo CEPE;

§ 1º A Universidade ministrará cursos presenciais e a distância, de acordo com a legislação vigente

§ 2º Aos alunos regulares é assegurada a orientação acadêmica sistemática,na forma definida no Regimento Geral e nas resoluções do Conselho de Ensino, Pesquisa e Extensão.

Art. 29. Os cursos de graduação têm como objetivo a formação de profissionais para o exercício de atividades que demandem estudos superiores.

Art. 30. Os cursos de pós-graduação têm como objetivo a formação de docentes, pesquisadores e profissionais de alto nível.

Art. 31. Os demais cursos terão os objetivos, a organização, a estrutura e as exigências previstos em cada caso.

Parágrafo único. As disposições referentes a esses cursos deverão ser estabelecidas em documento próprio registrado na Pró-Reitoria responsável.

CAPÍTULO IV
Da Pesquisa

Art. 32. A pesquisa é atividade básica da Universidade Federal de Lavras, devendo ser estimulada a aplicação de seus resultados através da extensão.

Parágrafo único. A elaboração e execução dos programas de pesquisa estarão a cargo dos Departamentos didático-científicos, isolada ou conjuntamente.

Art. 33. A Universidade incentivará a pesquisa por todos os meios possíveis, consoante os recursos e meios que dispuser e com os que conseguir atravésde convênios, acordos e ajustes.

Parágrafo único. A Universidade consignará, anualmente, em seu orçamento, recursos destinados à pesquisa.

CAPÍTULO V
Da Extensão

Art. 34. A extensão, atividade básica da Universidade Federal de Lavras,deverá alcançar toda a comunidade ou dirigir-se a pessoas e instituições públicas ou privadas, abrangendo cursos, estágios e serviços, que serão realizados no cumprimento de programas específicos.

§ 1º A extensão será realizada, com vistas à integração com a sociedade, emtodos os setores de atividade da Universidade e estará a cargo dos

Departamentos didático-científicos, dos docentes, dos discentes e de profissionais designados pela autoridade competente.

§ 2º A Universidade incentivará a extensão, através da reformulação permanente do seu programa orgânico específico, respondendo às iniciativas de fomento oficiais de extensão universitária e buscando parcerias com agentes sociais potenciais em cooperação na área de geração e difusão de ciência e tecnologia.

§ 3º A Universidade consignará, anualmente, em seu orçamento, recursos destinados à extensão.

TÍTULO IV
DA COMUNIDADE UNIVERSITÁRIA

Art. 35. A comunidade universitária é constituída por docentes, discentes e técnico-administrativos, diversificados em suas atribuições e funções eunificados nas finalidades e objetivos da Universidade.

Art. 36. Os requisitos exigidos dos membros da comunidade universitária, bem como seus direitos e deveres se pautam nos princípios de humanização, de respeito à pessoa, nas finalidades, objetivos, atribuições e competências expressos neste Estatuto, no Regimento Geral, nos regimentos internos, em normas e regulamentos pertinentes e na legislação superior vigente.

CAPÍTULO I
Do Corpo Docente

Art. 37. O Corpo Docente da Universidade é constituído por docentes que exerçam atividades de ensino, pesquisa e extensão ou que ocupem cargos administrativos ou técnicos, na qualidade de professor.

Art. 38. O ingresso, a nomeação, a posse, o regime de trabalho, a promoção, oacesso, a aposentadoria e a dispensa de docente são regidas pela legislação em vigor, pelo Regimento Geral, pelo Plano de Carreira da Universidade epelas resoluções dos Conselhos Universitário e de Ensino, Pesquisa e Extensão.

Art. 39. Haverá uma Comissão Permanente de Pessoal Docente - CPPD, com atribuições e constituição previstas em lei, neste Estatuto, no Regimento Geral e no seu Regimento Interno, destinada a assessorar os órgãos da Administração Superior, na formulação e execução da política referente ao pessoal docente.

CAPÍTULO II
Do Corpo Discente

Art. 40. O corpo discente é constituído por alunos regulares e especiais.

§ 1º Aluno regular é aquele matriculado em curso de graduação ou pós-graduação.

§ 2º Aluno especial é aquele inscrito em outros cursos, disciplinas isoladas ou atividades congêneres.

Art. 41. A Universidade prestará, de acordo com suas disponibilidades, assistência ao corpo discente, sem prejuízo de suas responsabilidades para com os demais membros da comunidade, fomentando, entre outras iniciativas: programas de alimentação, alojamento e saúde;

I. promoções de natureza cultural, artística, esportiva e recreativa;
II. programas de bolsas de estudo, de extensão, de iniciação científica, deestágio e monitoria;
III. orientação psicopedagógica e profissional;
IV. crescimento psicológico, político, cívico e democrático, pressupostosbásicos para a formação integral do cidadão.

CAPÍTULO III
Do Corpo Técnico-Administrativo

Art. 42. O corpo técnico-administrativo da Universidade é constituído por servidores integrantes do Quadro, que exerçam atividades técnicas, administrativas e operacionais, necessárias ao cumprimento dos objetivos institucionais.

Art. 43. O ingresso, a nomeação, a posse, o regime de trabalho, a promoção, oacesso, a aposentadoria e a dispensa de servidor técnico-administrativo são regidas pela legislação em vigor, pelo Regimento Geral, pelo Plano de Carreira da Universidade e pelas resoluções do Conselho Universitário e Conselho de Ensino, Pesquisa e Extensão.

Art. 44. Haverá uma Comissão Permanente de Pessoal Técnico-Administrativo – CPPTA, com atribuições e constituição previstas em lei, neste Estatuto e no Regimento Geral, destinada a assessorar os órgãos da Administração Superior, na formulação e execução da política referente ao pessoal técnico- administrativo.

TÍTULO V
DOS DIPLOMAS, CERTIFICADOS E TÍTULOS

Art. 45. Ao aluno regular que concluir curso de graduação ou de pós- graduação, com observância das exigências contidas na legislação em vigor, neste Estatuto e no Regimento Geral, a Universidade conferirá o grau e expedirá o correspondente diploma.

Art. 46. Ao aluno especial que concluir cursos de aperfeiçoamento, especialização e outros, disciplina isolada ou atividade de outra natureza, a Universidade expedirá o correspondente certificado.

Art. 47. A Universidade poderá conferir e expedir títulos de:

I. Mérito Universitário, a membro da comunidade que tenha se distinguido por relevantes serviços à Universidade;
II. Professor Emérito, a docente aposentado ou ex-docente daUniversidade Federal de Lavras, que tenha alcançado posição eminente em atividades universitárias;
III. Professor *Honoris causa*, a professor ou cientista ilustre não pertencente à Universidade Federal de Lavras, que a esta tenha prestado relevantes serviços;
IV. Doutor *Honoris causa*, a personalidade que tenha se distinguido pelo saber ou pela atuação em prol das ciências, artes e do bem-estar humano.

TÍTULO VI
DO PATRIMÔNIO, DOS RECURSOS E DO REGIME FINANCEIRO

Art. 48. O Patrimônio da Universidade administrado pelo Reitor, comobservância dos preceitos legais e regulamentares, é constituído:

I. pelos bens e direitos que integravam o patrimônio da Escola Superior de Agricultura de Lavras, os quais foram automaticamente transferidos,sem reservas ou condições, à Universidade Federal de Lavras, nos termos da Lei 8.956, de 15 de dezembro de 1994;
II. pelos bens e direitos que a Universidade vier a adquirir;
III. pelas doações ou legados que receber;
IV. por incorporações que resultem de serviços realizados pelaUniversidade.

