RAYSA MARQUES CARDOSO

ENDOPHYTIC MICROORGANISMS: IDENTIFICATION, PROSPECTION OF FUNCTIONAL GENES FOR PROMOTING GROWTH AND BIOLOGICAL CONTROL OF DISEASES IN COMMON BEANS

LAVRAS – MG

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Thesis presented to the Federal University of Lavras, as part of the requirements of the Postgraduate Program in Soil Science, area of concentration in Soil Biology, Microbiology and Biological Processes, to obtain the title of Doctor.

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RAYSA MARQUES CARDOSO

MICRORGANISMOS ENDOFÍTICOS: IDENTIFICAÇÃO, PROSPECÇÃO DE GENES FUNCIONAIS PARA PROMOÇÃO DE CRESCIMENTO E CONTROLE BIOLÓGICO DE DOENÇAS EM FEIJÃO COMUM

ENDOPHYTIC MICROORGANISMS: IDENTIFICATION, PROSPECTION OF FUNCTIONAL GENES FOR GROWTH PROMOTION AND BIOLOGICAL CONTROL OF DISEASES IN COMMON BEANS

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciência do Solo, área de concentração em Biologia do Solo, Microbiologia e Processos Biológicos, para obtenção do título de Doutor.

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Dedicatória

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“Tudo está interligado. Não existe ser humano sadio se o solo não for sadio e as plantas bem nutridas”

(Ana Maria Primavesi)

RESUMO

O feijão comum (*Phaseolus vulgaris* L.) é uma cultura de grande importância econômica e social no Brasil. Um dos desafios enfrentados é o surgimento de doenças causadas por fungos fitopatogênicos, como *Colletotrichum lindemuthianum*, *Sclerotinia sclerotiorum*, *Pseudocercospora griseola* e *Rhizoctonia solani*, que causam antracnose, mofo branco, mancha angular e tombamento, reduzindo a produtividade e a qualidade dos grãos. O controle biológico com microrganismos benéficos tem reduzido efetivamente esses patógenos, diminuindo a necessidade de fungicidas químicos. O estudo dos genomas de bactérias endofíticas envolvidas no controle biológico promete descobrir novas moléculas e aprofundar o conhecimento, contribuindo para a biotecnologia agrícola. Este estudo teve como objetivo avaliar estirpes de bactérias endofíticas de diferentes espécies quanto ao seu potencial no controle biológico de doenças fúngicas do feijão, relacionando-o ao conteúdo genético em seus genomas. O DNA das estirpes de *Pseudomonas* UFLA 02-281, UFLA02-293 e UFLA03-18 foi extraído para sequenciamento do genoma. A classificação em nível de espécie foi realizada calculando a ANI (Identidade Nucleotídica Média), considerando valores abaixo de 96% indicativos de novas espécies. Foi realizada a análise funcional dos genomas dessas estirpes e da estirpe UFLA03-10, previamente classificada como *Paenibacillus peoriae*. As estirpes UFLA 02-281, UFLA02-293, UFLA03-18 e UFLA03-10 foram testadas *in vitro* para observar o antagonismo com cepas dos fungos *Colletotrichum lindemuthianum*, *Sclerotinia sclerotiorum*, *Pseudocercospora griseola* e *Rhizoctonia solani*, e *in vivo* para avaliar o controle dessas cepas sobre antracnose, mofo branco, mancha angular e tombamento, inoculadas isoladamente e co-inoculadas com o fungo CML4019 (*Induratia coffeanna*). A estirpe UFLA 02-281 foi classificada como *Pseudomonas xanthosomatis*, UFLA 02-293 como *Pseudomonas cremoris* e UFLA 03-18 como *Pseudomonas bananamidigenes*. Genes relacionados à promoção do crescimento de plantas e ao controle biológico de doenças foram detectados nas quatro cepas bacterianas, como genes ligados à produção de metabólitos antimicrobianos, como *bce*, *hcn*, *toxA*, *kinB*, e indução de resistência, como *butA*, *metN*, *dacA*, para controle biológico, e genes relacionados à produção de hormônios, como os genes dos grupos *trpA*, *trpB*, *ald* e *ydcU*, e metabolismo de nutrientes, como os genes *pst*, *pho*, *fix*, *glnA*, *nit*, entre outros, que promovem o crescimento das plantas. Ensaios *in vitro* e *in vivo* mostraram que, entre as estirpes bacterianas estudadas, UFLA03-10 controlou mais eficientemente os fungos e as doenças. O fungo endofítico inoculado isoladamente, CML4019, controlou a doença, especialmente em ensaios que avaliaram o tombamento e a antracnose. Entre as co-inoculações, UFLA02-281 + CML4019 mostrou resultados favoráveis em pelo menos uma das cultivares avaliadas nos quatro ensaios *in vivo*. Os resultados dos testes *in vitro* e *in vivo* mostraram que, quando inoculadas ou co-inoculadas, essas estirpes apresentaram resultados promissores no controle de doenças causadas por fungos. Esses resultados mostram que as estirpes avaliadas neste estudo têm potencial para serem usadas como agentes de controle biológico em sistemas agrícolas.

Palavras chave: *Paenibacillus peoriae*, *Pseudomonas* sp., *Induratia coffeana*, análise funcional do genoma, co-inoculação, metabólitos secundários.

ABSTRACT

The common bean (*Phaseolus vulgaris* L.) is a crop of great economic and social importance in Brazil. One of the challenges faced is the emergence of diseases caused by phytopathogenic fungi, such as *Colletotrichum lindemuthianum*, *Sclerotinia sclerotiorum*, *Pseudocercospora griseola*, and *Rhizoctonia solani*, which cause anthracnose, white mold, angular leaf spot, and damping-off, reducing grain productivity and quality. Biological control with beneficial microorganisms has effectively reduced these pathogens, decreasing the need for chemical fungicides. The study of genomes of endophytic bacteria involved in biological control promises to discover new molecules and deepen knowledge, contributing to agricultural biotechnology. This study aimed to evaluate strains of endophytic bacteria of different species for their potential in the biological control of bean fungal diseases, relating it to the genetic content in their genomes. The DNA of *Pseudomonas* strains UFLA 02-281, UFLA02-293, and UFLA03-18 was extracted for genome sequencing. The species-level classification was performed by calculating the ANI (Average Nucleotide Identity), considering values below 96% indicative of new species. Functional analysis of the genomes of these strains and strain UFLA03-10, previously classified as *Paenibacillus peoriae*, was conducted. Strains UFLA 02-281, UFLA02-293, UFLA03-18, and UFLA03-10 were tested *in vitro* to observe antagonism with strains of the fungi *Colletotrichum lindemuthianum*, *Sclerotinia sclerotiorum*, *Pseudocercospora griseola*, and *Rhizoctonia solani*, and *in vivo* to assess the control of these strains on anthracnose, white mold, angular leaf spot, and damping-off, inoculated alone and co-inoculated with the fungus CML4019 (*Induratia coffeanna*). Strain UFLA 02-281 was classified as *Pseudomonas xanthosomatis*, UFLA 02-293 as *Pseudomonas cremoris* and UFLA 03-18 as *Pseudomonas bananamidigenes*. Genes related to plant growth promotion and biological control of diseases were detected in all four bacterial strains, as genes linked to the production of antimicrobial metabolites such as *bce*, *hcn*, *toxA*, *kinB* and induction of resistance such as *butA*, *metN*, *dacA* for biological control, and genes related to the production of hormones such as the genes of the *trpA*, *trpB*, *ald*, and *ydcU* and nutrient metabolism such as the genes *pst*, *pho*, *fix*, *glnA*, *nit* among others, which promote plant growth. *In vitro* and *in vivo* assays showed that among the bacterial strains studied, UFLA03-10 controled more efficiently the fungi and deseases. The endophytic fungus inoculated alone, CML4019, controlled the disease, especially in assays evaluating damping-off and anthracnose. Among the co-inoculations, UFLA02-281 + CML4019 showed favorable results in at least one of the cultivars evaluated in the four *in vivo* assays. The results of *in vitro* and *in vivo* tests showed that when inoculated or co-inoculated, these strains showed promising results in controlling diseases caused by fungi. These results show that the strains evaluated in this study have the potential to be used as biological control agents in agricultural systems.

Keywords: *Paenibacillus peoriae*., *Pseudomonas* spp., *Induratia coffeanna*., genoma mining. co-inoculation, secondary metabolites.

IMPACTOS SOCIAIS, TECNOLÓGICOS, ECONÔMICOS E CULTURAIS

O estudo das análises genômicas de bactérias endofíticas e sua inoculação em feijão comum representa um avanço significativo na agricultura moderna, com impactos profundos nos aspectos sociais, econômicos e tecnológicos. Essa pesquisa visa não apenas melhorar a promoção de crescimento das plantas, mas também controlar biologicamente doenças como mofo branco, antracnose, mancha angular do feijoeiro e tombamento, que frequentemente afetam severamente as colheitas. Do ponto de vista social, os benefícios são vastos. Com a redução do uso de agroquímicos, há menos impacto ambiental e riscos à saúde humana, o que é crucial em comunidades agrícolas. Além disso, ao promover práticas agrícolas mais sustentáveis e eficazes, a pesquisa pode melhorar a segurança alimentar, tornando cultivos mais resistentes e abundantes. Economicamente, a aplicação dessas tecnologias pode reduzir os custos de produção agrícola a longo prazo, uma vez que diminui a dependência de fungicidas e fertilizantes químicos. Isso não apenas beneficia diretamente os agricultores, aumentando sua rentabilidade, mas também pode ter um impacto positivo nos preços dos alimentos para os consumidores finais. No aspecto tecnológico, o uso de análises genômicas permite uma seleção mais precisa e eficaz de microrganismos endofíticos com características desejáveis, como capacidade de promoção de crescimento e supressão de patógenos. A integração de técnicas de biotecnologia com a agricultura tradicional abre caminho para inovações futuras, como o desenvolvimento de novas variedades de plantas mais resistentes e adaptadas a diferentes condições ambientais. O estudo das análises genômicas de bactérias endofíticas e sua aplicação no feijão comum para controle biológico de doenças não apenas representa um avanço científico significativo, mas também promete transformar positivamente a agricultura, influenciando aspectos sociais, econômicos e tecnológicos de maneira profunda e duradoura.

SOCIAL, TECHNOLOGICAL, ECONOMIC AND CULTURAL IMPACTS

The study of genomic analyses of endophytic bacteria and their inoculation in common beans represents a significant advancement in modern agriculture, profoundly impacting social, economic, and technological aspects. This research aims to enhance plant growth promotion and biologically control diseases such as white mold, anthracnose, angular leaf spot, and damping-off, which often severely affect crops. From a social perspective, the benefits are vast. By reducing agrochemicals, there is less environmental impact and fewer health risks, which is crucial in agricultural communities. Additionally, by promoting more sustainable and effective agricultural practices, the research can improve food security, making crops more resilient and abundant. Economically, these technologies can reduce long-term agricultural production costs by decreasing dependence on fungicides and chemical fertilizers. This directly benefits farmers, increasing their profitability and positively impacting food prices for end consumers. Technologically, genomic analyses allow for a more precise and effective selection of endophytic microorganisms with desirable traits, such as growth promotion and pathogen suppression. Integrating biotechnology techniques with traditional agriculture paves the way for future innovations, such as developing new plant varieties that are more resistant and better adapted to different environmental conditions. The study of genomic analyses of endophytic bacteria and their application in common beans for biological disease control represents a significant scientific breakthrough and promises to positively transform agriculture, profoundly and lastingly influencing social, economic, and technological aspects.

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO GERAL

O feijão comum (*Phaseolus vulgaris* L.) é um alimento de grande importância para a população brasileira, tanto do ponto de vista nutricional quanto econômico, destacando-se como uma cultura essencial para a segurança alimentar e o desenvolvimento agrícola do país. Um dos problemas encontrados em áreas de cultivo de feijão-comum é a presença de fungos fitopatogênicos. Entre esses fungos *Colletotrichum lindemuthianum* causador da antracnose, *Sclerotinia sclerotiorum* que causa o mofo branco, *Pseudocercospora griseola* que causa a mancha angular e *Rhizoctonia solani* que pode causar no feijão a podridão-radicular e em casos extremos o tombamento das plantas, estão entre os mais importantes. Esses fungos podem afetar uma ampla variedade de culturas e seus inóculos podem ficar no solo e também nos restos vegetais das culturas (Torres et al., 2016), dificultando o seu controle.

As principais formas de controle dessas doenças em campo são: aplicação de fungicidas químicos nas áreas e no tratamento de sementes para a redução do inóculo, uso de cultivares resistentes as doenças, desinfecção de sementes e rotação de culturas. No entanto, o desequilíbrio biológico resultante do uso de defensivos agrícolas químicos muda o ciclo de nutrientes e matéria orgânica, elimina organismos benéficos e reduz a biodiversidade, tornando-se necessário o desenvolvimento e a introdução de alternativas de manejo de doenças (Bending et al., 2007).

Nos últimos anos, há um interesse crescente no papel das rizobactérias no controle de doenças de plantas (García-Fraile et al., 2015). Muitos estudos demonstraram que certas estirpes de rizobactérias possuem mecanismos que lhes permitem suprimir doenças de plantas. Esses mecanismos incluem a produção de antibióticos e sideróforos, que podem inibir o crescimento de organismos patogênicos, bem como a indução de resistência sistêmica na planta hospedeira. (Sendi et al., 2020). Além disso, as rizobactérias podem melhorar o crescimento das plantas e a absorção de nutrientes, tornando as plantas mais resistentes a doenças. No geral, os mecanismos de controle de doenças de plantas por rizobactérias envolvem métodos diretos e indiretos. Os métodos diretos envolvem a produção de compostos que inibem diretamente o crescimento de patógenos, enquanto os métodos indiretos envolvem a melhoria da saúde das plantas e da resiliência a doenças (Beneduzi et al., 2012; Bogas et al., 2022).

Em um estudo de Martins e colaboradores (2018), que avaliou o efeito da inoculação com *Bacillus amylolicefaciens* ALB629 em sementes de feijão comum contra doenças causadas pelo fungo *R. solani*, observou-se controle da doença e aumento da produtividade do feijão. Em outros estudos realizados em casa de vegetação, as estirpes rizobacterianas endofíticas utilizadas controlaram o fungo *R. solani* em plantas de feijão comum e feijão fava quando inoculadas em conjunto com a estirpe rizobiana CIAT 899 (Ferreira et al., 2020, 2022).

Os fungos endofíticos se destacam pela quantidade de produtos naturais que produzem, suas características de atividade biológica e metabólitos secundários que podem se originar das interações metabólicas que ocorrem entre fungos e espécies vegetais. Isso faz com que espécies de fungos endofíticos tenham grande potencial como agentes antimicrobianos (Kusari et al, 2012). Com relação a promoção de crescimento vegetal, alguns fungos endofíticos apresentam essa função. Essa promoção de crescimento pode acontecer através da síntese de fitormônios e/ou tolerância aos estresses abióticos (Khan et al., 2015). No trabalho de Mota e colaboradores (2021) foram utilizados fungos endofíticos do gênero *Induratia* para a promoção de crescimento e controle biológico de mancha angular, mofo branco e antracnose no feijoeiro. Entre as cepas fúngicas utilizadas a CML4019 apresentou significativo controle sobre as doenças estudadas.

Os estudos genômicos permitem inferir os recursos que as bactérias utilizam durante a associação com as plantas. Tais informações podem revelar que substancias a bactéria produz, como antibióticos, compostos voláteis ou enzimas (Sundin et al., 2016). Enfim, dados que podem melhorar o entendimento das atividades das estirpes e, com isso, orientar a formulação de estratégias de controle de fitopatógenos. No entanto, a simples determinação da sequência do gene é de alto valor científico, mas não é suficiente para confirmar a verdadeira contribuição do mesmo para o comportamento bacteriano. Dessa forma experimentos são necessários para comprovar sua função, como e quando essas sequências são expressas e se realmente existe efeito da inoculação de estirpes de bactérias endofíticas para o controle biológico de doenças e para a promoção de crescimento em plantas (Sarethy & Saharan, 2021; Lahlali et al., 2022).

2 JUSTIFICATIVA

A redução da produtividade e qualidade dos grãos de feijão devido a doenças causadas por fungos fitopatogênicos é um problema significativo na agricultura. Atualmente, as práticas de controle dessas doenças com produtos químicos são limitadas, o que destaca a necessidade de encontrar alternativas sustentáveis. A utilização de microrganismos endofíticos, que promovem o crescimento das plantas e as protegem contra doenças, pode ser uma solução viável. No entanto, para implementar efetivamente o uso desses microrganismos, é fundamental compreender o funcionamento de seus genomas. O conhecimento do genoma das estirpes bacterianas pode fornecer insights sobre seus grupos funcionais no sistema solo-planta. Os genes presentes no genoma dessas estirpes podem desempenhar diversas funções, incluindo a promoção de crescimento em plantas e o controle biológico de patógenos. Portanto, é essencial identificar os genes envolvidos nessas funções para entender as circunstâncias em que são expressos e as funções que desempenham. Esse conhecimento permitirá o desenvolvimento de estratégias mais eficazes de manejo de doenças em campo, contribuindo para uma agricultura mais sustentável e produtiva

3 OBJETIVOS

Identificação a nível de espécie das estirpes do gênero *Pseudomonas* UFLA02-281, UFLA02-293 e UFLA 03-18.

Prospecção de genes relacionados ao antagonismo a patógenos e promoção de crescimento no genoma dessas estirpes.