Art. 49. Os recursos financeiros da Universidade serão provenientes de:

I. dotação que lhe for anualmente consignada no Orçamento da União;
II. dotações, auxílios, doações e subvenções que lhe venham a ser feitas ou concedidas pela União, Estados e Municípios, ou por quaisquer entidades, públicas ou privadas;
III. remuneração por serviços prestados a entidades públicas ou privadas, mediante contratos específicos;
IV. taxas, anuidades e emolumentos que forem cobrados pela prestação de serviços educacionais e outros, com observância da legislação pertinente;
V. resultado de operações de crédito e juros bancários, nos termos da lei;
VI. receitas eventuais;
VII. saldo de exercícios anteriores.

Art. 50. Os bens e direitos da Universidade serão utilizados ou aplicadosexclusivamente na realização de seus objetivos.

Art. 51. A movimentação de recursos financeiros e a sua contabilização ficarãoa cargo da Reitoria.

Parágrafo único. O produto de qualquer arrecadação na Universidade será recolhido conforme determina a Reitoria, sendo vedada a retenção de renda nos setores da Universidade.

Art. 52. O Reitor poderá delegar competência aos Pró-Reitores, Chefes de Departamento, Prefeito do Campus e Coordenadores de Cursos e de Convênio, para realização de despesas, den¬tro de limites e normas estabelecidas.

TÍTULO VII
DAS DISPOSIÇÕES GERAIS E TRANSITÓRIAS

Art. 53. As atividades relativas ao ensino, pesquisa, extensão, administração e outras decorrentes de eleição, designação, indicação, exercício de função oude atribuições, constituem deveres do corpo docente, técnico-administrativo e discente.

Parágrafo único. O não cumprimento das obrigações decorrentes de atividades de que trata este artigo torna o docente, o servidor técnico- administrativo e o discente sujeitos à atribuição de faltas, sem prejuízo de outras penalidades cabíveis.

Art. 54. O presente Estatuto poderá ser modificado pelo Conselho Universitário, mediante proposta aprovada por, no mínimo, dois terços de seus membros, devendo as alterações serem aprovadas pelo órgão superior competente.

Art. 55. Este Estatuto entra em vigor após a aprovação do Conselho Nacional de Educação e homologação pelo Ministério da Educação, revogadas as disposições em contrário.

Resolução CG nº 066, de 12/6/1995
Resolução CG nº 083, de 10/7/1995
Resolução CUNI nº 035, de 23/11/1998
Resolução CUNI nº 059, de 12/8/1999
Resolução CUNI nº 016, de 5/4/2006
Resolução CUNI nº 021, de 2/5/2007

Aprovado pela Portaria MEC nº 959, de 3/8/95, publicada no DOU de 4/8/95, alterado pelas Portarias MEC nº 1.591, de 28/10/99 (DOU de 3/11/99) e nº 66,de 17/1/07 (DOU de 19/1/07).

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REPÚBLICA FEDERATIVA DO BRASIL • IMPRENSA NACIONAL

Ano LXI Nº 83 Brasília - DF, segunda-feira, 4 de maio de 2020 SEÇÃO 2

Sumário

..... Esta edição completa do DOU é composta de 54 páginas.....

Atos do Poder Executivo

MINISTÉRIO DA EDUCAÇÃO

DECRETOS DE 30 DE ABRIL DE 2020

O PRESIDENTE DA REPÚBLICA, no uso da atribuição que lhe confere o art. 84, **caput**, inciso XXV, da Constituição, e tendo em vista o disposto no art. 16, **caput**, inciso I, da Lei nº 5.540, de 28 de novembro de 1968, e no art. 11 da Medida Provisória nº 914, de 24 de dezembro 2019, resolve:

NOMEAR

a partir de 11 de maio de 2020, MARCELO BRITO CARNEIRO LEÃO, Professor da Universidade Federal Rural de Pernambuco, para exercer o cargo de Reitor da referida Universidade, com mandato de quatro anos.

Brasília, 30 de abril de 2020; 199º da Independência e 132º da República.

JAIR MESSIAS BOLSONARO
Abraham Bragança de Vasconcellos Weintraub

NOMEAR

Brasília, 30 de abril de 2020; 199º da Independência e 132º da República.

JAIR MESSIAS BOLSONARO
Abraham Bragança de Vasconcellos Weintraub

Presidência da República

CASA CIVIL

PORTARIAS DE 30 DE ABRIL

DE 2020MINISTÉRIO DA SAÚDE

O MINISTRO DE ESTADO CHEFE DA CASA CIVIL DA PRESIDÊNCIA DA REPÚBLICA, no uso de suas atribuições e tendo em vista o disposto no art. 4º do Decreto nº 9.794, de 14 de maio de 2019, resolve:

Nº 208 - **EXONERAR**

DENIZAR VIANNA ARAÚJO do cargo de Secretário de Ciência, Tecnologia, Inovação e Insumos Estratégicos em Saúde do Ministério da Saúde, código DAS 101.6.

Nº 209 - **NOMEAR**

ANTONIO CARLOS CAMPOS DE CARVALHO, para exercer o cargo de Secretário de Ciência, Tecnologia, Inovação e Insumos Estratégicos em Saúde do Ministério da Saúde, código DAS 101.6.

WALTER SOUZA BRAGA NETTO

SECRETARIA EXECUTIVA

PORTARIA Nº 92, DE 30 DE ABRIL DE 2020

O SECRETÁRIO-EXECUTIVO DA CASA CIVIL DA PRESIDÊNCIA DA REPÚBLICA, no uso da competência que lhe foi subdelegada pela Portaria nº 2.207, de 22 de novembro de 2016, do Ministro de Estado Chefe da Casa Civil da Presidência da República, e tendo emvista o disposto no Decreto nº 9.794, de 14 de maio de 2019, resolve:

NOMEAR

MARIA JOSE GOMES MELLO RIBEIRO para exercer o cargo de Assessor Técnico, código DAS 102.3, no Gabinete do Ministro da Casa Civil da Presidência da República, ficando exonerada do que atualmente ocupa.

SERGIO JOSÉ PEREIRA

PORTARIA Nº 93, DE 30 DE ABRIL DE 2020

O SECRETÁRIO-EXECUTIVO DA CASA CIVIL DA PRESIDÊNCIA DA REPÚBLICA, no uso da competência que lhe foi subdelegada pela Portaria nº 2.207, de 22 de novembro de 2016, do Ministro de Estado Chefe da Casa Civil da Presidência da República, alterada pela Portaria nº 2.238, de 5 de dezembro de 2016, e tendo em vista o disposto no Decreto nº 9.794, de 14 de maio de 2019, resolve:

DESIGNAR

SYMONE OLIVEIRA LIMA para exercer a Função Comissionada do Poder Executivo, código FCPE 102.2, Assistente, na Subchefia de Análise e Acompanhamento de Políticas Governamentais da Casa Civil da Presidência da República, ficando exonerada do cargo que atualmente ocupa.

SERGIO JOSÉ PEREIRA

PORTARIA Nº 94, DE 30 DE ABRIL DE 2020

O SECRETÁRIO-EXECUTIVO DA CASA CIVIL DA PRESIDÊNCIA DA REPÚBLICA, no uso da competência que lhe foi subdelegada pela Portaria nº 2.207, de 22 de novembro de 2016, do Ministro de Estado Chefe da Casa Civil da Presidência da República, e tendo emvista o disposto no Decreto nº 9.794, de 14 de maio de 2019, resolve:

NOMEAR

RENATO HOLANDA DE ALCANTARA para exercer o cargo de Assessor, código DAS 102.4, no Gabinete do Ministro da Casa Civil da Presidência da República, ficando exonerado do que atualmente ocupa.

SERGIO JOSÉ PEREIRA

PORTARIA Nº 95, DE 30 DE ABRIL DE 2020

O SECRETÁRIO-EXECUTIVO DA CASA CIVIL DA PRESIDÊNCIA DA REPÚBLICA, no uso da competência que lhe foi subdelegada pela Portaria nº 2.207, de 22 de novembro de 2016, do Ministro de Estado Chefe da Casa Civil da Presidência da República, e tendo emvista o disposto no Decreto nº 9.794, de 14 de maio de 2019, resolve:

EXONERAR, a pedido,

FELIPE MORENO PARANHOS do cargo de Assistente Técnico, código DAS 102.1, da Coordenação do Gabinete da Subchefia de Articulação e Monitoramento da Casa Civil da Presidência da República, a contar de 29 de abril de 2020.

SERGIO JOSÉ PEREIRA

PORTARIA Nº 96, DE 30 DE ABRIL DE 2020

O SECRETÁRIO-EXECUTIVO DA CASA CIVIL DA PRESIDÊNCIA DA REPÚBLICA, no uso da competência que lhe foi subdelegada pela Portaria nº 2.207, de 22 de novembro de 2016, do Ministro de Estado Chefe da Casa Civil da Presidência da República, alterada pela Portaria nº 2.238, de 5 de dezembro de 2016, e tendo em vista o disposto no Decreto nº 9.794, de 14 de maio de 2019, resolve:

DESIGNAR

ROBERTO ENDRIGO ROSA para exercer a Função Comissionada do Poder Executivo, código FCPE 103.4, Gerente de Projeto, na Subchefia Adjunta de Finanças Públicas da Subchefia de Análise e Acompanhamento de Políticas Governamentais da Casa Civil da Presidência da República.

SERGIO JOSÉ PEREIRA

SECRETARIA DE GOVERNO

SECRETARIA EXECUTIVA

PORTARIA Nº 112, DE 30 DE ABRIL DE 2020

O SECRETÁRIO-EXECUTIVO DA SECRETARIA DE GOVERNO DA PRESIDÊNCIA DA REPÚBLICA, no uso da competência que lhe foi subdelegada pelo art. 3º da Portaria nº 117, de 31 de dezembro de 2015, do Ministro de Estado Chefe da Secretaria de Governo da Presidência da República, resolve:

NOMEAR

LOUISE CAROLINE CAMPOS LOW para exercer o cargo de Assessor Técnico na Coordenação-Geral de Acompanhamento Financeiro da Diretoria de Acompanhamento do Orçamento Impositivo da Secretaria Especial de Relações Institucionais da Secretaria de Governo da Presidência da República, código DAS 102.3.

JÔNATHAS ASSUNÇÃO DE CASTRO

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2 DECLARAÇÃO DE INVENTOR

DECLARAÇÃO DE INVENTOR

Biofungicida a base do fungo *Muscodor* sp. para o controle de podridões em frutos embalados

O abaixo assinado **Larissa Maia de Oliveira,** brasileiro, divorciada, CPF 081.278.746-39, residente à Rua Cesário Alvim, 188, Bairro Centro, cidade Patrocínio/ Minas Gerais, aluna do Curso de Agronomia, Departamento de Fitopatologia, tendo conhecimento do requerimento de Patente feito em nome da Universidade Federal de Lavras – UFLA, Caixa Postal 3037, CEP 37200-000, na cidade de Lavras, estado de Minas Gerais, CNPJ nº 22.078.679/0001-74, e da Lallemand Soluções Agrobiológicas LTDA, situada na Av. Julia Fernandes Caixeta, 555 Cidade Nova, Patos de Minas-MG, cnpj 07.983.734/0001-87, nos termos do parágrafo 2º do artigo 6º e do artigo 88 da Lei 9.279 de 14/05/1996, declara que nada tem a opor e dá pleno consentimento a que o Instituto Nacional da Propriedade Industrial conceda às aludidas Universidade Federal de Lavras e Lallemand Soluções Agrobiológicas LTDA, tal privilégio.

Lavras, 29 de outubro de 2020.

Larissa Maia de Oliveira

Larissa Maia de Oliveira

DECLARAÇÃO DE INVENTOR

Biofungicida a base do fungo *Muscodor* sp. para o controle de podridões em frutos embalados

O abaixo assinado **Jorge Teodoro de Souza,** brasileiro, casado, CPF 501667861-87, residente à Rua Goiânia, 72, Bairro Jardim Campestre III, Lavras, (Professor), do Curso de Agronomia, Departamento de Fitopatologia, tendo conhecimento do requerimento de Patente feito em nome da Universidade Federal de Lavras – UFLA, Caixa Postal 3037, CEP 37200-000, na cidade de Lavras, estado de Minas Gerais, CNPJ nº 22.078.679/0001-74, e da Lallemand Soluções Agrobiológicas LTDA, situada na Av. Julia Fernandes Caixeta, 555 Cidade Nova, Patos de Minas-MG, cnpj 07.983.734/0001- 87, nos termos do parágrafo 2º do artigo 6º e do artigo 88 da Lei 9.279 de 14/05/1996, declara que nada tem a opor e dá pleno consentimento a que o Instituto Nacional da Propriedade Industrial conceda às aludidas Universidade Federal de Lavras e Lallemand Soluções Agrobiológicas LTDA, tal privilégio.

Lavras, 08 de dezembro de 2020.

Jorge Teodoro de Souza

DECLARAÇÃO DE INVENTOR

Biofungicida a base do fungo Muscodor sp. para o controle de podridões em frutos embalados

O abaixo assinado Fernando Pereira Monteiro, brasileiro, Casado, CPF 017.359.951-69 residente na Rua das Palmeiras, Bairro Eldorado, Cidade Lavras – Minas Gerais, (Pesquisador), em pós-doutoramento no Departamento de Fitopatologia, tendo conhecimento do requerimento de Patente feito em 14 de Fevereiro de 2017, em nome da Universidade Federal de Lavras – UFLA, Caixa Postal 3037, CEP 37200-000, na cidade de Lavras, estado de Minas Gerais, CNPJ nº 22.078.679/0001-74, e da Lallemand Solucções Agrobiológicas LTDA, situada na Av. Julia Fernandes Caixeta, 555 Cidade Nova, Patos de Minas-MG, CNPJ nº 07.983.734/0001-87, nos termos do parágrafo 2º do artigo 6º e do artigo 88 da Lei 9.279 de 14/05/1996, declara que nada tem a opor e dá pleno consentimento a que o Instituto Nacional da Propriedade Industrial conceda às aludidas Universidade Federal de Lavras e Lallemand Soluções Agrobiológicas LTDA, tal privilégio.

Lavras, 14 de fevereiro de 2017.

Fernando Pereira Monteiro

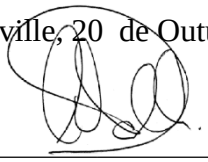
Fernando Pereira Monteiro

Digitalizado com CamScanner

Biofungicida a base do fungo *Muscodor* sp. para o controle de podridões em frutos embalados

O abaixo assinado Alan William Vilela Pomella, brasileiro, casado, CPF 819337206-97, residente à 7131 SW 80th terrace, Gainesville Florida- EUA, Suporte Tecnico e gerente comercial da Lallemand Specialties Inc., tendo conhecimento do requerimento de Patente feito em nome da Universidade Federal de Lavras – UFLA, Caixa Postal 3037, CEP 37200-000, na cidade de Lavras, estado de Minas Gerais, CNPJ nº 22.078.679/0001-74, e da Lallemand Solucções Agrobiológicas LTDA, situada na Av. Julia Fernandes Caixeta, 555 Cidade Nova, Patos de Minas-MG, cnpj 07.983.734/0001- 87, nos termos do parágrafo 2º do artigo 6º e do artigo 88 da Lei 9.279 de 14/05/1996, declara que nada tem a opor e dá pleno consentimento a que o Instituto Nacional da Propriedade Industrial conceda às aludidas Universidade Federal de Lavras e Lallemand Soluções Agrobiológicas LTDA, tal privilégio.

Gainesville, 20 de Outubro de 2020.