Determinar o efeito da inoculação das estirpes UFLA02-281, UFLA02-293, UFLA 03-18 e UFLA03-10 no controle doenças e promoção de crescimento no feijão comum.

Análise funcional e prospecção de genes relacionados ao antagonismo a patógenos e promoção de crescimento das estirpes UFLA02-281, UFLA02-293, UFLA 03-18 e UFLA03-10.

4 HIPÓTESES

i. As estirpes bacterianas estudadas podem promover o crescimento e reduzir os sintomas de doenças fúngicas em plantas de feijão comum quando inoculados ou co-inoculados com a cepa de fungo endofítico da espécie *Induratia coffeana*.

ii. O sequenciamento dos genomas das estirpes bacterianas estudadas permitirá sua identificação ao nível de espécie e verificar a presença de genes relacionados a promoção de crescimento vegetal e controle biológico de doenças

5 RERENCIAL TEÓRICO

5.1 Feijão no mundo

O feijão comum é uma planta anual nativa das Américas sem um centro de origem específico e com centros independentes de domesticação. A espécie é uma dicotiledônea, pertencente à família Leguminosae, com metabolismo C3 e sistema radicular pivotante.

A produção de feijão está sujeita a uma série de fatores, como riscos ambientais, estresses biológicos, políticas governamentais, hábitos alimentares de cada país entre outros. No panorama mundial, o Brasil ocupa a terceira posição no ranking de produção de grãos secos de feijão, apenas perde para a Índia e Myanmar. Com relação a importação, os países que mais importam feijão são Índia, China, Bangladesh, Estados Unidos e Egito e os países que mais exportam feijão são Myanmar, Estados Unidos, Argentina e Canadá (FAOSTAT, 2024)

Ao longo dos anos a demanda por alimentos provenientes de leguminosas tem aumentado devido aos vários benefícios a saúde, pois são alimentos ricos em micronutrientes, como potássio, magnésio, ferro e zinco. Outro fator que impulsionou o aumento da demanda de feijão no mundo são as dietas vegetarianas, tendo o feijão como uma importante fonte de proteína e carboidrato (Valenciano & Chávez, 2017).

5.2 Produção feijão no Brasil

No Brasil o feijão comum tem produção primordial, por fazer parte da alimentação básica da população em geral. O feijão é plantado em todas as regiões, sendo distribuído em três safras ao longo do ano. Os estados que produzem mais feijão, de acordo com a CONAB (2022) no último boletim anual sobre a cultura, são: Paraná, Minas Gerais, Bahia, Goiás e Mato Grosso. Paraná e Minas Gerais se destacam por apresentarem as maiores produções, 717,8 t e 474,3 t, respectivamente. Distrito Federal e Goiás, por sua vez, apresentam as maiores produtividades, 2.732 kg ha^{-1} e 2.548 kg ha^{-1}, sendo muito superiores à média nacional que foi de 1.046 kg ha^{-1} na safra 2021/22.

Além da alta produção anual, o feijão tem alta demanda de consumo no país, dessa forma mais de 90% do produto retorna para o mercado interno (CONAB, 2021). Na última década houve redução de área plantada de feijão em 12,5% ao longo dos anos, entretanto a produtividade média nacional manteve a faixa de aproximadamente 800 a 1200 kg ha-^{1}. A produção de feijão no Brasil ficou na faixa de 2.512,9 a 3.732,8 mil toneladas, essa variação

ocorreu principalmente por causa das variações edafoclimáticas e pelas perspectivas de mercado de cada safra (CONAB, 2022).

O consumo de feijão no Brasil é influenciado por preferências regionais, local de moradia e condição financeira. Classes menos favorecidas mantêm um consumo per capita mais elevado, desde que o poder aquisitivo não limite o acesso ao alimento. O consumo médio aparente per capita de feijão-comum foi de 12,2 kg/hab em 2021, com um declínio observado desde 1996, quando atingiu 18,8 kg/hab. A maioria dos feijões consumidos no Brasil é feijão-comum (80%) e feijão-caupi (20%) (CONAB, 2024). Dentre os feijões-comuns consumidos, 56% são do tipo carioca, 21% são pretos e 3% são especiais (Pereira et al., 2021). O declínio no consumo per capita ao longo dos anos pode ser atribuído a fatores como a substituição por alimentos mais acessíveis e de preparo mais conveniente. O feijão é uma fonte potencial de proteína vegetal, podendo ser uma alternativa para substituição de proteínas de origem animal. Além da diversidade de tipos de grãos, há variabilidade genética dentro de cada tipo, afetando os teores de proteína e minerais como ferro e zinco. Cultivares biofortificadas, com teores mais elevados desses nutrientes, podem contribuir para uma alimentação mais saudável em populações carentes (Silva et al., 2019).

5.3 Doenças importantes no feijoeiro

O feijão é afetado por diversas pragas e doenças que podem reduzir a produção da lavoura. Existem doenças que podem ser identificadas nas fases iniciais de desenvolvimento do feijoeiro, dessa forma o controle aplicado no início do aparecimento das doenças pode prevenir perdas futuras (Arf et al., 2015). Entre as principais doenças que causam danos no feijão se destacam as doenças causadas pelos fungos como *Rhizoctonia solani*, *Colletotrichum lindemuthianum*, *Sclerotinia sclerotiorum* e *Pseudocercospora griseola*.

O fungo *Rhizoctonia solani* afeta a cultura do feijão principalmente nas fases iniciais de desenvolvimento onde as plantas jovens são muito suscetíveis ao estrangulamento do colo podendo acarretar o tombamento conhecido como “damping off”, (Miranda et al., 2007; Vieira et al., 2016). O patógeno pode ser disseminado via semente e através de resíduos de solo em implementos agrícolas. Temperaturas amenas, entre 15 e 18°C, e alta umidade, são ideais para o desenvolvimento da doença. O controle convencional dessas doenças envolve o uso de variedades resistentes, fungicidas e uso de cobertura morta, porém são métodos que não

garantem o controle satisfatório, além de gerarem um custo adicional na produção (Canale et al., 2020).

A antracnose causada pelo fungo *Colletotrichum lindemuthianum* é uma doença cosmopolita na cultura do feijoeiro, ocorrendo principalmente nas épocas mais úmidas e com temperaturas mais amenas (em torno de 13 ºC a 26 ºC). É introduzida na área de cultivo pelas sementes e disseminada de uma planta para outra através das gotas de água da chuva ou da irrigação. Pode causar perdas de até 100% quando utilizadas sementes infectadas e plantios sob condições de ambiente favorável (Xavier et al., 2021). As lesões de antracnose podem atingir toda a parte aérea da planta. A presença de áreas necrosadas nas nervuras é um sintoma bem típico da doença. Nas folhas, as lesões, geralmente alongadas, de cor avermelhada ou marrom-escura, ocorrem principalmente na face inferior e, em menor frequência, na superior (Padder et al., 2017). Nas vagens as lesões de antracnose são deprimidas, circulares com a borda marrom mais escura que o centro, que tem cor acinzentada ou rosada. Em vagens recém-formadas, o grande número de lesões causa enrugamento e encurtamento das vagens. O patógeno também é capaz de infectar as sementes, provocando sintomas como a descoloração e a formação de lesões escuras no tegumento ou mesmo nos cotilédones (Wendland et al., 2016; Canale et al., 2020).

O mofo branco do feijoeiro é causado pelo fungo *Sclerotinia sclerotiorum*, em geral, os sintomas do mofo-branco iniciam-se na junção do pecíolo com a haste, aproximadamente de 10 cm a 15 cm acima do solo com a formação de micélio branco abundante. O início da infecção geralmente coincide com o final da fase vegetativa da cultura e o florescimento, quando pétalas de flores senescentes são colonizadas pelo fungo que, a seguir, invade outros órgãos da planta. (Machado et al., 2015). Dependendo do local e da extensão da necrose, a planta pode amarelecer e morrer. O controle desse patógeno é limitado pela ampla gama de hospedeiros e também pela capacidade do patógeno de sobreviver no solo por vários anos na forma de escleródios (Leite et al., 2014).

A mancha-angular causada pelo fungo *Pseudocercospora griseola* é uma doença da parte aérea, observada em todas as regiões produtoras de feijão. Os sintomas da doença estão presentes nos principais órgãos do feijoeiro-comum: folhas, caule e vagens. Nas folhas primárias, as lesões geralmente são circulares, de cor marrom ou castanha. Já nas folhas trifoliadas, as lesões adquirem coloração cinza a marrom-escuro, com halo amarelo ao redor, e são tipicamente angulares, delimitadas pelas nervuras. Esse sintoma é, inclusive, o que denomina a doença (Crous et al., 2006). Em campo os sintomas são mais evidentes nos estádios finais do ciclo das plantas do feijoeiro, período em que as lesões evoluem e coalescem, levando

a uma desfolha prematura. Nos caules e pecíolos, as lesões são alongadas e marrom-escuras. Nas vagens são circulares de aspecto marrom- -castanho, porém não deprimidas como as lesões de antracnose. A esporulação pode ocorrer tanto nas folhas, caules e pecíolos, quanto nas vagens (Pereira et al., 2015).

5.4 Uso de rizobactérias no controle biológico de doenças de feijão

A doença, do ponto de vista utilizado no controle biológico é a interação do patógeno x hospedeiro x ambiente. Dentro desta relação ainda existe a participação dos microrganismos não patogênicos presentes nos sítios de infecção, esses microrganismos podem apresentar efeitos positivos ou negativos sobre essa interação (Campanhola & Bettiol, 2003). Entre os microrganismos com o efeito negativo nesta interação, algumas espécies de bactérias endofíticas apresentam mecanismos de ação que podem reduzir o impacto de microrganismos fitopagênicos sobre plantas.

Os principais mecanismos de ação para o controle biológico são competição por espaço e por recursos do meio, produção de antibióticos e de outros metabólitos, até mesmo a promoção de crescimento e FBN são consideradas mecanismos de ação indiretos para o controle biológico, uma vez que a planta se desenvolve com mais vigor e os microrganismos fitopagênicos não conseguem causar danos as plantas (Lacava et al., 2013; Melo et al., 2020). Entre esses organismos agentes de biocontrole, as rizobactérias conseguem interagir com plantas utilizando os mecanismos citados e também por mecanismos de indução de resistência na planta a patógenos (Silveira & Freitas, 2007). Portanto, os pesquisadores buscam explorar o potencial existente nas bactérias endofíticas. No trabalho de Gholami e colaboradores (2014), utilizando estirpes endofíticas do gênero *Bacillus* e *Streptomyces* no controle biológico do fungo *Agroathelia rolfsii* foi observada redução da podridão radicular do feijoeiro maior nas sementes pré-tratadas com os isolados não identificados de *B. subtilis* subsp. *spizizenii*, *B. subtilis* subsp. *subtilis*, *B. atrophaeus*, *B. tequilensis* e *S. cyaneofuscatus*, o que resultou em 50–58,5% de redução na severidade da doença de podridão radicular. Em outro estudo com *Colletotrichum lindemuthianum* de acordo com o índice de severidade da doença, os isolados utilizados do gênero *Bacillus* e *Streptomyces* quando inoculados em plantas de feijão foi observada a redução da gravidade da doença de 40 – 76,8% em condições controladas de casa de vegetação (Gholami et al., 2013).

Bactérias endofiticas do gênero *Pseudomonas* também estão sendo estudadas com relação ao seu potencial antimicrobiano como agente de biocontrole em doenças do feijoeiro.

A estirpe ASU15 (*Pseudomonas putida*) proporcionou uma forte redução de germinação de esporos de *Uromyces appendiculatus in vitro* e redução no desenvolvimento da severidade da ferrugem em plantas de feijão em condições de casa de vegetação. Os tratamentos com ASU15 reduziram a severidade da doença em comparação com o tratamento controle em até 69.9% (Abo-Elyousr et al., 2021). Isolados de *Pseudomonas fluorescens* UTPF16 e UTPF26 diminuíram significativamente o número de plântulas com sintomas de tombamento causado por *R. solani* (Ahmadzadeh & Sharifi Tehrani et al., 2009). No trabalho de Sendi e colaboradores (2020) foi observada a redução de *Fusarium* sp. no feijoeiro quando inoculado com *Bacillus amyloliquefaciens*.

Em ensaios desenvolvidos no Brasil, os pesquisadores também estudam o potencial de biocontrole das rizobactérias. Estirpes da espécie *Bacillus subtilis* já fazem parte do desenvolvimento de produtos biológicos comerciais registrados para o controle de doenças em plantas. A estirpe UFLA168 (*Bacillus subtilis*) foi utilizada em um estudo onde controlou de forma eficiente a murcha bacteriana do feijoeiro em ensaios em casa de vegetação e ainda obteve índices de desenvolvimento vegetal semelhantes a estirpe ALB629 (*Bacillus amyloquifaciens*) que já foi estudada pela capacidade de produzir micotoxinas que controlam fungos causadores de doenças (Medeiros et al., 2012; Martins et al., 2015).

O feijoeiro cultivado em solo infestado com *Agrothelia rolfsii* e inoculado com bactérias do gênero *Rhizobium* SEMIA 4032, 4077, 4088, 4080 ou 4085 não apresentou sintomas de podridão do colo em ensaios de casa de vegetação. No ensaio de campo, a incidência da doença foi atenuada quando inoculada es estirpes SEMIA 4032, 4077, 4088, 4080, 4085 ou 439. Sendo que as estirpes mais eficientes identificadas foram SEMIA 439 e SEMIA 4088, que diminuíram 18,3 e 14,5% da incidência do mofo cinzento no feijoeiro (Volpiano et al., 2018).

No trabalho de Ferreira et al. (2020) foi avaliada a promoção de crescimento e produtividade do feijão na presença da *Rhizoctonia solani*, observando que as estirpes de bactérias endofíticas, UFLA 02-281/03-18 (*Pseudomonas* sp.) e UFLA 02-286 (*Bacillus* sp.), quando inoculadas na presença do fungo *R. solani* reduziram a severidade da doença na fase inicial de desenvolvimento das plantas.

5.5 Fungos endofíticos na agricultura e no controle biológico de doenças

Os fungos endofíticos habitam tecidos e órgãos vegetais sem gerar danos as plantas, e incluem uma grande diversidade de fungos filamentosos e leveduras com potencial de aumentar a produtividade agrícola. Alguns dos mecanismos envolvidos na promoção do crescimento de plantas por meio de fungos endofíticos incluem o aumento do acesso a nutrientes (nitrogênio, fósforo, potássio, zinco, ferro, etc.), produção de hormônios vegetais, redução da quantidade de etileno ou aumento na aquisição de água (Poveda, et al., 2021; Baron & Rigobelo, 2022; Bogas et al., 2022).

As interações entre fungos endofíticos e suas plantas hospedeiras são influenciadas por fatores bióticos e abióticos, resultando na produção de metabólitos secundários com atividades biológicas específicas, incluindo mecanismos de ação fitotóxicos como produção de antibióticos e bacterioricinas (Macías-Rubalcava & Garrido-Santos, 2022). Além disso, há consenso de que os compostos voláteis produzidos por fungos endofíticos podem inibir o crescimento de fungos patogênicos, oferecendo potencial para substituir defensivos químicos no controle de fitopatógenos (Kaddes et al., 2019).

Nos trabalhos de Santos e Silva (2014) foi demostrado que os fungos endofíticos podem ser utilizados como antagonistas no controle biológicos de patógenos de plantas. Os fungos endofíticos se destacam pela quantidade de produtos naturais que produzem, suas características de atividade biológica e metabólitos secundários que podem se originar das interações metabólicas que ocorrem entre fungos e espécies vegetais. Isso faz com que espécies de fungos endofíticos tenham grande potencial no desenvolvimento de agentes antibacterianos (Kusari et al, 2012).

Com relação a promoção de crescimento vegetal, alguns fungos endofíticos apresentam essa função. Essa promoção de crescimento pode acontecer através da síntese de fitormônios e/ou tolerância aos estresses abióticos (Khan et al., 2015).

No trabalho de Mota et al. (2021) foram utilizados fungos endofíticos do gênero *Induratia*, isolados das folhas do café, para a promoção de crescimento e controle biológico de mancha angular, mofo branco e antracnose no feijoeiro. Entre as cepas fúngicas utilizadas a CML 4019 apresentou significativo controle sobre as doenças estudadas. Isso mostra que fungos endofíticos podem ser utilizados no controle biológico para a redução de sintomas de doenças em plantas.

5.6 Mercado de insumos biológicos no Brasil

No Brasil apenas três estirpes de rizóbios apresentam autorização para serem utilizadas na formulação de inoculantes para a cultura do feijoeiro. Elas são CIAT 899 (*Rhizobium tropici),* H 12 (*Rhizobium leucaenae*) e PRF 81 (*Rhizobium freirei)* que podem ser encontradas também na nomenclatura SEMIA 4077, SEMIA 4088 e SEMIA 4080, respectivamente (MAPA, 2011). Essas estirpes usualmente são utilizadas como referência de comparação para estudos de inoculantes por já serem comercialmente utilizadas e apresentarem resultados positivos na inoculação do feijão com relação a fixação biológica de nitrogênio (Mercante et al., 2017), além da sua aprovação pelo MAPA como inoculante.

Com relação ao controle biológico, atualmente no mercado existem 119 produtos comerciais formulados classificados como fungicidas microbiológicos, entre eles 50 produtos com ingrediente ativo de fonte bacteriana registrados no Sistema de Agrotóxicos Fitossanitários (AGROFIT, 2024). As bactérias presentes nestes produtos são de diferentes gêneros e espécies, sendo que a grande maioria pertence ao gênero *Bacillus*, (*B. subtilis, B. amyloliquefaciens, B. pumilus, B. thuringiensis* e *B. velezensis),* sendo que não existe especificidade com relação a cultura apenas para o patógeno à ser controlado. Outras espécies de bactérias que foram registrados como ingrediente ativo de fungicidas microbiológicos são *Brevibacillus parabrevis* e *Paenibacillus azotofixans*, *Pseudomonas fluorescens*.

Os patógenos controlados pelos produtos biológicos já registrados variam entre as formulações, sendo que por exemplo o Protege controla apenas *Rhizoctonia solani*, enquanto que no registro do Serenade há 16 patógenos que podem ser controlados tais como *Rhizoctonia solani* e *Sclerotinia sclerotiorum*, entre outros. Entre os fungicidas microbiológicos registrados 19 produtos controlam o *Colletotrichum lindeamuthium*, como por exemplo o Fx Protection, Amanzi e Biagro Proteção; 44 produtos recomendados para o controle de *Rhizoctonia solani*, sendo que 39 deles apresentam bactérias em sua composição como Arvatico e o Bio Release e os outros 5, diferentes espécies de *Trichoderma*. Para *Sclerotinia sclerotiorum*, 61 produtos estão registrados no AGROFIT (MAPA) como fungicidas microbiológicos, sendo 37 deles com bactérias em suas formulações como o Bioshield, Ourotricx, Furatro e Native. Para as doenças causadas por *Pseudocercospora griseola* não existem produtos biológicos registrados (AGROFIT, 2024).

Tabela 1. Produtos comerciais de registrados para o controle biológico de doenças.

Nº Registro	**Marca Comercial**	**Ingrediente Ativo(Grupo Químico)**	**Patógeno**

24820	AgTecmmon	*B. amyloliquefaciens*, CPQBA 040-11DRM 01 + *B. amyloliquefaciens*, CPQBA 040-11DRM 04	*Colletotrichum truncatum; Corynespora cassiicola; Ramularia areola; Colletotrichum lindemuthianum; Phaeosphaeria maydis*
21721	Amanzi	*B. amyloliquefaciens*	*Colletotrichum gloeosporioides; Colletotrichum lindemuthianum*
6721	Amitrix SC	*B. amyloliquefaciens*	*Colletotrichum gloeosporioides; Colletotrichum lindemuthianum*
27021	Amylo-X SL; Bacilo-X;	*B. amyloliquefaciens*	*Botrytis cinérea; Colletotrichum gloeosporioides; Pythium ultimum; Mycosphaerella fijiensis; Sclerotinia sclerotiorum; Sphaeroteca fuliginea; Alternaria porri; Rhizoctonia solani*
3221	Arvatico	*B. velezensis*, CNPSo 3602	*Fusarium solani f. sp. glycines; Rhizoctonia solani;*
8121	Ataplan; Aratel;	*B. subtilis* + *B. velezensis*, CNPSo 3602	*Colletotrichum truncatum; Fusarium oxysporum f.sp. vasinfectum; Fusarium oxysporum f.sp. phaseoli; Rhizoctonia solani; Pythium aphanidermatum; Pythium ultimum*
22620	Bactel; DuoBac Meta;	*B. amyloliquefaciens*	*Colletotrichum gloeosporioides; Colletotrichum lindemuthianum*
19121	Baktillis	*B. subtilis*	*Colletotrichum gloeosporioides; Fusarium solani f. sp. Glycines; Rhizoctonia solani*
25720	Biagro Proteção	*B. amyloliquefaciens*	*Colletotrichum gloeosporioides; Colletotrichum lindemuthianum*
34123	Bio Release	*Pseudomonas fluorescens*, cepa CCT 7997	*Rhizoctonia solani*
26816	Biobac; Tacap;	*B. subtilis*	*Hemileia vastatrix; Neofabraea perennans; Alternaria porri; Rhizoctonia solani; Botrytis cinerea*

43418	Bio-imune; Multi-Attack; Multi-Guard;	*B. subtilis*	*Alternaria solani; Colletotrichum acutatum; Colletotrichum gloeosporioides; Colletotrichum lindemuthianum; Colletotrichum truncatum ; Hemileia vastatrix ; Phakopsora pachyrhizi ; Pseudomonas syringae ;Pseudomonas syringae pv. garcae; Sclerotinia sclerotiorum; Uncinula necator; Xanthomonas vesicatoria*
23123	Biolucro	*Bacillus circulans*, isolado CCT0026 + *Bacillus licheniformis*, isolado CCTB07 + *Bacillus subtilis*, isolado CCTB04 + *Paenibacillus azotofixans*, isolado CCT4719	*Macrophomina phaseolina; Rhizoctonia solani*
11422	Biomagno	*B. amyloliquefaciens*, CNPSo3202 + *B. thuringiensis*, CNPSo3915 + *B. velezensis*, CNPSo 3602	*Rhizoctonia solani; Pratylenchus brachyurus*
19822	Bioshield	*Bacillus pumilus*, CNPSo 3203 + *Bacillus subtilis*, CNPSo2720 + *Bacillus velezensis*, CNPSo 3602	*Septoria glycines; Colletotrichum acutatum; Sclerotinia sclerotiorum; Sphaeroteca fuliginea; Colletotrichum gloeosporioides; Sphaerotheca pannosa; Botrytis cinerea; Sclerotium rolfsii; Mycosphaerella fijiensis; Colletotrichum lindemuthianum.*
27321	Bombardeiro; Lastro;	*B. pumilus*, CNPSo 3203 + *B. subtilis*, CNPSo2720 + *B. velezensis*, CNPSo 3602	*Septoria glycines; Puccinia triticina; Corynespora cassiicola; Cercosporidium personatum; Colletotrichum acutatum; Exserohilum turcicum; Sclerotinia sclerotiorum; Colletotrichum falcatum; Ramularia areola; Stenocarpella maydis; Cercospora zeae-maydis; Hemileia vastatrix; Sphaeroteca fuliginea; Colletotrichum truncatum; Cercospora kikuchii*

4621	BTP 005-19	*B. pumilus*, CNPSo 3203	*Cercospora kikuchii; Corynespora cassiicola; Septoria glycines*
31022	BTP 007-19	*Bacillus velezensis*, CNPSo 3602	*Fusarium solani*
14724	BTP 167-21A	*Bacillus circulans*, CCT0026 + *Bacillus licheniformis*, CCTB07 + *Bacillus subtilis*, CCTB04 + *Paenibacillus azotofixans*, isolado CCT4719	*Macrophomina phaseolina; Rhizoctonia solani* .
4721	Caravan	*B. pumilus*, CNPSo 3203	*Cercospora kikuchii; Corynespora cassiicola; Septoria glycines*
3121	Certano	*B. velezensis*, CNPSo 3602	*Fusarium solani f. sp. glycines; Rhizoctonia solani;*
22718	Duravel	*B. amyloliquefaciens*	*Botrytis cinerea; Botrytis squamosa; Cryptosporiopsis perennans; Phyllosticta citricarpa; Pythium ultimum; Rhizoctonia solani; Xanthomonas campestris pv. campestris*
26616	Eco-Shot	*B. amyloliquefaciens*	*Alternaria solani; Botrytis cinerea; Colletotrichum gloeosporioides; Cryptosporiopsis perennans; Erysiphe polygoni; Phyllosticta citricarpa; Sclerotinia sclerotiorum; Sphaeroteca fuliginea; Uncinula necator*
21424	Égide	*Bacillus amyloliquefaciens* CCT8135 + *Bacillus velezensis* CCT8136	*Botrytis cinerea*
16923	FrontierControl	*Bacillus velezensis* Cepa Labim 40	*Septoria glycines; Colletotrichum truncatum; Corynespora cassiicola; Pantoea ananatis*
15220	Furatro	*B. subtilis*	*Sclerotinia sclerotiorum; Rhizoctonia solani*

30820	Fx Protection	*B. amyloliquefaciens*	*Colletotrichum gloeosporioides; Colletotrichum lindemuthianum*
30918	Lalstop I32 SC	*B. amyloliquefaciens*	*Botrytis cinerea*
20323	Milarum	*Bacillus subtilis*, IAB/BS03	*Pseudoperonospora cubensis; Phytophthora infestans*
22123	Nacillus Max	*Bacillus licheniformis*, cepa Copihue + *Bacillus subtilis*, N5 + *Brevibacillus parabrevis*, cepa N4	*Alternaria solani*
21220	Native	*B. amyloliquefaciens*, CPQBA 040-11DRM 01 + *B. amyloliquefaciens*, CPQBA 040-11DRM 04 + *Trichoderma harzianum*	*Rhizoctonia solani; Sclerotinia sclerotiorum*
34518	No-Nema; Nema-Attack; Nema-Guard; Bio Baciens; Cropwinner Eco Nemaxy; Vitalforce Bio Amilofaciens; Bionova Sanus S-Nema; Nematha; Klaatu; Nemavale;	*Bacillus amyloliquefaciens*	*Fusarium verticillioides; Macrophomina phaseolina*
22323	Ospo Vi55	*Bacillus subtilis*, IAB/BS03	*Sphaeroteca fuliginea; Leveillula taurica; Uncinula necator*
420	Ourotricx	*B. amyloliquefaciens*, CCT 7901 + *Trichoderma asperellum* URM 8120 + *Trichoderma harzianum*, URM 8119	*Colletotrichum lindemuthianum; Rhizoctonia solani; Sclerotinia sclerotiorum*
520	Pardella	*B. amyloliquefaciens*, CCT 7901 + *Trichoderma asperellum* URM 8120 + *Trichoderma harzianum*, URM 8119	*Colletotrichum lindemuthianum; Rhizoctonia solani; Sclerotinia sclerotiorum*
10822	Protege	*B. amyloliquefaciens*, CNPSo3202 + *B. thuringiensis*, CNPSo3915 + *B. velezensis*, CNPSo 3602	*Rhizoctonia solani*

522	Provilar	*B. subtilis* + *B. velezensis*, CNPSo 3602	*Sclerotinia sclerotiorum*
19922	Quorum	*Bacillus amyloliquefaciens*, CNPSo3202 + *Bacillus thuringiensis*, CNPSo3915 + *Bacillus velezensis*, CNPSo 3602	*Rhizoctonia solani*
35419	Restrict; Zigurat; Vitanica Duo Protect;	*B. amyloliquefaciens*, CPQBA 040-11DRM 01 + *B. amyloliquefaciens*, CPQBA 040-11DRM 04	*Colletotrichum lindemuthianum; Ramularia areola; Phaeosphaeria maydis; Corynespora cassiicola; Colletotrichum truncatum*
27221	Reverb	*Bacillus pumilus* + *Bacillus subtilis* + *Bacillus velezensis*, isolado CNPSo 3602	*Cercospora kikuchii; Septoria glycines; Cercospora zeae-maydis; Cercosporidium personatum; Colletotrichum acutatum; Colletotrichum lindemuthianum; Sclerotinia sclerotiorum;*
14023	Row-Vispo	*Bacillus subtilis*, IAB/BS03	*Hemileia vastatrix; Phakopsora pachyrhizi; Colletotrichum lindemuthianum; Septoria glycines; Corynespora cassiicola;*
3911	Serenade	*B. subtilis* QST 713	*Alternaria dauci; Alternaria porri; Botrytis cinerea; Colletotrichum acutatum; Colletotrichum gloeosporioides; Cryptosporiopsis perennans; Fusarium oxysporum f.sp. lycopersici; Mycosphaerella fijiensis; Pythium ultimum; Rhizoctonia solani; Sclerotinia sclerotiorum; Sphaeroteca fuliginea; Sphaeroteca macularis; Streptomyces scabies; Xanthomonas citri subsp. citri; Xanthomonas vesicatoria*
33918	Shocker; Peak; Bio Venci;	*B. amyloliquefaciens* + *B. amyloliquefaciens* + *Trichoderma harzianum*	*Rhizoctonia solani; Sclerotinia sclerotiorum*

29623	Sinsmart	*Bacillus amyloliquefaciens*, CL3	*Botrytis cinérea; Fusarium solani; Xanthomonas campestris pv. campestris; Rhizoctonia solani; Sphaerotheca fuliginea; Pythium ultimum*
4311	Sonata	*B. pumilus*	*Alternaria porri; Alternaria solani; Botrytis cinerea; Colletotrichum lindemuthianum; Cryptosporiopsis perennans; Sphaeroteca macularis; Sphaerotheca fuliginea; Uncinula necator*
824	Superguard	*Bacillus amyloliquefaciens* BAC076 + *Bacillus amyloliquefaciens* BAC077 + *Bacillus subtilis* BAC059 + *Bacillus subtilis* BAC116	*Fusarium oxysporum; sclerotinia sclerotiorum; Rhizoctonia solani*
12523	Taegro e Krivesta	*Bacillus amyloliquefaciens*, FZB24	*Alternaria solani; Botrytis cinerea; Colletotrichum gloeosporioides; Uncinula necator; Sclerotinia sclerotiorum; Sphaeroteca fuliginea; Rhizoctonia solani.*
1820	Tanus	*B. amyloliquefaciens*, CCT 7901 + *Trichoderma asperellum* URM 8120 + *Trichoderma harzianum*, URM 8119	*Colletotrichum lindemuthianum; Rhizoctonia solani; Sclerotinia sclerotiorum*
21120	Torpeno; Trichosmart; Bellator;	*B. amyloliquefaciens*, CPQBA 040-11DRM 01 + *B. amyloliquefaciens*, CPQBA 040-11DRM 04 + *Trichoderma harzianum*	*Rhizoctonia solani; Sclerotinia sclerotiorum*
25621	Tricozak	*B. amyloliquefaciens*, CCT 7901 + *Trichoderma asperellum* URM 8120 + *Trichoderma harzianum*, URM 8119	*Colletotrichum lindemuthianum ; Rhizoctonia solani ; Sclerotinia sclerotiorum*
23523	Tryba	*Bacillus amyloliquefaciens*, SVG-0036-B + *Bacillus subtilis*, SVG-0037-B + *Trichoderma asperellum*, SVG-00124-F + *Trichoderma harzianum*, SVG-00003-F	*Rhizoctonia solani*

25120	TWIXX-A; Bio Braza; Amosbio;	*B. amyloliquefaciens,* CPQBA 040-11DRM 01 + *B. amyloliquefaciens*, CPQBA 040-11DRM 04	*Colletotrichum lindemuthianum; Colletotrichum truncatum; Corynespora cassiicola; Phaeosphaeria maydis*
21021	Velez	*B. amyloliquefaciens*	*Colletotrichum gloeosporioides; Colletotrichum lindemuthianum*

Fonte: AGROFIT (2024)

5.7 Genoma e prospecção de genes de interesse de bactérias endofíticas

O genoma consiste em todo material genético retido nos organismos, isso inclui toda a informação necessária para o ciclo e manutenção de vida do indivíduo avaliado. Existem bancos de dados onde genomas dos organismos sequenciados são depositados e podem ser acessados pela comunidade (Kaye & Wasserman, 2021). O GenBank contém sequências de nucleotídeos de todos os tipos de organismos, incluindo bactérias e o RefSeq fornece sequências de referência de alta qualidade e anotadas para diversos organismos, incluindo bactérias, ambos são mantidos pelo NCBI (National Center for Biotechnology Information, 2024) dos Estados Unidos. O European Nucleotide Archive (ENA) é um banco de dados abrangente de sequências de nucleotídeos mantido pelo European Bioinformatics Institute (EBI, 2024). Ele contém sequências de genomas bacterianos e de outros organismos e o DNA Data Bank of Japan (DDBJ, 2024), assim como o GenBank e o ENA, o DDBJ é um banco de dados de sequências de nucleotídeos que inclui genomas bacterianos e de outros organismos.

Na bacteriologia, o sequenciamento do genoma foi utilizado inicialmente para a identificação e diferenciação de novas espécies, em novos agrupamentos. Além da identificação dos microrganismos da microbiota os cientistas também utilizam o sequenciamento do genoma de bactérias para analisar a presença de genes que expressam diferentes funções no ambiente que a bactéria está inserida (Kisand & Lettieri, 2013; Land et al., 2015).

A promoção de crescimento em plantas por microrganismos pode ser prospectada por uma grande quantidade de genes ligados a diversas funções nas plantas, entre os mecanismos de ação associados a promoção de crescimento temos principalmente o metabolismo de nutrientes, entre eles o nitrogênio e o fósforo, produção de hormônios vegetais e proteção das plantas contra fitopatógenos. Entre os genes já prospectados em outros trabalhos temos genes associados a síntese de AIA como *ysnE*, *trpA* e *trpB*, gene para a síntese de óxido nítrico (*nos*); transportadores de nitrato com *Nar* e *Nas*; genes relacionados a fixação biológica

de nitrogênio como os *nifs* e *fixs* transportadores de potássio como *ktrA,* genes que prospectam fosfatases como por exemplo o gene *ppa* e transportadores de fosfatos como *phoU* e *pit* (Eida et al., 2020; Gu et al., 2020).

Entre os genes prospectados para o controle biológico temos, genes envolvidos na rota metabólica de 2,3- Butanediol como *ilvA*, *ilvD*, *ilvC*, *ilvN*, *ilvB* e *ilvE*, genes precursores de Isoprene como *ispG* e *lytB* e genes precursores de Methanethiol como *metH*, *mmuM*, *mtnE*, compostos estudados para a indução de resistência sistêmica em plantas (Yi et al., 2016; Zhao et al., 2022). Outro mecanismo envolvido no controle biológico é a degradação de compostos voláteis e temos genes como *acoA* e *acoB,* e genes correlacionados com a colonização como *minD*, sendo a competição por espaço e recursos um mecanismo de ação diretamente relacionado ao biocontrole (Adekele et al., 2021).