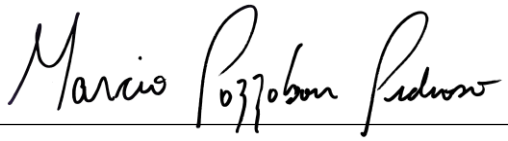


Alan William Vilela Pomella

Biofungicida a base do fungo *Muscodor* sp. para o controle de podridões em frutos embalados

O abaixo assinado **Marcio Pozzobon Pedroso,** brasileiro, casado, CPF 828.435.200-10, residente à Av. Pref. Silvio Damaso de Castro, nº. 231, Bairro Jardim Campestre 3, Cidade Lavras, Professordo Curso de Química, Departamento de Química, tendo conhecimento do requerimento de Patente feito em nome da Universidade Federal de Lavras – UFLA, Caixa Postal 3037, CEP 37200-000, na cidade de Lavras, estado de Minas Gerais, CNPJ nº 22.078.679/0001-74, e da Lallemand Solucções Agrobiológicas LTDA, situada na Av. Julia Fernandes Caixeta, 555 Cidade Nova, Patos de Minas-MG, cnpj 07.983.734/0001-87, nos termos do parágrafo 2º do artigo 6º e do artigo 88 da Lei 9.279 de 14/05/1996, declara que nada tem a opor e dá pleno consentimento a que o Instituto Nacional da Propriedade Industrial conceda às aludidas Universidade Federal de Lavras e Lallemand Soluções Agrobiológicas LTDA, tal privilégio.

Lavras, 29 de outubro de 2020.



Marcio Pozzobon Pedroso

UNIVERSIDADE FEDERAL DE LAVRAS
Sistema Integrado de Gestão de Recursos Humanos Pró-Reitoria de Gestão e Desenvolvimento de Pessoas

D E C L A R A Ç Ã O

Declaramos que JORGE TEODORO DE SOUZA, matrícula SIAPE 1551053, ocupante do cargo de PROFESSOR DO MAGISTÉRIO SUPERIOR, classe C - Adjunto, nível 002, do quadro de pessoal do(a) UFLA, foi admitido(a) a partir de 25/09/2014, sendo lotado(a) no(a) DEPARTAMENTO DE FITOPATOLOGIA/ESAL, em regime de Dedicação Exclusiva.

Lavras/MG, 09 de Dezembro de 2020.

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PROCURAÇÃO

Por este instrumento particular de Procuração, a **Lallemand Soluções Agrobiológicas LTDA, situada na Av. Julia Fernandes Caixeta, 555 Cidade Nova, Patos de Minas-MG, CNPJ 07.983.734/0001-87**, neste ato representada por FERNANDO LUCAS URBAN, brasileiro, solteiro, empresário, diretor presidente, portador da Cédula de Identidade RG n°11.843.117 SSP/MG inscrito no CPF sob n° 156.028.066-10, confere poderes especiais à **UNIVERSIDADE FEDERAL DE LAVRAS**, autarquia federal, com sede no Campus Universitário, Caixa postal 3037, Lavras – MG, CEP 37.200-000, Brasil, inscrita no CNPJ sob o n° 22.078.679/0001-74, neste ato representada por seu Reitor João Chrysostomo de Resende Júnior, de acordo com Decreto de 30 de abril de 2020, publicado no Diário Oficial da União de 04 de maio de 2020, Seção 2, página 1, Professor Titular do Magistério Superior, inscrito no CPF sob o nº 512.259.806-15 e de RG MG-3.215.010, brasileiro, casado, Rua Francisco Narciso, 100, Bairro Santa Filomena, CEP: 37.200-000, Lavras – MG, para representar a outorgante perante o **INSTITUTO NACIONAL DE PROPRIEDADE INDUSTRIAL – INPI** a quem confere poderes para nos termos da Lei 9.279/96 (Lei da Propriedade Industrial), dos atos normativos, das portarias e das resoluções baixadas pelo INPI, a requerer e obter patente de invenção, modelos de utilidades, modelos de desenhos industriais, e respectivas prorrogações, apresentarem desistências dos mesmos, tudo em conformidade com a legislação em vigor, podendo preencher todas as formalidades legais, assinar petições de desistências, requerer o arquivamento e desarquivamento de processos, cumprir exigências, comprovar o pagamento de anuidade de pedidos de privilégios e patentes, taxas ou impostos, requerer caducidade, restaurações, anotações de transferências, alterações de nome, de sede, de cancelamento, juntar e retirar documentos, bem como fazer prova de uso efetivo e patentes, exclusivamente relativos ao invento intitulado: "**Biofungicida a base do fungo Muscodor sp. para o controle de podridões em frutos embalados"** a ser solicitado junto ao INPI, para mantê-lo em vigor com amplos poderes para assinar petições e documentos, pagar taxas, anotar transferências, fazer prova de uso da invenção patenteada, apresentar oposições, recursos, réplicas, anotar, elaborar notificações extrajudiciais, e praticar para os fins mencionados todos os atos necessáriosperante as autoridades administrativas competentes no Brasil e no exterior, em benefício dos Outorgantes, ratificando os atos já praticados.

Patos de Minas, 06 de novembro de 2020

FERNANDO LUCAS URBAN:05602806610

Assinado de forma digital por FERNANDO LUCAS URBAN:05602806610
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FERNANDO LUCAS URBAN
Lallemand Soluções Agrobiológicas LTDA

RESUMO

Biofungicida a base do fungo Muscodor sp. para o controle de podridões em frutos embalados

A presente invenção provê um conjunto de compostos orgânicos voláteis com ação fungicida que é produzido naturalmente pelo isolado *Muscodor* sp. FTB01. Mais particularmente, a invenção refere-se a uma formulação de *Muscodor* e método para preparar e usar esta formulação. Esta invenção inclui umprocesso e produto para controle do fungo fitopatogênico *Botrytis cinerea* em morangos embalados. No entanto, a técnica pode ser expandida para o controle deoutros fungos fitopatogênicos e o procedimento também pode ser aplicado em outros frutos e vegetais em pós-colheita. Esta invenção é considerada sustentável, pois é de origem biológica e não interfere no meio ambiente.