A comparação de genomas tem como objetivo obter um entendimento da evolução das espécies, genes funcionais e de regiões não codificantes do genoma (Malard et al., 2022). Nesses estudos comparativos é possível observar a enorme diversidade entre isolados da mesma espécie. A diversidade genômica dentro da mesma espécie de bactéria é resultado de múltiplos mecanismos genéticos e interações ecológicas. Transferência horizontal de genes, mutação, pressões seletivas, recombinação, elementos genéticos móveis, variação de fase e antigênica, dinâmica populacional, adaptação ambiental e interações microbianas. Esses fatores contribuem coletivamente para a variabilidade observada em genes e regiões codificantes entre bactérias (Greenlon et al., 2019; Ryall et al., 2012; Soucy et al., 2015; Lin et al., 2022).

As bactérias endofíticas produzem várias moléculas e têm uma gama de atividades relacionadas à quimiotaxia, motilidade, adesão, propriedades da parede celular bacteriana, secreção, regulação da transcrição e utilização de substrato para estabelecer uma interação bem-sucedida. No genoma também podem ser encontrados genes envolvidos em mais de uma função. A presença de determinados genes não configura que está acontecendo a sua expressão, todavia a prospecção de genes relacionadas com determinadas funções benéficas em plantas já é um indicativo do potencial que as bactérias ali presentes podem gerar (Pinski et al., 2019). Dessa forma, é importante explorar com sucesso as interações benéficas entre plantas e bactérias endofíticas na agricultura e os principais aspectos que governam as interações bem-sucedidas ainda precisam ser definidos.

5.8 Metabolismo secundário dos microrganismos endofíticos

Os organismos vivos são a fonte de uma enorme diversidade de mais de 1.000.000 de metabólitos diferentes. A maioria desses metabólitos é encontrada em plantas, mas os microrganismos são uma fonte particularmente rica, com mais de 20.000 compostos biologicamente ativos que afetam o desempenho e a sobrevivência de outros organismos. Os metabólitos secundários não possuem funções específicas conhecidas no metabolismo primário dos organismos, mas sua alta diversidade reflete em papéis biológicos, principalmente nas interações entre organismos em seu ambiente, e mostram seu papel de importância na sinalização de atividades e na produção de toxinas (Demain & Sanchez, 2009; Brader et al., 2014).

A análise do genoma de bactérias pode revelar agrupamentos de genes potencialmente envolvidos na produção de metabólitos, sendo que essas descobertas ainda são recentes e abrangem estruturas e ocorrência de metabólitos com funções conhecidas e desconhecidas (Saati-Santamaría et al., 2022).

A identificação de conjuntos de genes para a produção de compostos peptídicos antimicrobianos, conhecidos como bacteriocinas, está relacionada com a capacidade de certas bactérias em controlar fitopatógenos devido à sua capacidade de mirar e matar especificamente microrganismos patogênicos. Além disso, as bacteriocinas têm um alto grau de seletividade para seus alvos, minimizando os efeitos fora do alvo. (Vu et al., 2020; Subramanian & Smith, 2015). Esta abordagem de manejo de doenças pode ajudar a mitigar o desenvolvimento de resistência a antibióticos e reduzir os impactos ambientais. Essas interações aumentam a tolerância das plantas às mudanças ambientais, seja em relação ao solo ou às plantas com as quais as bactérias estão associadas (Macías-Rubalcava & Garrido-Santos, 2022).

Na atualidade entendemos os endófitos como organismos não fitopatogênicos, que colonizam os tecidos vegetais pelo menos em uma parte de sua vida (Oliveira et al., 2003). Vários estudos indicam que as bactérias endofíticas quando colonizam o ambiente vegetal precisam ser adaptáveis, dessa forma seu potencial metabólico pode ser diferente quando utilizados ambientes diferentes e com plantas distintas, sendo a rizosfera um ambiente altamente competitivo, rico em predadores e compostos antibióticos (Manhaes & Francelino, 2013).

Entre essas bactérias, várias espécies do gênero *Bacillus* e *Pseudomonas* produzem uma série de metabólitos como peptídeos sintetizados ribossomicamente e modificados pós-tradução (RiPPs) que na literatura são descritos como importantes para nas funções de antibiose

e para induzir mecanismos de defesa das plantas (Hetrick & Van Der Donk, 2017), peptídeos sintetizados não-ribossomais (NRPS), que apresentam uma diversidade de produtos naturais com ampla atividade biológica e propriedades farmacológicas geralmente são toxinas, sideróforos ou pigmentos (Tajbakhsh et al., 2017) e sintetases de policetídeos (PKS) que são metabólitos que possuem diversas aplicações na indústria (Jenke-Kodama et al., 2005 Wang et al., 2020; Gaete et al., 2022; Adeleke et al., 2021).

Muitas bactérias produzem metabólitos secundários como agentes necessários para absorção, em particular, sideróforos envolvidos na aquisição de ferro, sendo que essa aquisição de sideróforos também está ligada aos aspectos de promoção de crescimento das plantas e solubilização de fósforo no solo (Verma et al., 2022). Os metabólitos secundários podem promover a formação de biofilme, produção de bacteriocinas, redução do fator de virulência (Raaijmakers & Mazzola, 2010; Munakata et al., 2022) e sinalização hormonal em plantas sendo essas funções ligadas ao controle biológico de doenças em plantas (Boro et al., 2022).

Metabólitos do grupo das surfactinas estimulam a colonização, a formação de biofilme, facilitam a motilidade e atuam como ativadores dos mecanismos de defesa das plantas contra vários patógenos microbianos. Outro metabólito do tipo NPRS, a bacilibactina é conhecida por exercer uma forte atividade antifúngica e/ou desencadear as defesas da planta hospedeira contra patógenos (Nifakos et al., 2021).

Outra observação importante é que pode haver heterogeneidade nos agrupamentos de genes biossintéticos (BCGs) em microrganismos da mesma espécie. Nem todos os BCGs são igualmente conservados e apresentam diferenciações a nível modular. Isso mostra que, mesmo que uma espécie bacteriana tenha estirpes com capacidade antimicrobiana e que promovem o crescimento das plantas, outras estirpes da mesma espécie podem não ter a mesma capacidade (Nanjani et al., 2022).

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SEGUNDA PARTE – ARTIGOS

ARTIGO 1- *In vitro, in vivo,* and genomic analyses show the potential of *Paenibacillus peoriae* UFLA03-10 for biocontrol of diseases and as a biofertilizer in common bean

In vitro, in vivo and genomic analysis demonstrate potential of *Paenibacillus peoriae* UFLA03-10 for biocontrol of diseases and biofertilizer in common-beans

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Data availability All data analyzed during this study are included in this article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abstract

Bacteria belonging to the genus *Paenibacillus* have been shown potential as plant growth promoters and biocontrol agents. This study aimed to assess the efficacy of the UFLA03-10 strain *in vitro* and *in vivo* against four phytopathogenic fungi known to cause diseases in common beans. Additionally, the study sought to identify genes and gene clusters within the strain's genome that are responsible for its growth-promoting and disease-controlling properties in plants. *In vitro* experiments demonstrated the UFLA03-10 strain's ability to inhibit the growth of fungal strains belonging to *Colletotrichum lindemuthianum*, *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, and *Pseudocercospora griseola*, which are causative agents of anthracnose, white mold, damping-off, and angular spot diseases, respectively, in common beans. Similar results were observed in greenhouse trials, where inoculation with UFLA03-10 reduced disease symptoms by 73-84% in common beans. Functional annotation of the genome revealed the presence of genes associated with promoting plant growth, such as those involved in hormone and siderophore production, nutrient availability, and volatile compounds production. Additionally, genes related to biological control were identified, including those responsible for antimicrobial compound production and resistance induction. Genome analysis using antiSMASH identified gene clusters responsible for the biosynthesis of secondary metabolites. Given its ability to promote plant growth, broad-spectrum antimicrobial activity, and genomic characteristics, the *P. peoriae* UFLA03-10 strain shows potential for use as a biocontrol agent and biofertilizer.

Keywords: endophytic bacteria, biocontrol, functional genomic analysis, secondary metabolites

Key points

• Average nucleotide identity showed UFLA03-10 belongs to the *Paenibacillus peoriae* species.

• Inoculation with UFLA03-10 in common beans lessened fungal disease symptoms.

• Inoculation with UFLA03-10 in common beans promotes common bean growth

• Genome and *in vivo* tests confirm UFLA03-10 as an effective common bean inoculant.

Introduction

Paenibacillus genus comprises a diverse group of gram-positive bacteria that have been gaining attention in recent years for their potential applications in agriculture (Rybakova et al., 2016). Genomic analysis of *Paenibacillus* provides valuable insights into their genetic makeup and functional capabilities, allowing for a better understanding of their potential as biocontrol agents and plant growth promoters (Grady et al., 2016). The genera *Paenibacillus* currently comprises 389 species and 8 subspecies cataloged in the LPSN (List of Prokaryotic Names with Standing in Nomenclature) (Parte et al., 2020).

Within this genus, species such as *P. polymyxa* (Li et al., 2020), *P. alvei* (Fatouros et al., 2018), *P. terrae* (Yu et al., 2018), and *P. peoriae* (Yuan et al., 2022; Zhao et al., 2022), were identified as beneficial to plants by enhancing growth, producing phytohormones such as indole-acetic acid (IAA), solubilizing phosphates, and fixing nitrogen. Regarding plant pathogen control, some strains within the genus *Paenibacillus* exhibit mechanisms for induction of systemic resistance and production of bacteriocins and antimicrobial substances (Weselowski et al., 2016).

Among the species most commonly found as antimicrobial agents, *P. polymyxa* stands out. For instance, the strain ZYPP18 inhibited *in vitro* development of the fungus *Rhizoctonia cerealis*, the causative agent of wheat sheath rust, by over 92%. Wheat plants inoculated with ZYPP18 in the presence of the fungus *R. cerealis* showed mild to no infection on the leaves (Li et al., 2023). Other *Paenibacillus* species have been used for biological control, such as *Paenibacillus tianmuensis* YM002, which can effectively combat the bacterium *Acidovorax citrulli*, the causal agent of bacterial fruit blotch in cucumber plants, reducing the symptoms caused by the bacterium and promoting plant development (Koo et al., 2023).

In strain ZF390 (*P. peoriae*), the biosynthesis of metabolites such as brevicidin, fusaricidin B, octapeptin, paenilan, paenilipoheptin, paeninodine and tridecaptin was identified, with verified control of cucumber soft rot. (Zhao et al., 2022). Furthermore, the *P. peoriae* strain ZBFS16, isolated from wheat roots, suppressed white rot disease in grapes caused by *Coniella vitis* (Yuan et al., 2022). The *P. peoriae* strain HJ-2, isolated from the rhizosphere of *Paris polyphylla*, native to China, can act as a control agent for stem rot caused by *Fusarium concentricum* in plants of the same species (Jiang et al., 2022).

The strain UFLA03-10 was recently classified as *Paenibacillus peoriae* (Leite et al., 2024 submitted). This strain was isolated from cowpea nodules and tested *in vitro* for phosphate solubilization assays (Marra et al., 2012). Another characteristic analyzed in this strain was its ability to control *Rhizoctonia solani* in common beans and lima beans, as it exhibited a disease

index and disease progress curve lower than the non-inoculated controls used in the trials (Ferreira et al., 2020; Ferreira et al., 2023).

Paenibacillus peoriae UFLA03-10 was also efficient in solubilizing rock phosphate-based biochar fertilizer, making nutrients accessible for uptake by plants and other microorganisms, thus showing promise as a future alternative biofertilizer to achieve benefits similar to soluble phosphate fertilizers or to progressively reduce the application of soluble phosphate fertilizers on the soil (Leite et al., 2020). For these reasons, UFLA03-10 was selected for genome analysis and *in vitro* and *in vivo* tests to verify its antagonism against four fungal species that cause diseases in common beans as well as its ability to promote the growth of common beans.

Material and methods

Obtaining the strain

Paenibacillus peoriae strain UFLA03-10 is part of the Collection of plant growth-promoting bacteria in the laboratory of Biology, Microbiology, and Soil Biological Processes at the Soil Science department in the Federal University of Lavras. It was isolated from cowpea nodules (*Vigna unguiculata*) in culture medium 79, showing rapid growth (2 to 3 days) with an average diameter of over 2 mm, medium acidification, and abundant production of exopolysaccharides (Marra et al., 2012). The UFLA03-10 strain has been tested for phosphate solubilization, biological control, and growth promotion in cowpea, common bean, and lima bean plants (Marra et al., 2012; Costa et al., 2015; Marra et al., 2015; Marra et al., 2019; Ferreira et al., 2020; Leite et al., 2020; Ferreira et al., 2023).

Rhizobium tropici The CIAT899 approved by the Ministry of Agriculture, Livestock, and Food Supply (MAPA), has also been used as a symbiotic N2-fixing inoculant for common bean cultivation. This strain was shown to reduce symptoms of *Rhizoctonia solani* in common bean plants when co-inoculated with the UFLA03-10 (Ferreira et al, 2020; Ferreira et al., 2023). The UFLA02-127, isolated from common bean nodule, was selected because it did not control pathogenic fungi *in vitro*, although it is efficient in biological nitrogen fixation (Ferreira et al., 2009).

The complete genome of the UFLA03-10 strain has been made available on GenBank (National Center for Biotechnology Information, NCBI) (http://www.ncbi.nlm.nih.gov/). The genome sequence has been deposited in GenBank under the accession code JAUBWL000000000 (Leite et al., 2024).

In vitro assays testing the antagonism of the UFLA03-10 strain against fungi that cause diseases in bean

The antagonism of the UFLA03-10 strain was evaluated against the fungal strains: Lv134 race 65 (*Colletotrichum lindemuthianum*), UFLA 44 (*Sclerotinia sclerotiorum*), CML1846 (*Rhizoctonia solani*), and race 63-63 (*Pseudocercospora griseola*), responsible for the following common bean diseases: anthracnose, white mold, damping-off, and angular leaf spot, respectively.

For the tests with *Colletotrichum lindemuthianum* Lv134 race 65, *Sclerotinia sclerotiorum* UFLA 44, and *Rhizoctonia solani* CML1846, a 5 mm diameter disk of each fungal culture was placed in the center of Petri dishes (9 cm in diameter). Each fungal inoculum was previously cultivated on a PDA (potato-dextrose-agar) medium for seven days. The bacterial strain UFLA03-10 was cultivated in liquid culture medium 79 under agitation at 110 rpm and 28 °C for three days. The culture medium used was common bean exudates. The seeds were immersed in water at the proportion of 0.5 kg of seeds for 1 L of distilled water and placed in a refrigerator for three days. After this period, bacteriological agar (16 g L^{-1}) was added, autoclaved for 30 min, and poured into Petri dishes. (Ferreira et al., 2020). In the plates, 20 µL of the bacterial inoculant were pipetted at four points around the fungal culture. Evaluation was based on measuring the fungal colony diameter (DF) in cm and the size of the inhibition halo formed by the bacterium when visualized.

For the test with *Pseudocercospora griseola* race 63-63, the inoculum was previously cultivated in tubes with inclined PDA medium for ten days. After this period, 5 mL of autoclaved distilled water was added and agitated to obtain a spore suspension. An aliquot of 50 µL of this suspension was spread on a PDA medium with a Drigalski loop. Then, 20 µL of the bacterial inoculant was pipetted at each of the four points in different quadrants of the Petri dish.

Biocontrol trials assays of diseases in common bean plants by UFLA03-10

Greenhouse trials evaluated the control of anthracnose, angular spot, damping-off, and white mold diseases of the bean plant from June 2022 to January 2023. For the anthracnose, angular spot, and damping-off tests, 500 mL plastic cups were used, and two common bean cultivars, BRS Notável and Pérola, were used. Treatments included inoculation with UFLA03-10 and CIAT 899 and a negative control without any inoculation.

Only the Pérola variety was used for the white mold test in 2 L pots. Treatments included inoculation with UFLA03-10, CIAT 899, and negative control without disease to control. In

the control treatment and the UFLA03-10 inoculation, two sources of nitrogen were used: mineral nitrogen from urea and inoculation with strain UFLA02-127 (*Rhizobium* sp.) for biological fixation purposes of nitrogen. The tests were carried out once, with a completely randomized experimental design and three replications for each treatment.

Bacterial inoculants (UFLA03-10, CIAT 899, and UFLA02-127 strains) were prepared in medium 79 for three days. Inoculation was done at the time of seed planting, with 1 ml per seed and five seeds for each cup or pot. The trials used a mixture of soil (Oxisol) (pH = 5.7; P = 0.11 mg dm^{-3}; K = 10.48 mg dm^{-3}; Al = 0.0 cmolc dm^{-3}; Ca = 0.16 cmolc dm^{-3}; Mg = 0.08 cmolc dm^{-3}; Na = 3.00 mg dm^{-3}; S.B. = 0.27 cmolc dm^{-3}; t = 0.27 cmolc dm^{-3}; T = 1.57 cmolc dm^{-3}; M.O. = 0.28 dag/kg; P-Rem = 7.20 mg/L; V = 17.0%; Clayey texture; clay = 49 dag/kg; silt = 37 dag/kg; sand = 14 dag/kg) collected on the UFLA campus (21°13'49.3"S 44°58'02.3"W) and Tropstrato HT substrate (composition: pine bark, vermiculite, pg mix 14.16.18, potassium nitrate, simple superphosphate, and peat), in a 2:1 ratio, with two parts of soil and 1 part of substrate.

The phytopathogenic fungal strain inoculated, its preparation, application, and disease index evaluation differed for each disease.

For damping-off caused by the *Rhizoctonia solani* CML 1846 strain, the fungus was previously grown on a PDA medium for seven days. Afterward, four disks of 5 mm of the fungus were transferred to 100 grams of previously autoclaved rice husk with 40 mL of distilled water. After ten days, this material was dried in paper bags in the oven (48 h, 60-65°C), and the dry material was crushed. Twenty-four hours before planting, 50 mg of this dry material per kg of soil was added to the soil. The plants were kept in the greenhouse at 16°C at night and 24°C during the day, with 70% relative humidity. The final evaluation was performed on the 15th day after sowing. For this trial, the germination rate, dry mass weight, and disease index, according to Noronha et al (1995).

The fungus responsible for anthracnose - *Colletotrichum lindemuthianum* LV134 race 65 strain- was grown on PDA plates for ten days. Then, a portion of the fungal mycelium was placed in pods inserted into test tubes and previously autoclaved for 1 hour. This material was incubated for 8-12 days for spore multiplication. Next, spores were scraped with a blade and distilled water. The Neubauer chamber was used to adjust the inoculum concentration to 1.2 x 10^6 spores per mL-1The spores were inoculated on plants ten days after emergence by spraying until run-off. Inoculated plants were kept in a humid chamber (95%) for 72 hours and then in the greenhouse, with 80% relative humidity and a temperature of around 24°C. Evaluation was performed ten days after pathogen inoculation using the scale proposed by Rava et al. (1996).

The inoculum preparation for *Pseudocercospora griseola* race 63-63 was done in PDA tubes. After ten days, the mycelium mass in the tubes was suspended with distilled water and filtered with gauze, and the inoculum concentration to be applied was 2×10^4 spores per mL^{-1}, as adjust by the Neubauer chamber. Ten days after sowing, the spore suspension was inoculated on plants by spraying on both sides of the leaves until run-off. After inoculation, plants were kept in the greenhouse, with 80% relative humidity and a temperature of around 16°C at night and 24°C during the day. Twelve days after inoculation, disease severity was assessed using the scale proposed by Librelon et al. (2015).

The UFLA 44 isolate of *S. sclerotiorum* was used for the white mold experiment. A mycelium disk, grown on PDA at 23°C for seven days, was taken from the colonized medium using plastic micropipette tips. The straw test method proposed by Petzoldt and Dickson (1996) was used for inoculation. Inoculation was done 28 days after sowing. Pots were kept in the greenhouse at 16°C at night and 25°C during the day, with 70% relative humidity. Seven days after pathogen inoculation, disease severity was assessed using a diagrammatic scale from 1 to 9, proposed by Singh et al. (2014). The data from *in vitro* and *in vivo* trials were subjected to normality tests and analysis of variance of means using the Tukey test with a significance level of 5% in the R software (R Core Team, 2020).

Genome Mining for Genes Encoding Plant Beneficial and Biocontrol Traits

The functional genes involved in biocontrol and plant growth promotion, such as genes related to nutrient metabolism, phytohormone production, volatile compounds production, induction of resistance, colonization, and bacteriocin production, were identified using the RASTtk (Rapid Annotations using Subsystems Technology toolkit—version 2.0) (https://rast.nmpdr.org/rast.cgi) (Aziz et al., 2008) and the KEGG database on the BlastKOALA platform (Kanehisa & Goto, 2000; Kanehisa et al., 2016). KEGG's database on the KofamKOALA were used for identified metabolic pathways (Kanehisa & Sato, 2020).

Analysis of gene clusters involved in the biosynthesis of secondary metabolites

The prediction of gene clusters involved in secondary metabolism was performed using antiSMASH (version 7.1.0) (Blin et al., 2024), where biosynthetic gene clusters sharing less than 60% amino acid identity with known clusters were considered novel. Comparative analysis of secondary metabolites identified in the UFLA03-10 strain with the type strain *P. peoriae* KCTC 3763^T was conducted using BLASTp (Basic Local Alignment Search Tool).

The orthologous gene clusters within the genus *Paenibacillus* were identified using OrthoVenn3 (https://orthovenn3.bioinfotoolkits.net) (Xu et al., 2019). Genomic sequence comparison included the UFLA03-10 strain with the genome sequences of closest strains UY79^T (*P. farraposensis*), KACC 13842^T (*P. brasilensis*), AM 49^T (*P. kribbenses*), ZF129^T (*P. polymyxa*), and KCTC 3763^T (*P. peoriae*) of the genera *Paenibacillus*.

Results

Control of pathogenic fungi by UFLA03-10 *in vitro*

The UFLA03-10 strain almost completely inhibited the phytopathogenic fungi LV134 race 65 (*Colletotrichum lindemuthianum*), race 63-63 (*Pseudocercospora griseola*), and UFLA 44 (*Sclerotinia sclerotiorum*). Progression of the fungus CML 1846 (*Rhizoctonia solani*) was not inhibited; however, the bacterium created an inhibition halo, as shown in figure 1.

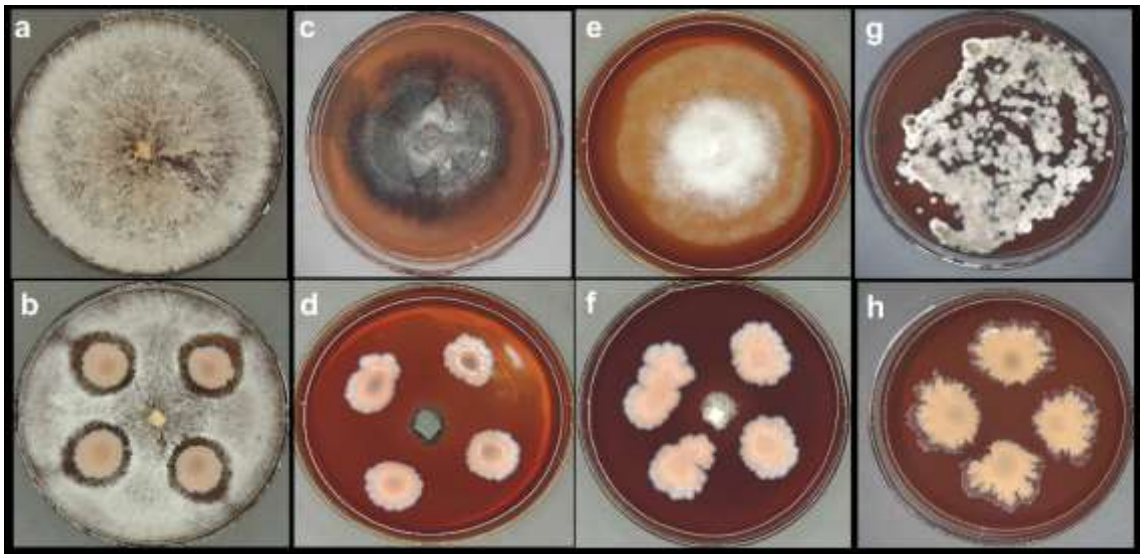

Fig 1. Antifungal activity of strain UFLA03-10 against phytopathogenic fungal strains **(a)** CML1846 (*Rhizoctonia solani*). **(b)** UFLA03-10 × CML1846 inhibition. **(c)** LV134 race 65 (*Colletotrichum lindemuthianum*). **(d)** Inhibition UFLA03-10 × LV134 race 65. **(e)** UFLA 44 (*Sclerotinia sclerotiorum*). **(f)** UFLA03-10 × UFLA44 inhibition**. (g)** Race 63-63 (*Pseudocercospora griseola*). (h) UFLA03-10 inhibition × run 63-63.

Control of fungal diseases in common bean by UFLA03-10

Regarding anthracnose, the analysis of variance revealed no statistically significant difference between the shoot dry weight and root dry weight indices for the cultivars Pérola and BRS Notável. Regarding the disease index (DI), UFLA03-10 reduced anthracnose symptoms in common bean plants by 77.8% for the susceptible cultivar Pérola. The DI scores were up to 2 grades, while the average DI of the control was greater than 6. For the moderately resistant cultivar BRS Notável, the difference between the average DI scores was 1.5 for inoculation

with UFLA03-10, while the control was 3.83. However, the DI averages were lower than in the trial with the Pérola cultivar (Fig 2).

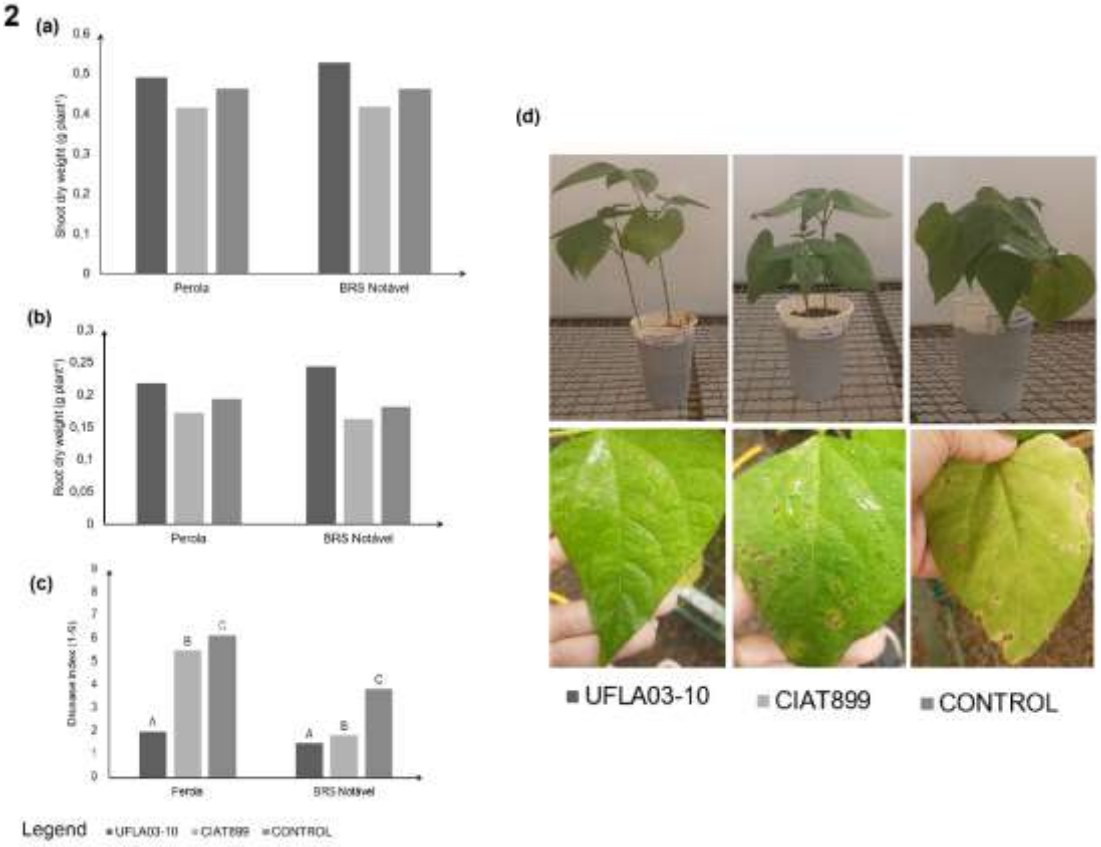

Fig 2. Results of the inoculation assay with the strain UFLA03-10 in common bean plants, evaluating growth promotion and biological control of anthracnose in the initial phase of common bean development. **a)** Shoot dry weight of the treatments on the two common bean cultivars. **b)** Root dry weight of the treatments on the two common bean cultivars. **c)** Disease index of the treatments on the two common bean cultivars. **d)** Photographs of common bean plants of the Perola cultivar and of their symptoms 23 days after sowing.

Regarding angular leaf spot in common bean, for BRS Notável, there was only significant difference for the parameters of root dry weight (RDW) and DI, with the UFLA03-10 inoculation treatment showing the lowest DI average and highest root dry weight. For Pérola, differences in plant height and DI were significant, and the treatment with UFLA03-10 better controlled angular leaf spot symptoms (Fig 3).

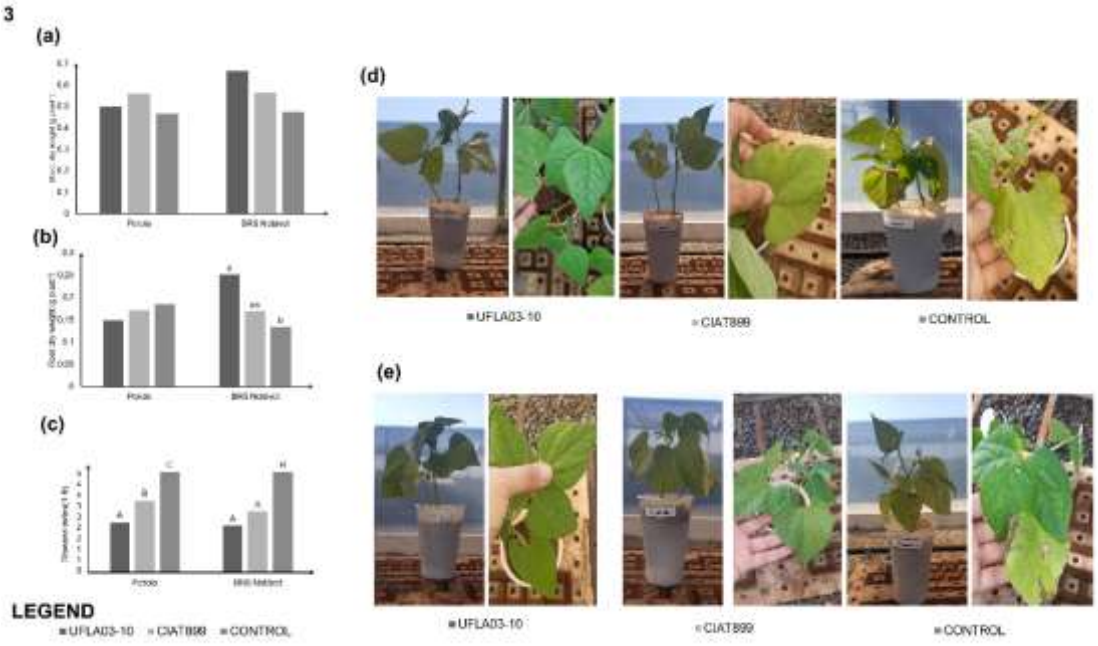

Fig 3. Result of the inoculation assay of the strain UFLA03-10 on common bean plants, evaluating growth promotion and control of angular leaf spot symptoms in the early development phase of the common bean plant. **a)** Shoot dry weight of the treatments on the two common bean cultivars. **b)** Root dry weight of the treatments on the two common bean cultivars. **c)** Disease index of the treatments on the two common bean cultivars. **d)** Photographs of common bean plants of the Perola cultivar and of their symptoms 22 days after sowing. **e)** Photographs of common bean plants of the BRS Notável cultivar and of their symptoms 22 days after sowing.

Regarding *Rhizoctonia solani*, all the parameters evaluated showed significant differences between the control and the UFLA03-10 inoculation treatment for both cultivars. The results of UFLA03-10 inoculation for all the parameters indicated that plants inoculated with UFLA03-10 were less susceptible to damage caused by *Rhizoctonia solani* and exhibited enhanced growth. For instance, inoculation with the strain resulted in a 100% germination rate for both cultivars. In the disease index, both cultivars showed susceptibility to *Rhizoctonia*. The index used by Noronha et al. (1995) ranges from 0 to 5. The negative controls averaged 4.67 for BRS Notável and 5 for Pérola. However, the index for plants inoculated with UFLA03-10 did not exceed 2 for either cultivar (Fig 4). Plants inoculated with CIAT899 also showed better disease control than the control plants.

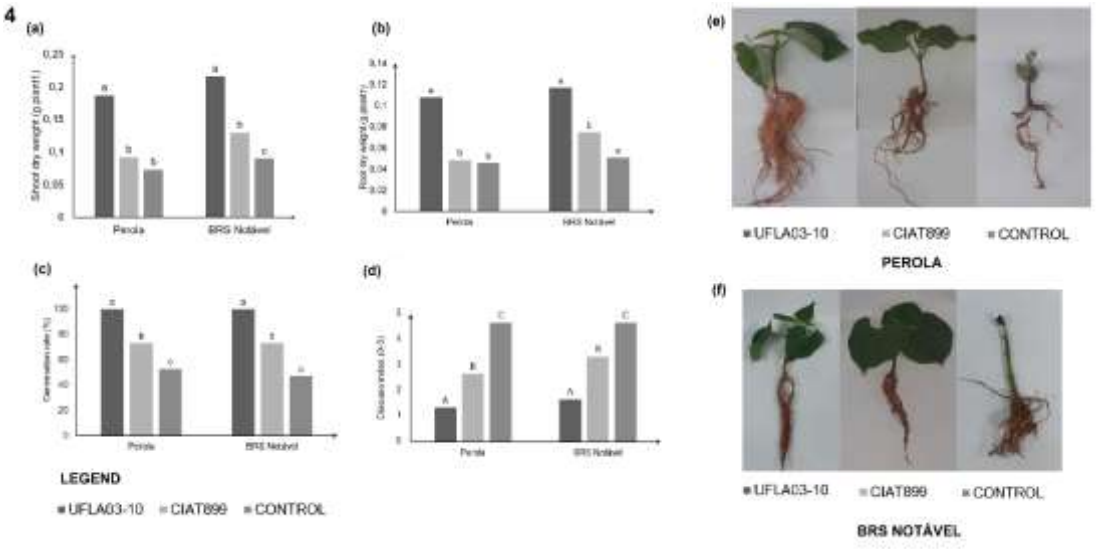

Fig 4. Effect of inoculation with the strain UFLA03-10 on common bean plants growing in soil infested with *Rhizoctonia solani*, evaluating growth promotion and controlling damping-off symptoms in the early development phase. **a)** Shoot dry weight of the treatments on the two common bean cultivars. **b)** Root dry weight of the treatments on the two common bean cultivars. **c)** Germination rate of the treatments on the two common bean cultivars. **d)** Disease index of the treatments on the two common bean cultivars. **e)** Photographs of common bean plants of the Perola cultivar and of their symptoms 15 days after sowing. **f)** Photographs of common bean plants of the BRS Notável cultivar and of their symptoms 15 days after sowing.