Figura 1

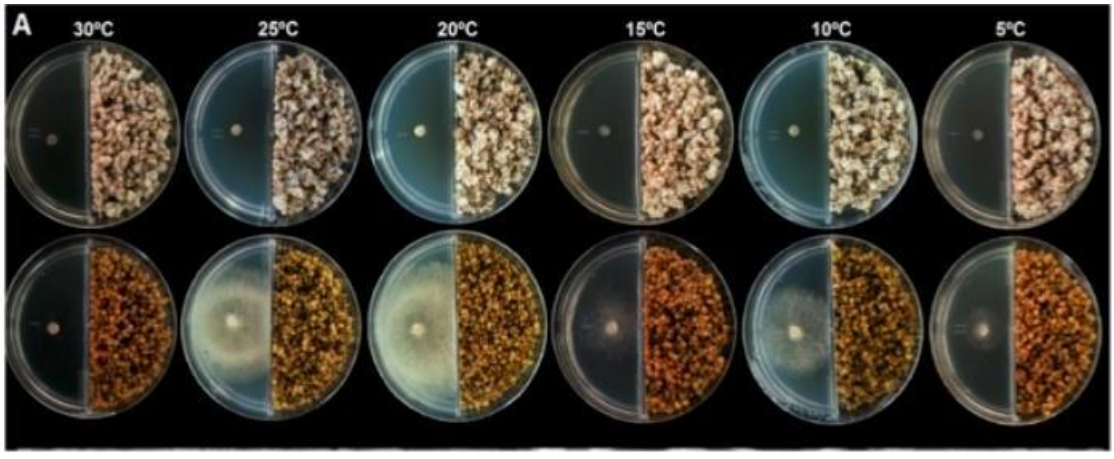

Figura 2

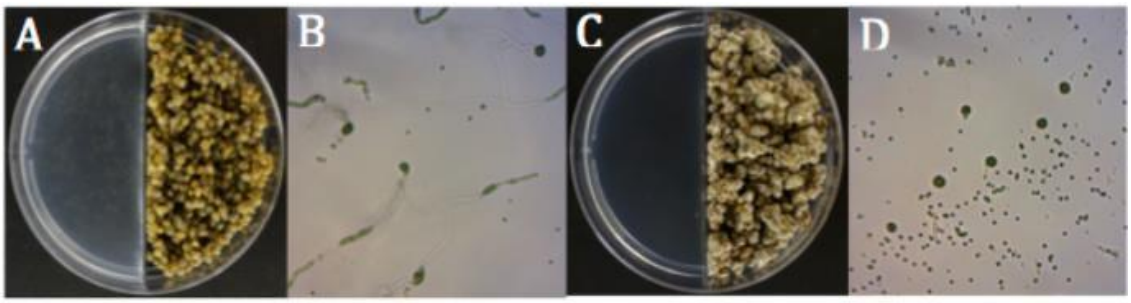

Figura 3

Figura 4

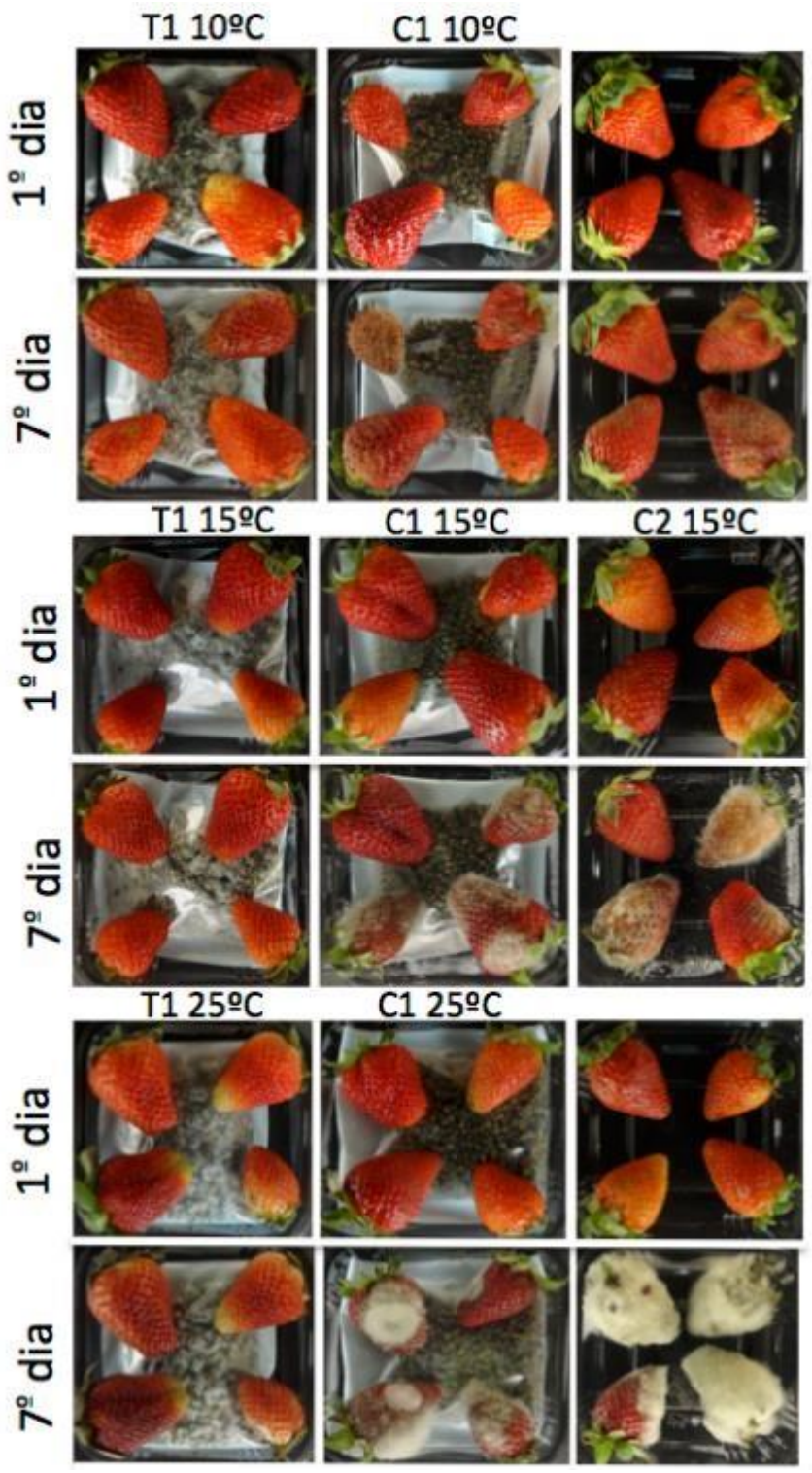
T1 10ºC
C1 10ºC
1º dia
7º dia
T1 15ºC
C1 15ºC
C2 15ºC
1º dia
7º dia
T1 25ºC
C1 25ºC
1º dia
7º dia

Figura 5

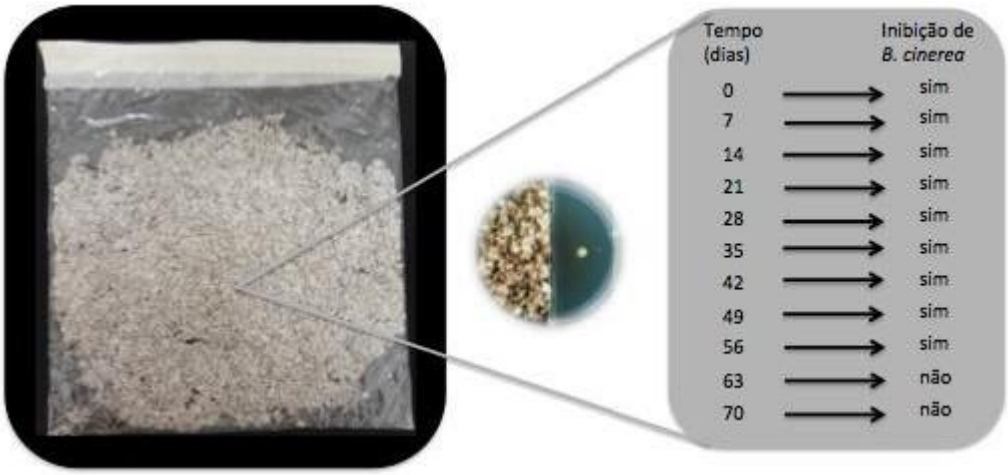
Tempo (dias)
Inibição de B. cinerea
0 sim
7 sim
14 sim
21 sim
28 sim
35 sim
42 sim
49 sim
56 sim
63 não
70 não

Figura 6

3 RELATÓRIO DESCRITIVO

Biofungicida a base do fungo Muscodor sp. para o controle de podridões em frutos embalados

Campo de aplicação

[01] A presente invenção refere-se aos campos de micologia e fungicidas e fornece um método para impedir o crescimento de fitopatógenos que causam deterioração de frutos após a colheita. Mais especificamente a invenção refere-se a uma formulação à base de milheto como substrato para *Muscodor* sp. e modo deusar este produto como fungicida em frutos colhidos e prontos para o consumo humano.

Estado da técnica

[02] Atualmente, *Muscodor* sp. é o gênero de fungo mais estudado em relação aos compostos orgânicos voláteis. Isolamento, identificação e testes para verificar a ação dos seus voláteis tem sido foco de muitas pesquisas. Existem diversas publicações e patentes relacionadas a este fungo como agente de biocontrole. Na revisão de Gomes et al. (GOMES, A.A.M; QUEIROZ, M.V; PEREIRA, O.L - Mycofumigation for the biological control of postharvest diseases in fruits and vegetables: a review. Austin J. Biotchnol. Bioeng. 2:2-8, 2015) os autores citam algumas espécies de fungos do gênero *Muscodor* com capacidade de inibir patógenos de plantas, mas não fazem referência sobre produtos à base de *Muscodor*. No trabalho publicado por Gomes et al. (GOMES, A.A.M; PINHO, D.P; CUSTÓDIO, F.A; PEREIRA, O.L. – *Muscodor* species isolated from coffee plants in brazil, with the potential for mycofumigation of post-harvest phytopathogenic fungi. VIII Congresso Brasileiro de Micologia. Anais, 2016), os autores isolaram diferentes espécies de *Muscodor* e testaram *in vitro* a ação dos compostos voláteis contra *Botrytis cinerea* e *Arpergillus ochraceus*, mas também não fazem referência à formulação de produtos à base de *Muscodor*, tampouco o uso de milheto como substrato.

[03] No livro “Defensivos Agrícolas Naturais: usos e perspectivas” (Livro Defensivos agrícolas naturais: uso e perspectivas. Embrapa, 2016), mais uma vez, os autores relatam a efetividade dos voláteis produzidos por *Muscodor* em controlar patógenos de plantas, principalmente após a colheita. Citam também queexistem formulações comerciais a base de microrganismos para controle de doenças em pós-colheita, no entanto, não citam formulação de produto à base de *Muscodor*, muito menos o uso de milheto como substrato efetivo para produçãode voláteis com ação antagonista a *B. cinerea* em morangos.

[04] A propriedade intelectual número PI 0412701-3A trata de mistura sintética e formulação carreadora de *Muscodor* para serem usados como pesticidas. Busca abranger uma ampla gama de atividades do produto desde insetos, nematoides e microrganismos fitopatogênicos até materiais de construção. A formulação comercial a base de *Muscodor*, que contém nutrientes e agente estabilizante é também produzida em uma forma encapsulada. Grãos de centeio, grãos de arroz integral e ágar de dextrose batata são utilizados como substrato. Não foi estudado milheto como substrato e vale ressaltar que a eficiência dos voláteis produzidos por *Muscodor* está diretamente ligado ao tipo de substrato, temperatura, isolado usado, entre outros fatores. Constatando que os isolados citados nesta PI 0412701-3A são diferentes dos isolados da patente que está sendo solicitada, bem como o substrato, os compostos orgânicos voláteis produzidos são diferentes, o que leva oproduto final a ter diferentes eficiências e finalidades de uso.

[05] As patentes US 20120058058A1 e US 20140086879A1 citam o uso de um isolado de *Muscodor* sp. para controle de *Botrytis cinerea*, porém não exploraram a produção de voláteis pelo fungo em meio de cultura utilizando milheto como substrato. As patentes WO 2014052354A1 e PI 0208931-9A2, exploraram a produção de voláteis pelo fungo, mas não o milheto como o meio de cultura e não contempla o uso em pós-colheita para estender a vida de prateleira dos frutos.

Problemas do estado da técnica

[06] Diversos alimentos são embalados em bandejas e caixas ou são transportados em ambiente fechado, como frutas e vegetais. Estes alimentos não estão

protegidos do ataque de fitopatógenos que causam uma rápida deterioração. Devido a segurança alimentar, a aplicação de fungicidas não é permitida para os alimentos prontos para serem consumidos.

[07] A nossa proposta para superar esse problema é a utilização do controle biológico, uma tecnologia considerada segura e sustentável. A presente invenção aumenta a vida de prateleira de morangos em pós-colheita atacados pelo fungo patogênico *Botrytis cinerea*. Ao multiplicar o fungo *Muscodor* sp. FTB01 em milheto, em uma atmosfera fechada e no mesmo ambiente que os alimentos, as moléculas voláteis produzidas pelo fungo como metabólitos secundários impedem o crescimento do fungo fitopatogênico e preserva o alimento por um tempo maior que o normal, estendendo o que é conhecido como vida de prateleira do produto.

[08] Vale ressaltar que os frutos não entram em contato com o produto a base de *Muscodor* e milheto, e apenas os voláteis produzidos são responsáveis pela proteção e controle de fitopatógenos. Desse modo o processo aqui proposto possibilita que o alimento seja transportado a longas distâncias mantendo a sua qualidade e prolongando a viabilidade do alimento, garantindo a segurança alimentar, além de evitar perdas econômicas.

Objeto da invenção

[09] A presente invenção apresenta uma solução para um problema de ordem fitossanitária na fase de pós-colheita de frutos. A falta de agrotóxicos que sejam seguros para aplicação após a colheita coloca em risco a segurança alimentar, visto que o uso de produtos inadequados e/ou fitopatógenos produtores de toxinas podem afetar negativamente a saúde humana.

[10] A invenção é suscetível de aplicação industrial uma vez que o processo de produção massal do fungo é totalmente aplicável utilizando o milheto como substrato sólido. O milheto utilizado na formulação do produto é encontrado facilmente e tem um baixo custo, e o agente de biocontrole, *Muscodor* sp. FTB01, mantem-se estável desde que preservado adequadamente.

[10] Mais particularmente a invenção provê um método seguro de proteção e controle de doenças em pós-colheita de frutos, podendo estender-se a vegetais.

Este efeito é factível porque os compostos orgânicos voláteis produzidos por *Muscodor* sp. FTB01 são as moléculas responsáveis pela ação fungicida. O produto inventado não entra em contato direto com o alimento e não deixa resíduos. Cabe destacar que alguns dos compostos produzidos são encontrados naturalmente, como por exemplo 2-metil butanol e fenetil álcool presentes em uvas e suco de maçã.

Descrição da invenção

[11] O fungo *Muscodor* sp. FTB01 utilizado como agente de controle biológico foi isolado como endófito de erva-de-passarinho (*Struthanthus flexicaulis*), planta hemiparasita, coletada em Ijaci, Minas Gerais, Brasil. Para correta identificação, o sequenciamento genômico parcial da região ITS foi realizado. Uma cultura isolada de *Muscodor* sp. FTB01 foi depositada em 05 de setembro de 2018 na Coleção Micológica de Lavras, localizada no Departamento de Fitopatologia, na Universidade Federal de Lavras, Lavras, Minas Gerais, Brasil. *Muscodor* sp.FTB01 recebeu o número de acesso CML 4023. A disponibilidade de um depósitonão concebe licença para executar a presente invenção em detrimento dos direitos de patente reconhecidos.

[12] *Muscodor* sp. FTB01 produz como metabólitos secundários compostos orgânicos voláteis que inibem e matam diversos fitopatógenos. Verificou-se também que esses voláteis são efetivos *in vitro* e *in vivo*.

Fomulação de *Muscodor*

[13] A presente invenção é direcionada para uma formulação estável de *Muscodor* para a produção de compostos voláteis e método de usar esta composição como fungicida em pós-colheita de frutos.

[14] A prática da presente invenção emprega o uso de grãos de milheto como substrato sólido para crescimento e multiplicação de *Muscodor* sp. FTB01. Os grãos de milheto utilizados no estudo contêm em sua composição diversos nutrientes, como mostrado na tabela 01.

Tabela 01. Composição química dos grãos de milheto usados no estudo.