There was no significant difference in root dry weight values regarding white mold, whereas for shoot dry weight, the plants inoculated with UFLA03-10 showed the highest averages. Concerning plant height, the Tukey test separated the averages into three groups: the first and tallest group was for the averages of plants inoculated with UFLA03-10, the second was for the averages of plants inoculated with CIAT899, and the third was for the controls. Regarding the disease index, the scores given to the symptoms were low, considering that the index ranges from 1-9. The treatments of UFLA03-10 inoculation along with inoculation with UFLA02-127 rhizobia and with mineral N fertilization showed the lowest disease index averages (Fig 5).

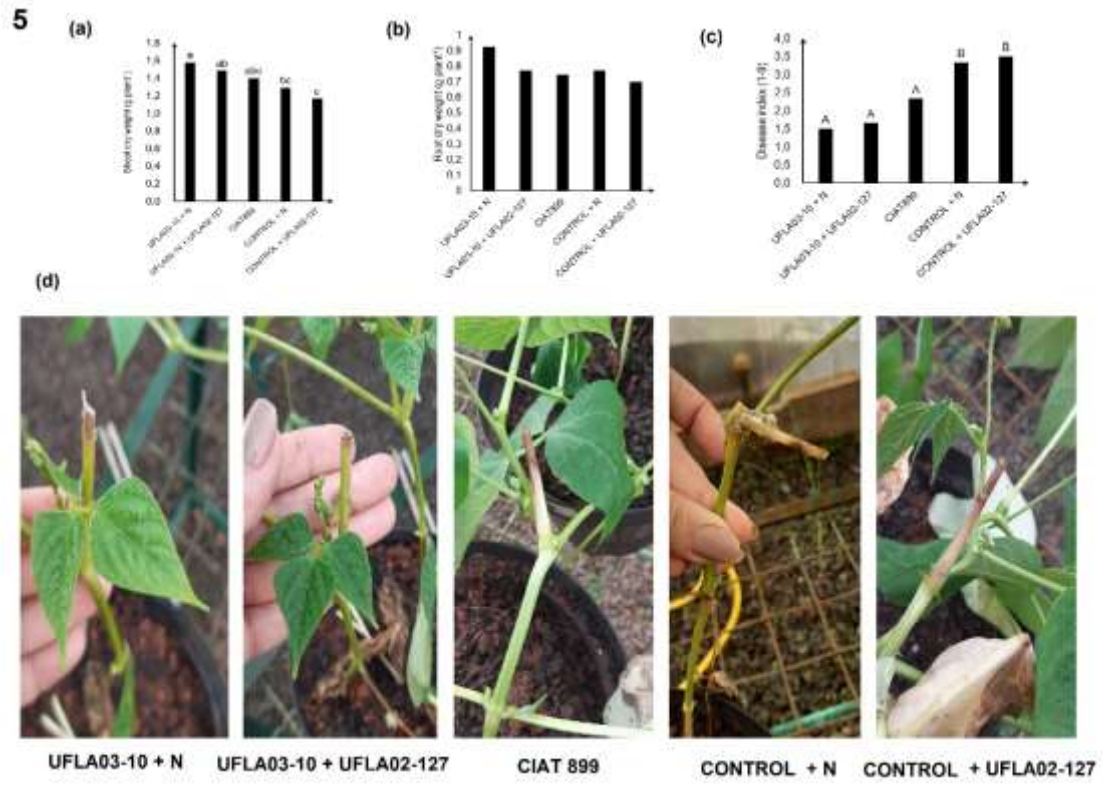

Fig 5. Effect of inoculation with the UFLA03-10 strain on common bean plants, evaluating growth promotion and control over white mold symptoms in the early development phase. **a)** Shoot dry weight of the treatments on the two common bean cultivars. **b)** Root dry weight of the treatments on the two common bean cultivars. **c)** Disease index of the treatments on the two common bean cultivars. **d)** Photographs of common bean plants of the Perola cultivar and their symptoms 35 days after sowing.

Functional annotation of the *P. peoriae* UFLA 03-10 genome for biological control and growth promotion in plants

In the UFLA03-10 genome, 5,989 coding sequence (CDS) regions were identified, according to RASTk annotation, comprising 1,846 genes classified into 27 functional groups. Additionally, using the KOALABlast platform (KEGG), 2842 genes were identified and classified into 21 functional groups.

The UFLA03-10 genome revealed several genes associated with plant growth promotion features, such as nutrient metabolism and production of plant hormones and volatile compounds, as well as forms of biological control, including colonization, induction of resistance, and production of bacteriocins and ribosomally synthesized antibacterial peptides. The metabolic pathways, genes, their products, and the codes in the KEEG (KO) and NCBI (COG) databases can be found in the supplementary tables (Table S1-S7).

Clusters of genes involved in secondary metabolite synthesis

In antiSMASH analysis, the UFLA03-10 exhibited secondary metabolites that have not been identified in other species, except for node 57, where the NRPS-type (Non-ribosomal

peptide synthetase) gene cluster showed 87% similarity to fusaricidin B found in *Paenibacillus polymyxa* (BGC0001152.1) (Supplementary material, Table S8). In node 14, a type of NPRS was identified with 90% similarity to bacillibactin (BGC0000401.1) found in *Paenibacillus kribbensis*.

In antiSMASH ClusterBlast, a 60% identity threshold is typically used to define whether a secondary metabolite biosynthetic gene cluster (BGC) is the same as or highly similar to a known BGC. In this analysis, the identified metabolites showed high similarity with metabolites identified in strains. As shown in Figure 6, this analysis showed that of the 23 clusters identified overall by antiSMASH, 9 showed more similarity than 60% compared to other strains, with the highest similarity values being with strains belonging to the genus *Paenibacillus*.

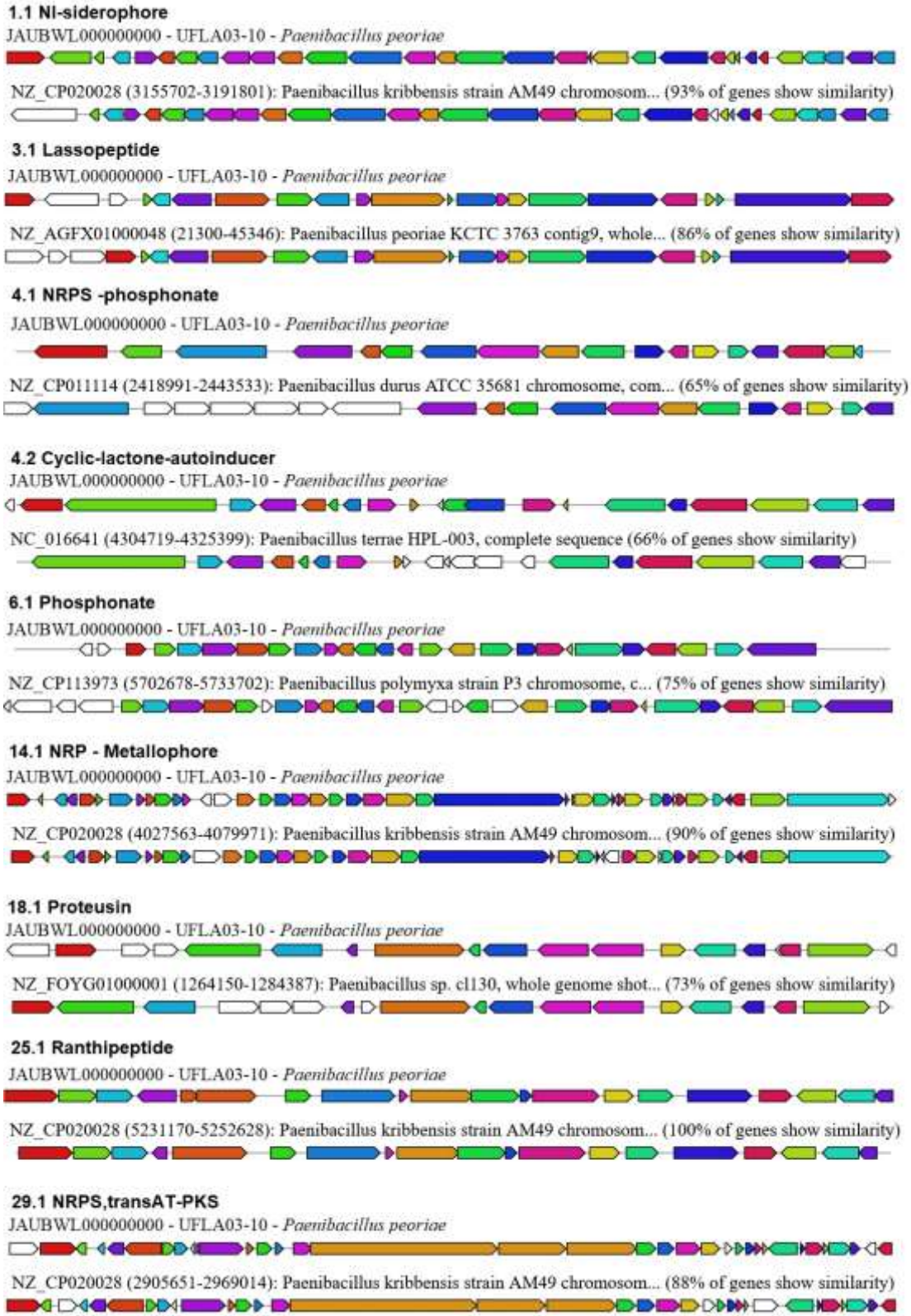

Fig 6. Output of ClusterBlast with the higher percentage of similarity between the query biosynthetic gene cluster (BGC) in UFLA03-10 and the known BGC, taking into account factors such as sequence identity, gene content, and organization.

Comparative analysis of biosynthetic gene clusters of UFLA03-10 with those of KCTC 3763^{T}

The analysis of antiSMASH for secondary metabolites of the *Paenibacillus peoriae* KCTC 3763^{T} type strain revealed a total of 23 clusters. Only the NRPS classified as Tridecaptin was exclusive to the type strain. The UFLA03-10 strain had 2 clusters of Fusaricidin B and Lichenysin. The similarity of these clusters to those of the same type is described in Table 3 below.

Table 3. Similarity between metabolites of the strains UFLA03-10 and KCTC3763T identified in antiSMASH analysis.

Metabolite	Activity	Location		S*(%)
		UFLA 03-10	KCTC 376^{T}	
Bacillibactin	Antibacterial and antifungal properties and hemolytic activities	18,082 - 65,551	446,817 - 494,268	94.54
Fusaricidin B	Broad antimicrobial activity against *Fusarium* sp.; also suppresses G+ bacteria	1 - 10,928	50,838 - 99,424	97.24
		1 - 10,259		96.11
Lichenysin	Exerted a similar biological effect on boar spermatozoa, such as loss of motility, damaged plasma membrane, and swelling of the acrosome	1 - 25,563	1 - 36,32	94.77
		1 - 10,74		95.69
Paenilipoheptin	Antimicrobial activity spectrum remains to be Elucidated	3,28 - 66,596	1 - 46,081	94.79
Paeninodin	Broad antimicrobial activity against G+ and G− bacteria	116,797 - 140,888	21,301 - 45,346	97.50
Staphylobactin	Siderophore biosynthesis	14,081 - 32,157	178,545 - 196,627	95.59
Tauramamide	Antimicrobial activity against bacteria and fungi	69,467 - 110,366	124,065 - 164,964	95.32
Tridecaptin	Suppresses G− bacteria	_	60,111 - 153,931	_

S – Similarity (%)

Comparative analysis of UFLA03-10 with *Paenibacillus* strains

The KCTC 3763^{T} type strain of *Paenibacillus peoriae* was the most phylogenetically similar to UFLA03-10. This strain was initially isolated from soil and classified as *Bacillus*, and later reclassified as *Paenibacillus* (Heyndrickx et al., 1996).

Complete genome sequences of these strains were also compared with genome sequences of the type strains UY79^{T} (*P. farraposensis*), AM 49^{T} (*P. kribbensis*), and ZF129^{T} (*P. polymyxa*) to identify specific orthologs in *Paenibacillus*. Gene families evolve through speciation processes, creating orthologs. As shown in Fig 1, there are 3000 core clusters present in *Paenibacillus* strains. These orthologous protein-coding clusters are relatively conserved in *Paenibacillus*.

Strains belonging to the same species, UFLA03-10 and KCTC 3763^{T}, share 4735 genes, with 123 clusters exclusively shared between them. However, UFLA03-10 has 94 unique clusters and shares 4722 genes with *P. farraposensis* UY79^{T}, 4714 genes with AM 49^{T} (*P. kribbensis*), and 4667 genes with ZF129^{T} (*P. polymyxa*).

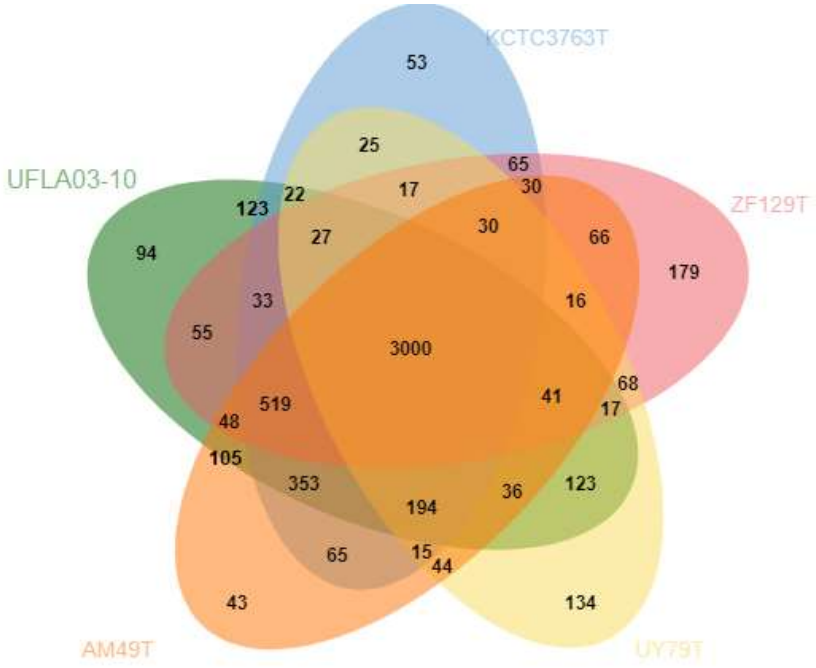

Fig 1. Venn diagram depicting clusters of orthologous genes (COGs) in UFLA03-10 (*Paenibacillus peoriae*) and types strains KCTC3763^{T} (*P. peoriae)*, UY79^{T} (*P. farraposensis*), AM49^{T} (*P. kribbensis*), and ZF129^{T} (*P. polymyxa*).

Discussion

In this study, *P. peoriae* UFLA03-10 notably attenuated disease symptoms in common bean plants infected with different genera of phytopathogenic fungi under greenhouse conditions (Fig 2-5), representing a potential biofungicide and plant growth promoter for use as an inoculant. Regarding co-inoculation with rhizobia in the white mold assay, the results show synergism between UFLA03-10 and rhizobia, specifically UFLA02-127, corroborating

data from Ferreira et al. (2020; 2023), who tested CIAT899, also belonging to *Rhizobium* genus.

The UFLA03-10 strain has been used in other studies with *Rhizoctonia solani*; this strain slightly reduced the average disease incidence in common bean (Ferreira et al., 2020) as well as in lima beans (Ferreira et al., 2023). Although UFLA03-10 was not the best agent for controlling *Rhizoctonia solani* in common bean, it showed better results than the control. The negative control had a DI of 95.67%, while the treatment inoculated with UFLA03-10 had an average DI of 71.83% (Ferreira et al., 2023). During the experiment, the plants inoculated with UFLA03-10 showed low scores on the indices evaluated, indicating a lower incidence of the disease caused by *Rhizoctonia solani*.

Bacillus is the genus most assessed for biological control of these diseases, with strains already used and registered for biological control of *Colletotrichum lindemuthianum*. Gholami and colleagues (2013) evaluated the application of endophytic bacteria from different species (*Bacillus subtilis* subsp. s*ubtilis, B. atrophaeus, B. tequilensis, B. subtilis* subsp. *spizizenii, Streptomyces cyaneofuscatus, S. flavofuscus, S. parvus, S. acrimycini*) for biocontrol of *C. lindemuthianum* in greenhouse trials. They obtained disease control rates ranging from 40 to 76.80 % under greenhouse conditions without adverse effects on growth performance and obtained average plant biomass production without significant differences. The UFLA03-10 strain presented control levels greater than or similar to those of *Bacillus* species, which are the most used and studied as biological control.