Macronutrientes (g.Kg-1)					
Nitrogênio	Fósforo	Potássio	Cálcio	Magnésio	Enxofre
30.33±0.81	6.20±0.95	4.93±0.81	0.77±0.15	1.93±0.15	0.93±0.06
Micronutrientes (mg.Kg-1)					
Zinco	Cobre	Ferro	Manganês	Boro	
53.30±1.11	6.47±0.70	110.40±5.43	15.10±0.61	6.83±1.59	
Proteína Total (g.kg-1)					
191.58±6.78					

[15] *Muscodor* spp. pode ser cultivado em diferentes substratos. Sabe-se que o substrato, temperatura, pH, tempo de incubação e isolado de *Muscodor* utilizado interferem na produção do conjunto de voláteis produzidos e consequentemente, na sua finalidade de uso e eficiência.

[16] A presente invenção projetou uma formulação comercialmente utilizável de *Muscodor* sp. FTB01 que provê nutrientes apropriados para a produção de compostos orgânicos voláteis com ação fungicida.

[17] Para a formulação, o milheto colonizado pelo fungo endófito *Muscodor* sp. FTB01, usou-se 100 g de grãos de milheto e 100 mL de água destilada que foram colocados em saco plástico transparente medindo 30 cm x 40 cm (Figura 01). Posteriormente, os sacos contendo o milheto e a água foram autoclavados por 20 minutos a 120ºC (15 lbs de pressão). Depois de retirados da autoclave, esperou-se o resfriamento dos grãos e então adicionou-se 15 discos de 5mm de micélio de *Muscodor* sp. FTB01 e foram incubados à 25ºC por 21 dias. Nas condições citadas acima, *Muscodor* sp. FTB01 produz os seguintes compostos orgânicos voláteis: etanol; acetato de etila; 3-metil butanol; 2-metil butanol; isobutirato de etila; 2-metilbutanoato de etila; 3-octanol; fenetil álcool;dehydroaromandendrene; epiglobulol (Tabela 02).

Tabela 02. Compostos orgânicos voláteis produzidos por *Muscodor* sp. FTB01 colonizando grãos de milheto após 21 dias e incubados a 25ºC. A análise foi feita por cromatografia gasosa acoplada a espectrometria de massas - GC/MS.

Pico	KI Calculado	KI Literatura	Tempo	Compostos	Similaridade
1			1.97	Etanol	96
2			2.61	Acetato de etila	97
3	733	731	4.21	3-metil Butanol	96
4	737	739	4.30	2-metil Butanol	96
5	753	756	4.61	Isobutirato de etila	94
6	846	848	7.12	2-metilbutanoato de etila	95
7	1000	1000	13.30	3-octanol	91
8	1123	1122	19.04	Fenetil alcool	88
9	1456	1456	34.18	Dehydroaromandendrene	94
10	1523	1532	36.92	Epiglobulol	88

KI = constante de ionização.

[18] Os grãos de milheto colonizados por *Muscodor* sp. FTB01 foram utilizados para experimentos de inibição de *Botrytis cinerea*. Para ensaios *in vitro*, os grãos de milheto colonizados por *Muscodor* sp. FTB01 foram inseridos em um dos lados da placa de Petri bipartida e no outro lado vertido meio de cultura batata dextrose ágar (BDA) para crescimento do fitopatógeno *B. cinerea.* Foi adicionadoum disco do meio de cultura BDA contendo micélio do fungo fitopatogênico em estudo. As placas foram incubadas em diferentes temperaturas (5, 10, 15, 20, 25 e 30ºC). O crescimento do micélio do fitopatógeno foi medido diariamente até queo fungo colonizasse completamente a placa controle, que não tinha milheto colonizado por *Muscodor* (Figura 02). O mesmo experimento foi realizado, porémcom conídios de *B. cinerea e* observou-se completa inibição dos conídios em todas as temperaturas testadas (Figura 03).

[19] Para os ensaios *in vitro* com outros fitopatógenos, placas de *Petri* bipartidas também foram utilizadas. Em uma das metades da placa *Muscodor* sp. FTB01 cultivado em milheto por 21 dias e na outra metade, meio de cultivo apropriado para crescimento dos fitopatógenos (BDA – batata dextrose ágar ou CSA –

cenoura sacarose ágar). Como controle foi utilizado milheto sem *Muscodor* sp. FTB01. A avaliação foi feita quando o fitopatógeno atingiu a borda da placa. Avaliou-se o crescimento micelial do fitopatógeno e, posteriormente, o disco de micélio do fitopatógeno foi retirado e colocado separadamente em nova placa contendo meio de cultura. A viabilidade foi avaliada pela capacidade do fitopatógeno crescer novamentente após a exposição aos voláteis (vivo) ou não (morto) (Tabela 03). Para ver a viabilidade, as placas foram observadas por 30 dias. A formulação carreadora de *Muscodor* sp. FTB01 foi eficiente para inibir e/ou matar os seguintes fitopatógenos: *Rhizoctonia solani*, *Botrytis cinerea*, *Phytophthora sp.*, *Colletotrichum lindemuthianum*, *Sclerotinia sclerotiorum*, *Fusarium verticillioides*, *Rhizopus stolonifer*, *Pectobacterium carotovorum* (Tabela 03).

Tabela 03. Atividade antimicrobiana dos voláteis produzidos por *Muscodor* sp. FTB01 cultivado por 21 dias em milheto. Teste *in vitro* para verificar o crescimento e a viabilidade de diversos patógenos expostos aos voláteis.

Fitopatógeno	Avaliação	Tratamento	
		Controle	*Muscodor*?sp.?FTB01
Rhizoctoniasolani	Crescimento(?cm)	4	0
	Viabilidade	Vivo	Morto
Botrytis cinerea	Crescimento(?cm)	4	0
	Viabilidade	Vivo	Morto
Phytophthora sp.	Crescimento(?cm)	4	0
	Viabilidade	Vivo	Morto
Rhizopus stolonifer	Crescimento(?cm)	4	0
	Viabilidade	Vivo	Vivo
Colletotrichum lindemuthianum	Crescimento(?cm)	4	0
	Viabilidade	Vivo	Vivo
Sclerotinia sclerotiorum	Crescimento(?cm)	4	0
	Viabilidade	Vivo	Morto
Fusarium verticilioides	Crescimento(?cm)	4	0.8
	Viabilidade	Vivo	Vivo
Pectobacterium carotovorum	Crescimento(?cm)	4	2.05
	Viabilidade	Vivo	Vivo

[20] Para os ensaios *in vivo*, foram feitos *sachets* com uma face de plástico e a outra face de papel e adicionou-se 20 g de grãos de milheto colonizado por *Muscodor* sp. FTB01 em cada *sachet* (Figura 04). Foram utilizadas embalagens plásticas, contendo quatro morangos. Os *sachets* foram colocados com a face plástica voltada para cima, no fundo da embalagem, evitando o contato direto com

os morangos (Figura 05). As bandejas foram incubadas a 10, 15, 20 e 25ºC e sete dias depois foi feita contagem do número de frutos com incidência de *B. cinerea* (Tabela 04). Mais de 60% dos frutos nos controles tinham incidência de *B.cinerea* enquanto que nos frutos tratados com *Muscodor* sp. FTB01 não foi observado a presença de *B. cinerea*. No controle a 20ºC, a incidência do patógeno foi observada em todos os frutos. O experimento pode ser repetido também em outros recipientes, desde estes sejam mantidos fechados.

[21] Para verificar o período máximo que *Muscodor* sp. FTB01 estava produzindo voláteis com ação fungicida, um teste *in vitro* foi realizado. A cada 7 dias, uma amostra de *Muscodor* sp. FTB01 cultivado em milheto foi colocado em uma metade da placa bipartida e na outra metade, foi colocado um disco de micélio de *B. cinerea* em meio BDA. Como controle, foi utilizado milheto sem a colonização de *Muscodor* sp. FTB01. Após 63 dias de cultivo em milheto, *Muscodor* sp. FTB01 deixou de inibir completamente *B. cinerea* (Figura 06). Este teste confirma que durante os 7 dias do experimento com os frutos, a formulação carreadora de *Muscodor* sp. FTB01 produzia compostos voláteis com capacidade de inibir completamente *B. cinerea.*

Tabela 04. Incidência de *B. cinerea* em morangos nas diferentes temperaturas testadas após sete dias. Tratamento1: morangos tratados com *sachet* contendo grãos de milheto colonizado por *Muscodor* sp. FTB01. Controle1: *sachet* com grãos de milheto sem *Muscodor* sp. FTB01. Controle2: sem *sachet* de milheto e sem *Muscodor* sp. FTB01. Os valores se referem a médias de 4 repetições contendo 4 frutos em cada repetição.

Temperatura ºC	**Incidência de *B. cinerea* (%)**		
	Tratamento1	**Controle1**	**Controle2**
10	0	62.5	56.25
15	0	87.5	93.75
20	0	100	100
25	0	81.25	81.25

Método de usar a formulação de *Muscodor*

[22] Verificou-se que a formulação de *Muscodor* sp. FTB01 inibe e/ou mata os seguintes fitopatógenos: *Rhizoctonia solani*, *Botrytis cinerea, Phytophthora sp., Colletotrichum lindemuthianum, Sclerotinia sclreotiorum, Fusarium verticilioides*, *Rhizopus stolonifer, Pectobacterium carotovorum* (Tabela 03). Logo, a invenção apresenta um método para inibir e/ou inviabilizar o crescimento de fitopatógenos expondo-os a uma quantidade eficaz dos compostos orgânicos voláteis produzidos por *Muscodor* sp. FTB01 cultivado na formulação a base de milheto.

[23] Os *sachets* confeccionados, contendo milheto colonizado por *Muscodor* sp. FTB01, devem ser colocados com a face plástica voltada para cima, evitando o contato direto com os alimento (Figura 05). A quantidade testada no estudo foi um *sachet* com 20 g para um recipiente fechado com aproximadamente 700 cm^3.

[24] Para utilização agrícola, a invenção fornece um método para proteger e/ou tratar morangos embalados. O uso desta invenção evita a rápida deterioração causada por fitopatógenos de pós-colheita, podendo ser expandida a outros frutos e vegetais desde que estejam em ambiente fechado, expondo o alimento aos voláteis descritos na tabela 02.

Descrição das Figuras e Desenhos

[25] A figura 01 mostra o saco plástico transparente mendindo 30 cm x 40 cm utilizado para crescimento de *Muscodor* sp. FTB01 cultivado em milheto. É possível observar o micélio esbranquiçado de *Muscodor* sp. FTB01 crescido a 25ºC por 21 dias sobre os grãos de milheto .

[26] A figura 02 mostra as placas de Petri bipartidas do experimento *in vitro* para avaliar a inibição do crescimento micelial de *B. cinerea* por *Muscodor* sp. FTB01 cultivado em grãos de milheto e incubados a diferentes temperaturas. O painel de cima mostra a completa inibição de *B. cinerea* por *Muscodor* sp. FTB01 em todas as temperaturas de incubação (5, 10, 15, 20, 25 e 30ºC). Para os tratamentos foram usados os grãos de milheto colonizados por *Muscodor* sp. FTB01. Para o controle foi usado apenas grãos de milheto autoclavados. O painel de baixo

mostra o crescimento micelial de *B. cinerea* no controle. Observou-se no experimento, que independente da temperatura testada (5, 10, 15, 20, 25 e 30ºC) houve completa inibição do crescimento micelial de *B. cinerea*.

[27] A figura 03 mostra o experimento *in vitro* para avaliar a inibição da germinação de conídios de *B. cinerea* por *Muscodor* sp. FTB01 utilizando placa de Petri bipartida. A figura 03 A e B mostra conídios germinados de *B. cinerea* no controle. A figura 03 C e D mostram conídios não germinados expostos aos voláteis produzidos por *Muscodor* sp. FTB 01 cultivados em grãos de milheto. As imagens são de placas incubadas a 20ºC. Observou-se no experimento, que independente da temperatura testada (5, 10, 15, 20, 25 e 30ºC) houve completa inibição da germinação dos conídios quando foram expostos aos voláteis.

[28] A figura 04 mostra o *sachet* medindo 7cm x 9 cm, com uma face de plástico e a outra face de papel contendo 20 g de grãos de milheto colonizado por *Muscodor* sp. FTB01 após 21 dias. Este *sachet* foi colocado dentro da embalagemcom os morangos, com a face plástica voltada para cima.

[29] A figura 05 mostra o aspecto dos morangos tratados com *sachet* contendo grãos de milheto colonizado por *Muscodor* sp. FTB01 (T1), *sachet* com grãos de milheto mas sem *Muscodor* sp. FTB01 (C1) e sem *sachet* (C2). As imagens são de morangos incubados a 10, 15 e 25ºC. Os painéis superior e inferior referem-se ao primeiro e sétimo dia do experimento, como descrito na figura.

[30] A figura 06 mostra um esquema para exemplificar o teste feito para observar por quantos dias *Muscodor* sp. FTB01 era capaz de produzir voláteis que inibiam completamente *B. cinerea*. A cada 7 dias foi retirado uma amostra do mesmo saco e colocado em placa de Petri bipartida em uma metade, e na outra metade, um disco de micélio de *B. cinerea*. O dia 0 é o dia em que foi feita a inoculação com *Muscodor* sp. FTB01 e já é possível observar a completa inibição de *B. cinerea*. Ao 63º dia foi observado que não havia mais inibição completa do fitopatógeno.

4 REINVINDICAÇÕES

01. Formulação de *Muscodor* sp. FTB01 em milheto, caracterizada por possuir quantidades de compostos orgânicos voláteis eficazes para proteção e controle de patógenos em pós-colheita de frutos embalados.

02. Formulação, de acordo com a reivindicação 01, caracterizada pelo fato de que os compostos orgânicos voláteis compreendem etanol; acetato de etila; 3-metil butanol; 2-metil butanol; isobutirato de etila; 2-metilbutanoato de etila; 3-octanol; fenetil álcool; dehydroaromandendrene; epiglobulol.

03. Método para inibição de fitopatógenos em pós-colheita de frutos, caracterizado pelo fato de expor os fitopatógenos à compostos voláteis com ação fungicida.

04. Método para inibição e controle de fitopatógenos em pós-colheita de frutos, caracterizado pelo fato de expor os frutos a uma quantidade eficaz do conjunto de voláteis compreendendo em etanol; acetato de etila; 3-metil butanol; 2-metil butanol; isobutirato de etila; 2-metilbutanoato de etila; 3-octanol; fenetil álcool; dehydroaromandendrene; epiglobulol.

05. Formulação carreadora de *Muscodor* sp. FTB01 caracterizada por possuir um carreador, água e um isolado de *Muscodor* sp. FTB01, em que a cultura é aderida ao carreador.

06. Formulação carreadora de *Muscodor,* de acordo com a reivindicação 05,caracterizada pelo fato de que o carreador é o grão.

07. Formulação carreadora de *Muscodor,* de acordo com a reivindicação 06,caracterizada pelo fato de que o grão selecionado é o milheto.

08. Formulação carreadora de *Muscodor,* de acordo com a reivindicação 07,caracterizada pelo fato de que o carreador é um grão contendo fontes de nitrogênio, fósforo, potássio, cálcio, magnésio, enxofre, zinco, cobre, ferro, manganês e boro.

09. Produção de compostos orgânicos voláteis de maneira natural, caracterizado pelo fato de que o isolado *Muscodor* sp. FTB01 na formulação carreadora produz etanol; acetato de etila; 3-metil butanol; 2-metil butanol; isobutirato de etila; 2-metilbutanoato de etila; 3-octanol; fenetil álcool; dehydroaromandendrene;epiglobulol.