The UFLA03-10 genome contains several identified metabolic pathways. Incomplete pathways were also found in this genome, with implications related to nutritional requirements. Bacteria with incomplete metabolic pathways may be auxotrophic for certain nutrients, unable to synthesize essential compounds, and reliant on external sources. These bacteria may depend on interactions with other organisms, such as symbiotic relationships, to obtain essential nutrients or metabolic intermediates (McCutcheon et al., 2012; Pál et al., 2003).

When plants are less affected by phytopathogenic microorganisms, they have more excellent vegetative development and higher yield. Plant protection and development are often associated with the production of nutrient-mobilizing enzymes, hormones, and antibiotics (Ajijah et al., 2023; Beneduzi et al., 2012; Harman et al., 2021; Ahemad et al., 2014; Gu et al., 2017). Genes involved in the expression of plant growth-promoting phytohormones, such as the *trp* gene group involved in the production of indole-3-acetic acid (IAA) and auxins, and genes associated with ethylene modulation, such as *speE, arcC, ydcU, speB, afuC,* and *aguA*, were annotated in the genome of UFLA03-10 (Supplementary Table S4).

Genes present in the metabolic pathway of 2, 3-Butanediol, an essential compound for inducing resistance in plants, such as *butA*, *budC*, *butB*, and *ilvNA*, were also identified in the genome of UFLA03-10 (Supplementary Table S1). These genes are related to synthesizing 2,3-butanediol, associated with induced systemic resistance (ISR). The presence of genes such as *flgL* and *tuf* in the genome of UFLA03-10 suggests a potential mechanism for biological control involving the induction of resistance in plants. Effective colonization is a prerequisite for growth-promoting bacteria to implement their biocontrol functions. Exudates, environmental factors, and other factors affect colonization of endophytic bacteria. The UFLA03-10 strain, an endophyte isolated from cowpea nodules, can become established and live inside plant tissues, adjusting gene expression while residing in plants (Abo-Koura, 2023).

Among the genes identified about the metabolic pathways of nutrient absorption, *fix* genes involved in nitrogen fixation and *nod* were not noted for the nitrogen pathways. These genes are present in metabolic pathways of nodulation induction in plants (Black et al., 2012;). This suggests that the strain UFLA03-10 may not directly involve nitrogen fixation or nodulation induction in plants. However, the *nifs* genes were identified in UFLA03-10, demonstrating the potential of bacteria for biological nitrogen fixation, even though this index was not evaluated in this work (Supplementary Table S5).

The secondary metabolite production was assessed using antiSMASH, revealing 23 gene clusters. Notably, seven (bacillibactin, fusaricidin B, lichenysin, paenilipoheptin, paeninodin, staphylobactin, and tauramamide) were classified as antimicrobial metabolites previously identified within the MIBig (Minimum Information about a Biosynthetic Gene cluster) database. These secondary metabolites can directly induce plant defense mechanisms, promote plant development, or reduce a pathogen's ability to cause diseases (Matilla & Krell, 2018).

The ability to produce or capture siderophores is an advantage that makes bacteria competitive in colonizing plant tissues (Uwaremwe et al., 2022). The metabolite bacillibactin, a type of siderophore, was identified in gene cluster 12. The bacillibactin metabolite identified in the genome of UFLA03-10 showed 90% similarity to that identified in *Paenibacillus kribbensis*. *P. peoriae* is closely related to *P. kribbensis*, with an ANI between their type strains (*P. kribbensis* AM49T; *P. peoriae* KCTC3763T) of 94.64%. The ANI of UFLA03-10 with *P. kribbensis* was 95.04%. Among these species, gene clusters involved in the biosynthesis of antifungal and antibacterial peptides, such as fusaricidin, paenilan, and paeninodin, are encoded in the genomes of both *P. peoriae* and *P. kribbensis* (Yang et al., 2018; Ali et al., 2021).

Two clusters were identified as fusaricidin B in the antiSMASH annotation, with one showing high similarity (87%) to that metabolite identified in *Paenibacillus polymyxa*. Strains belonging to this species have already been studied for the biological control of plant diseases (Padda et al., 2017). Recent extensive genome sequencing and bioinformatic analysis have shown that terpene synthesis is widely distributed in bacteria used for biological control of diseases (Gupta et al., 2022; Hamaoka et al., 2021). In cluster 18, a sequence of this type of metabolite was identified in UFLA 3-10 (Supplementary table S8), although it showed no similarity to other terpenes in the database used by the tool.

The UFLA03-10 strain of *Paenibacillus peoriae* has potential applications both as a biocontrol agent and as a biofertilizer, as it improved plant growth and demonstrated broad-spectrum microbial activity. Furthermore, identifying genes and gene clusters in this strain's genome attests to its genetic potential and capabilities. Once important genes in metabolic pathways are identified, they can express essential substances for plant growth and antimicrobial substances for plant protection, with which UFLA03-10 may be associated.

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Supplementary material

Table S1. Metabolic pathways involved in the synthesis of resistance inducers

	GENE	PRODUCT	KO	COG
map00650	**Butanoate metabolism (complete 4/4)**			
	butA	2,3-butanediol dehydrogenase, S-alcohol forming, (R)-acetoin-specific (EC 1.1.1.4) / Acetoin (diacetyl) reductase (EC 1.1.1.304)	K03366	COG1028
	budC	2,3-butanediol dehydrogenase, S-alcohol forming, (S)-acetoin-specific (EC 1.1.1.76)	K03366	COG1028
	butB	2,3-butanediol dehydrogenase, R-alcohol forming, (R)- and (S)-acetoin-specific (EC 1.1.1.4)	K00004	COG1063
	ilvN	Acetolactate synthase small subunit (EC 2.2.1.6)	K01653	COG0440
map00900	**Isoprenoid biosynthesis, bacteria (complete 5/5)**			
	idi	Isopentenyl-diphosphate delta-isomerase, FMN-dependent (EC 5.3.3.2)	K01823	COG1443
	lytB	4-hydroxy-3-methylbut-2-enyl diphosphate reductase (EC 1.17.7.4)	K03527	COG0761
	gcpE	(E)-4-hydroxy-3-methylbut-2-enyl-diphosphate synthase (flavodoxin) (EC 1.17.7.3)	K03526	COG0821
	ispF	2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (EC 4.6.1.12)	K01770	COG0245
	ispE	Small acid-soluble spore protein SspF	K00919	COG1947
map00660	**Acetoin (complete 1/1)**			
	alsD	acetolactate decarboxylase [EC:4.1.1.5]	K01575	COG3527
map00270	**Methionine biosynthesis (complete 7/7)**			
	mmuM	Homocysteine S-methyltransferase (EC 2.1.1.10)	K00547	COG2040
	metN	Methionine ABC transporter ATP-binding protein	K02071	COG1135
	mtnE	Glutamine-dependent 2-keto-4-methylthiobutyrate transaminase	K08969	COG0436
	metE	Methionine synthase II (cobalamin-independent)	K00549	COG0620
	hom	homoserine dehydrogenase [EC:1.1.1.3]	K00003	COG0460
	asd	aspartate-semialdehyde dehydrogenase [EC:1.2.1.11]	K00133	COG0136
map02040	**Flagellar assembly (incomplete 3/8)**			
	dacA	Diadenylate cyclase spyDAC	K18672	COG1624
	flgL	Flagellar hook-associated protein FlgL	K02397	COG1344
	tuf	Translation elongation factor Tu	K02358	COG0050

Table S2. Metabolic pathways involved in involved in colonization

	GENE	PRODUCT	KO	COG
map02020	**Biofilm formation and quorum sensing (complete 8/8)**			
	wecB	UDP-N-acetylglucosamine 2-epimerase (EC 5.1.3.14)	K01791	COG0381
	dacA	D-alanyl-D-alanine carboxypeptidase (EC 3.4.16.4)	K07258	COG1686
	kinB	Sporulation kinase B (EC 2.7.13.3)	K07697	COG0642

	ldcA	Muramoyltetrapeptide carboxypeptidase (EC 3.4.17.13)	K01297	COG1619
	oppB	Oligopeptide ABC transporter, permease protein OppB (TC 3.A.1.5.1)	K15581	COG0601
	riba	GTP cyclohydrolase II (EC 3.5.4.25)	K01497	COG0807
	tasA	Secreted and spore coat-associated protein 1, similar to biofilm matrix component TasA and to camelysin	K06336	COG5884
	sinR	XRE family transcriptional regulator, master regulator for biofilm formation	K19449	COG1396
map02030	**Motility and Chemotaxis (complete 10/10)**			
	cheC	Chemotaxis protein CheC -- inhibitor of MCP methylation	K03410	COG1776
	cheW	Positive regulator of CheA protein activity (CheW)	K03408	COG0835
	fliM	Flagellar motor switch protein FliM	K02416	COG1868
	fliN	Flagellar motor switch protein FliN	K02417	COG1886
	msmE	Maltose/maltodextrin ABC transporter, substrate binding periplasmic protein MalE	K10117	COG1653
	fliG	Flagellar motor switch protein FliG	K02410	COG1536
	cheB	Chemotaxis response regulator protein-glutamate methylesterase CheB (EC 3.1.1.61)	K03412	COG2201
	cheX	Chemotaxis protein CheX	K03409	COG1406
	cheR	Chemotaxis protein methyltransferase CheR (EC 2.1.1.80)	K00575	COG1352
	cheD	Chemotaxis protein CheD	K03411	COG1871
map01250	**Colanic acid biosynthesis (complete 5/5)**			
	Wzb	Low molecular weight protein tyrosine phosphatase (EC 3.1.3.48)	K25307	COG0394
	Gmd	GDP-mannose 4,6-dehydratase (EC 4.2.1.47)	K01711	COG1089
	Fcl	GDP-L-fucose synthetase (EC 1.1.1.271)	K02377	COG0451
	manC	Sugar-phosphate guanylyltransferase / Sugar-phosephate isomerase	K00971	COG0662
	manB	Beta-mannosidase (EC 3.2.1.25)	K01192	COG0836

Table S3. Metabolic pathways involved in involved in Biosynthesis of secondary metabolites, bacteriocins and antibacterial peptides synthesized by ribosomes

	GENE	**PRODUCT**	**KO**	**COG**
map00997	**Staphyloferrin B biosynthesis (incomplete 4/7)**			
	sbnC	staphyloferrin B synthase [EC:6.3.2.56]	K23375	COG4264
	sbnH	2-[(L-alanin-3-ylcarbamoyl)methyl]-2-hydroxybutanedioate decarboxylase [EC:4.1.1.117]	K23373	COG0019
	sbnF	2-[(L-alanin-3-ylcarbamoyl)methyl]-3-(2-aminoethylcarbamoyl)-2-hydroxypropanoate synthase [EC:6.3.2.55]	K23374	COG4264
	sbnE	L-2,3-diaminopropanoate---citrate ligase [EC:6.3.2.54]	K23372	COG4264

map02010		**Bacitracin stress response (complete 5/5)**		
	bceB	Bacitracin export permease protein BceB	K11632	COG0577
	bceA	Bacitracin export ATP-binding protein BceA	K11631	COG1136
	bceR	Two-component response regulator BceR	K11630	COG0745
	bceS	Two-component sensor histidine kinase BceS	K11629	COG0642
	pspA	Protein LiaH, similar to phage shock protein A	K03969	COG1842
	liaF	Transporter LiaF	K11622	COG4758

Table S4. Metabolic pathways involved production of phytohormones and volatile organic compounds

	GENE	**PRODUCT**	**KO**	**COG**
map00130	**4-hydroxybenzoate (PBH) (incomplete 3/5)**			
	ubiA	Menaquinone via futalosine polyprenyltransferase	K03179	COG0382
	bsdC	Hydroxyaromatic non-oxidative decarboxylase protein C (EC 4.1.1.-)	K01612	COG0043
	ubiD	UbiD family decarboxylase associated with menaquinone via futalosine	K16239	COG0043
map01120	**Hydrogen sulfide (H2S) (complete 6/6)**			
	cysC	Adenylylsulfate kinase (EC 2.7.1.25)	K00860	COG0529
	cysE	Serine acetyltransferase (EC 2.3.1.30)	K00640	COG1045
	mccB	Cystathionine gamma-lyase (EC 4.4.1.1)	K17217	COG0626
	mccA	Cystathionine beta-synthase (EC 4.2.1.22)	K17216	COG0031
	met3	Sulfate adenylyltransferase (EC 2.7.7.4)	K00958	COG2046
	cysH	Phosphoadenylyl-sulfate reductase [thioredoxin] (EC 1.8.4.8)	K00390	COG0175
map00250	**Indole-3-acetic acid (IAA) (complete 4/4)**			
	trpC	Indole-3-glycerol phosphate synthase (EC 4.1.1.48)	K01609	COG0134
	trpE	Anthranilate synthase, aminase component (EC 4.1.3.27)	K01657	COG0147
	ald	Alanine dehydrogenase (EC 1.4.1.1)	K00259	COG0686
	aspA	Aspartate ammonia-lyase (EC 4.3.1.1)	K01744	COG1027
map00400	**Auxin Biosynthesis (incomplete 4/6)**			
	trpA	Tryptophan synthase alpha chain (EC 4.2.1.20)	K01695	COG0159
	trpB	Tryptophan synthase beta chain (EC 4.2.1.20)	K01696	COG0133
	trpF	Phosphoribosylanthranilate isomerase (EC 5.3.1.24)	K01817	COG0135
	trpD	Anthranilate phosphoribosyltransferase (EC 2.4.2.18)	K00766	COG0547
map00220	**Polyamine/ethylene modulation (complete 6/6)**			
	speE	Spermidine synthase (EC 2.5.1.16)	K00797	COG0421
	arcC	Carbamate kinase (EC 2.7.2.2)	K00926	COG0549
	ydcU	Spermidine Putrescine ABC transporter permease component PotB (TC 3.A.1.11.1)	K02054	COG1176
	speB	Agmatinase (EC 3.5.3.11)	K01480	COG0010

	afuC	Putrescine transport ATP-binding protein PotA (TC 3.A.1.11.1)	K02010	COG3842
	aguA	Agmatine deiminase (EC 3.5.3.12)	K10536	COG2957

Table S5. Metabolic pathways involved in nitrogen metabolism

	GENE	**PRODUCT**	**KO**	**COG**
map00910	**Nitrate and nitrite ammonification (3) (1 block missing 1/2)**			
	fdhC	Nitrite transporter from formate/nitrite family	K21993	COG2116
	nirD	Nitrate and nitrite ammonification	K00363	COG2146
	nirB	Nitrate and nitrite ammonification	K00362	COG1251
map00910	**Denitrifying reductase (8) (2 blocks missing 1/3)**			
	nxrA	Respiratory nitrate reductase alpha chain (EC 1.7.99.4)	K00370	COG5013
	nxrB	Respiratory nitrate reductase beta chain (EC 1.7.99.4)	K00371	COG2180
	narW	Respiratory nitrate reductase delta chain (EC 1.7.99.4)	K00373	COG2180
	narV	Respiratory nitrate reductase gamma chain (EC 1.7.99.4)	K00374	**COG2181**
	narI	nitrate reductase gamma subunit [EC:1.7.5.1 1.7.99.-]	K00374	**COG2181**
	narJ	nitrate reductase molybdenum cofactor assembly chaperone NarJ	K00373	COG2180
	narH	nitrate reductase / nitrite oxidoreductase, beta subunit [EC:1.7.5.1 1.7.99.-]	K00371	COG2180
	narG	nitrate reductase / nitrite oxidoreductase, alpha subunit [EC:1.7.5.1 1.7.99.-]	K00370	COG5013
map00910	**Nitrogen fixation – Nitrogenase (complete 10/10)**			
	nifB	Nitrogenase FeMo-cofactor synthesis FeS core scaffold and assembly protein NifB	K02585	COG0535
	nifH	Nitrogenase (molybdenum-iron) reductase and maturation protein NifH	K02588	COG1348
	nifD	Nitrogenase (molybdenum-iron) alpha chain (EC 1.18.6.1)	K02586	COG2710
	nifK	Nitrogenase (molybdenum-iron) beta chain (EC 1.18.6.1)	K02591	COG2710
	nifE	Nitrogenase FeMo-cofactor scaffold and assembly protein NifE	K02587	COG2710
	nifN	Nitrogenase FeMo-cofactor scaffold and assembly protein NifN	K02592	COG2710
	nifX	Nitrogenase FeMo-cofactor carrier protein NifX	K02596	-
	nifV	homocitrate synthase NifV [EC:2.3.3.14]	K02594	COG0119
	nifU	nitrogen fixation protein NifU and related proteins	K04488	COG0822
	nifF	Flavodoxin	K03839	COG0716
map00910	**Ammonia assimilation, Glutamine, Glutamate, Aspartate and Asparagine Biosynthesis, Glutamine synthetases (8) (1 block missing 1/2)**			
	glnQ	ABC transporter, ATP-binding protein (cluster 3, basic aa/glutamine/opines)	K10038	COG1126
	glnH	Glutamine ABC transporter, substrate-binding protein GlnH / Glutamine ABC transporter, permease protein GlnP	K10036	COG0834
	glnP	glutamine transport system permease protein	K10037	COG0765
	glnA	Glutamine synthetase type I (EC 6.3.1.2)	K01915	COG0174
	glnL	Two-component system, response regulator GlnL	K07719	-

	glnK	Two-component system, sensor histidine kinase GlnK [EC:2.7.13.3]	K07717	COG0642
	glnP	Glutamine ABC transporter, permease protein GlnP	K10040	COG0765
	glnS	Glutaminyl-tRNA synthetase (EC 6.1.1.18)	K01886	COG0008

Table S6. Metabolic pathways involved in phosphorus metabolism

	GENE	**PRODUCT**	**KO**	**COG**
map01100	**Phosphate metabolismo (complete 6/6)**			
	ppx	Exopolyphosphatase (EC 3.6.1.11)	K01524	COG0248
	ppk1	Polyphosphate kinase (EC 2.7.4.1)	K00937	COG0855
	phoU	Phosphate transport system regulatory protein PhoU	K02039	COG0704
	phoH	Phosphate starvation-inducible protein PhoH, predicted ATPase	K06217	COG1702
	ppaX	Inorganic pyrophospatase PpaX (EC 3.1.3.18)	K06019	COG0546
	phoA	Alkaline phosphatase (EC 3.1.3.1)	K01077	COG1785
map02010	**Phosphate solubilization**			
	phnE	ABC transporter, permease protein (cluster 12, methionine/phosphonates)	K02042	COG0581
	phnC	phosphonate transport system ATP-binding protein [EC:7.3.2.2]	K02041	COG3639
	pstB	phosphate transport system ATP-binding protein [EC:7.3.2.1]	K02036	COG3638
	pstA	Phosphate ABC transporter, permease protein PstA (TC 3.A.1.7.1)	K02038	COG1117
	pstC	Phosphate ABC transporter, permease protein PstC (TC 3.A.1.7.1)	K02037	COG0581
	gnd	6-phosphogluconate dehydrogenase, decarboxylating (EC 1.1.1.44)	K00033	COG0573
	gntK	Gluconokinase (EC 2.7.1.12)	K25031	COG0362
	ppc	Phosphoenolpyruvate carboxylase (EC 4.1.1.31)	K01595	COG1070
	gltB	Glutamate synthase [NADPH] large chain (EC 1.4.1.13)	K00265	COG2352
	acnA	Aconitate hydratase (EC 4.2.1.3)	K01681	COG0070
	sucD	Succinyl-CoA ligase [ADP-forming] alpha chain (EC 6.2.1.5)	K01902	COG1048
	sucC	Succinyl-CoA ligase [ADP-forming] beta chain (EC 6.2.1.5)	K01903	COG0074
	sdhA	Succinate dehydrogenase flavoprotein subunit (EC 1.3.5.1)	K00239	COG0045
	sdhB	Succinate dehydrogenase iron-sulfur protein (EC 1.3.5.1)	K00240	COG1053
	fumC	Fumarate hydratase class II (EC 4.2.1.2)	K01679	COG0479
	mdh	Malate dehydrogenase (EC 1.1.1.37)	K00024	COG0114
	pdhA	Pyruvate dehydrogenase E1 component alpha subunit (EC 1.2.4.1)	K00161	COG0039
	poxB	Pyruvate dehydrogenase (quinone) (EC 1.2.5.1)	K00156	COG1071
map02010	**Fosfonatos (complete 7/7)**			
	phnE	Pyridoxamine 5'-phosphate oxidase (EC 1.4.3.5)	K02042	COG3639
	phnC	phosphonate transport system ATP-binding protein [EC:7.3.2.2]	K02041	COG3638

	phnD	phosphonate transport system substrate-binding protein	K02044	COG3221
	phnB	PhnB protein; putative DNA binding 3-demethylubiquinone-9 3-methyltransferase domain protein	K04750	COG2764
	phnA	Protein PhnA	K06193	COG2824
	phnP	phosphoribosyl 1,2-cyclic phosphate phosphodiesterase [EC:3.1.4.55]	K06167	COG1235
	phnX	phosphonoacetaldehyde hydrolase(EC:3.11.1.1)	K05306	-
map02010	**Specific phosphate transport (complete 4/4)**			
	pstB	Phosphate ABC transporter, ATP-binding protein PstB (TC 3.A.1.7.1)	K02036	COG1117
	pstA	Phosphate ABC transporter, permease protein PstA (TC 3.A.1.7.1)	K02038	COG0581
	pstC	Phosphate ABC transporter, permease protein PstC (TC 3.A.1.7.1)	K02037	COG0573
	pstS	Phosphate ABC transporter, substrate-binding protein PstS (TC 3.A.1.7.1)	K02040	COG0226

Table S7. Metabolic pathways involved in potassium metabolism and iron acquisition.

	GENE	**PRODUCT**	**KO**	**COG**
map02020	**Potassium metabolismo (incomplete 5/6)**			
	kdpA	Potassium-transporting ATPase A chain (EC 3.6.3.12) (TC 3.A.3.7.1)	K01546	COG2060
	kdpB	Potassium-transporting ATPase B chain (EC 3.6.3.12) (TC 3.A.3.7.1)	K01547	COG2216
	kdpC	Potassium-transporting ATPase C chain (EC 3.6.3.12) (TC 3.A.3.7.1)	K01548	COG2156
	ybiO	Potassium efflux system KefA protein	K22044	COG0668
	mscL	Large-conductance mechanosensitive channel	K03282	COG1970

Table S8. Secondary metabolites were noted in the genome of the UFLA03-10 strain by antiSMASH.

Cluster	**Type**	**From**	**To**	**Most similar known cluster**	**% of genes show similarity**	**MIBiG BGC-ID**
Cluster 1	siderophore	14,081	32,157	staphylobactin	25%	BGC0000939
Cluster 2	cyclic-lactone-autoinducer	361,378	381,689	_	_	_
Cluster 3	NRPS-like	383,911	427,606	_	_	_
Cluster 4	lassopeptide	116,797	140,888	paeninodin	40%	BGC0001634.1
Cluster 5	cyclic-lactone-autoinducer	199,092	219,649	_	_	_
Cluster 6	NRPS	1	21,855	_	_	_
Cluster 7	cyclic-lactone-autoinducer	41,255	61,806	_	_	_
Cluster 8	phosphonate	69,467	110,366	tauramamide	9%	BGC0001859.1
Cluster 9	NRPS-like	223,475	245,304	_	_	_
Cluster 10	terpene,cyclic-lactone-autoinducer	135,61	155,913	_	_	_
Cluster 11	NRPS	1	25,563	lichenysin	28%	BGC0001615.1
Cluster 12	NRPS	18,082	65,551	bacillibactin	90%	BGC0000401.1

Cluster 13	proteusin	89,856	109,212	_	_	_
Cluster 14	ranthipeptide	30,023	51,48	_	_	_
Cluster 15	transAT-PKS,NRPS	3,28	66,596	paenilipoheptin	19%	BGC0000426.1
Cluster 16	cyclic-lactone-autoinducer	1	16,885	_	_	_
Cluster 17	NRPS	1	21,293	_	_	_
Cluster 18	terpene	1	13,241	_	_	_
Cluster 19	NRPS	1	14,762	_	_	_
Cluster 20	NRPS	1	10,928	fusaricidin B	25%	BGC0001615.1
Cluster 21	NRPS	1	10,74	lichenysin	14%	BGC0001826.1
Cluster 22	NRPS	1	10,259	fusaricidin B	87%	BGC0001152.1
Cluster 23	NRPS	1	3,403	_	_	_

ARTIGO 2- Pseudomonas strains: IDENTIFICATION, PROSPECTION OF FUNCTIONAL GENES, GROWTH PROMOTION AND BIOLOGICAL CONTROL OF DISEASES IN COMMON BEANS

Pseudomonas strains: IDENTIFICATION, PROSPECTION OF FUNCTIONAL GENES, GROWTH PROMOTION AND BIOLOGICAL CONTROL OF DISEASES IN COMMON BEANS

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Key points
• Inoculation with UFLA02-281/02-293/03-18 in common beans reduced fungal disease symptoms.
• Inoculation with UFLA02-281/02-293/03-18 promotes common bean growth.
• Genes linked to biocontrol and plant growth promotion were identified in all three strains.
• Genomic analysis revealed biocontrol-associated secondary metabolites in the three strains.
• UFLA02-281/02-293/03-18 were identified as *P. xanthosomatis*, *P. cremoris*, *P. bananamidigenes*.

Abstract

Bacteria of the genus *Pseudomonas* can have several applications in agriculture. The objective of this study was to sequence the genomes for species-level identification and to prospect for genes associated with biological disease control and growth promotion, followed by *in vitro* and *in vivo* evaluations in common bean plants. The strains UFLA02-281/02-293/03-18, selected from previous works, were analyzed through ANI and dDDH; UFLA02-281 was classified as P. xanthosomatis, UFLA02-293 as P. cremoris and UFLA03- 18 as P. bananamidigenes. In vitro tests, the UFLA02-281 strain more efficiently inhibited the growth of fungi such as Colletotrichum lindemuthianum, Sclerotinia sclerotiorum, Rhizoctonia solani, and Pseudocercospora griseola. In in vivo tests, all three strains achieved disease index averages lower than the controls used. Functional annotation revealed the presence of genes involved in plant growth promotion, such as trp related to the production of plant hormones and pst related to phosphor solubilization and biocontrol traits genes like antimicrobial production like hcn, toxA, and chiA. The analysis carried out with AntiSMASH identified clusters of genes responsible for producing several types of secondary metabolites, such as ranthipeptide, betalactone, RiPP-like, and NRPS, which have several functions, including biological control and growth promotion in plants. By correlating the in vitro and in vivo findings with the genomic functional analysis, we can conclude that the Pseudomonas strains UFLA02-281, UFLA02-293, and UFLA03-18 exhibit the potential to be developed as biofungicides.

Keywords: endophytic bacteria, common bean, fungal diseases, secondary metabolites.

Introduction

The use of endophytic bacteria in agriculture is a growing trend aiming at sustainable and environmentally friendly management practices. The application of these bacteria as biofertilizers, biodefensives, and biostimulants is being explored to enhance crop productivity while minimizing the ecological impact of traditional agricultural practices. One of the most significant scientific advancements in recent decades has been the realization that diverse and active microbial communities play a crucial role in the development and survival of plants (Firdous et al., 2019; Liu et al., 2017).

Among the bacterial genera suitable for agricultural applications, the *Pseudomonas* genus stands out as one of the most diversified groups in various environments. According to the List of Prokaryotic Names with Standing in Nomenclature (LPSN), there are currently 423 validly published species of *Pseudomonas*, including synonyms (Parte et al., 2020). Some *Pseudomonas* species can enhance plant growth by promoting nutrient availability. They can solubilize mineral phosphates, fix atmospheric nitrogen, and produce plant growth-promoting hormones. These activities contribute to increased nutrient absorption by plants and overall plant health (Kaushal & Wani, 2016; Oteino et al., 2015).

One of the primary functions of *Pseudomonas* bacteria in agriculture is biological control, wherein the bacteria produce antimicrobial compounds such as antibiotics and enzymes that inhibit the growth of plant pathogens, making them valuable for integrated disease management strategies and reducing dependence on chemical pesticides (Rana et al., 2020). These products are commonly employed for the control of soil-borne pathogens. *P. fluorescens* strains are renowned for producing secondary metabolites with antimicrobial properties, aiding in suppressing the growth of plant pathogens in the soil (Ganeshan & Kumar, 2005; Tayyarcan & Boyaci, 2023). *P. chlororaphis* strains can produce antifungal compounds and enzymes, contributing to their biocontrol capabilities against pathogens (Raio & Puopolo et al., 2021). While some *P. syringae* strains may be plant pathogens, certain non-pathogenic strains are exploited for their capacity to induce systemic resistance in plants against diseases. These species are used in formulations that trigger plant defense mechanisms for disease control (Meena et al., 2022; Skliros et al., 2023). In organic agriculture, many bioproducts containing *Pseudomonas* spp. are approved by the Organic Materials Review Institute (OMRI, 2024), with the restriction of using only non-pathogenic strains that do not pose risks to the health of other living beings.

In Brazil, according to AGROFIT (2024), there are four phytosanitary products registered with strains of *Pseudomonas* in their composition as active ingredients. These include

two microbiological insecticides, *Pseudomonas chlororaphis* strain CCTB19 and *Pseudomonas fluorescens* strain CCTB03, one microbiological nematicide strain SYM23945 from the species *Pseudomonas oryzihabitans*, and one microbiological fungicide strain CCT7997 *Pseudomonas fluorescens*.

Ferreira et al. (2028, 2020) investigated *Pseudomonas* strains UFLA02-281 and UFLA02-293, isolated from common bean nodules, and UFLA03-18, isolated from cowpea nodules, for plant growth promotion and damping-off control. Their results showed compatibility when co-inoculated with the CIAT899 strain (*Rhizobium tropici*) as well as potential for growth promotion and biocontrol of damping- off caused by *Rhizoctonia solani*. However, they have not been studied these strains for other diseases (Ferreira et al., 2018; Ferreira et al., 2020; Ferreira et al., 2023).

The objectives of this study concerning the UFLA02-281, UFLA02-293, and UFLA03-18 strains were: 1- to identify them at species level through genomic sequencing and comparison with type strains of known species of this genus; 2- to prospect genes related to growth promotion and biological disease control; 3- to evaluate their antagonistic effect *in vitro* and *in vivo* in common bean plants against phytopathogenic fungal strains; and 4- to verify the effect of inoculating these strains on common bean plants in promoting growth.

Material and methods

Origin of strains and cultural characteristics

The three bacterial strains belong to the Collection of Plant Growth-Promoting Bacteria at the the laboratory of Biology, Microbiology, and Soil Biological Processes at the Soil Science department in the Federal University of Lavras.. They were selected based on their growth-promoting abilities and antagonism towards *Rhizoctonia solani* (Ferreira et al., 2020, 2023). These strains were isolated from nodules of common beans - UFLA02-281, UFLA02-293 (Ferreira et al., 2018) - and cowpea - UFLA03-18 (Oliveira-Longatti et al., 2014), all originating from the state of Amazonas and were identified by 16S rRNA sequencing as belonging to the *Pseudomonas* genus. In 79 medium, these strains exhibit rapid growth rate, acidified pH, and low gum production. The strains CIAT899 (*Rhizobium tropici*) approved by the Ministry of Agriculture, Livestock, and Supply (MAPA) and UFLA02-127 (*Rhizobium* sp.) were used as controls because both are efficient in N2 fixation in symbiosis with common bean.

Extraction of DNA, sequencing, and genome assembly

The genomic DNA extraction from the strains was performed using the Wizard® Genomic DNA Purification kit from Promega, following the manufacturer's recommendations. The quality and concentration of the extracted DNA were analyzed on the Colibri spectrometer, and agarose gel electrophoresis was conducted to assess the purity of the extraction. The DNA library was constructed using 1 ng of the total extracted DNA, and Illumina sequencing was carried out.

Quality assessment of the sequences and genome assembly was conducted on the Kbase platform using SPAdes 3.13.0 (Bankevich et al., 2012) for assembly, and the quality check was performed using checkM (Parks et al., 2015). The genomes were deposited in GenBank (National Center for Biotechnology Information, NCBI) (https://www.ncbi.nlm.nih.gov/).

Taxonomic investigations of strains of the genus *Pseudomonas*

The Average Nucleotide Identity (ANI) of each strain was assessed on the Kostas Lab platform (http://enve-omics.ce.gatech.edu/) (Rodriguez & Konstantinidis, 2016) using the ANI Matrix tool. This tool estimates the genome similarity against all genomes in a collection. To construct this matrix, 368 genomes of type strains from different species of the genus *Pseudomonas* available on NCBI were used, considering that strains with ANI greater than 96% belong to the same species (Ciufo et al., 2018). Genome sequence data were uploaded and taxonomically analyzed by digital DNA-DNA hybridization (dDDH) using TYGS (Type Genome Server) (https://tygs.dsmz.de/), where strains with a dDDH value greater than 70% are considered to belong to the same species, and >79% likely belong to the same subspecies (Meier-Kolthoff & Göker, 2019).

Genome Mining for Genes Encoding Plant Beneficial and Biocontrol Traits

The functional genes involved in biocontrol and plant growth promotion, such as genes associated with nutrient metabolism, phytohormone production, volatile compound production, resistance induction, colonization, and bacteriocin production, were identified using the RASTtk (Rapid Annotations using Subsystems Technology toolkit—version 2.0) (https://rast.nmpdr.org/rast.cgi) (Aziz et al., 2008) and the KEGG database on the BlastKOALA platform (Kanehisa & Goto, 2000; Kanehisa et al., 2016).

Analysis of gene clusters involved in the biosynthesis of secondary metabolites

The prediction of secondary metabolism gene clusters was performed using antiSMASH (version 7.0.0) (Blin et al., 2023), where biosynthetic gene clusters sharing less than 70% amino acid identity with known clusters were considered novel.

Orthologous gene clusters within the *Pseudomonas* genus were identified using OrthoVenn3 (https://orthovenn3.bioinfotoolkits.net/home) (Sun et al., 2023).

The genomic sequence comparison included the strains UFLA02-281/02-293 and 03-18 with the genomic sequences of the strains ATCC13525^{T} (*P. fluorescens*), ATCC9446^{T} (*P. chlororaphis*), NBRC102199^{T} (*P. oryzihabitans*), which are species within the genus *Pseudomonas* extensively studied for the biological control of plant diseases.

In vitro assays testing the antagonism of strains of the genus *Pseudomonas* against fungi that cause diseases in common bean

The antifungal activity of strains UFLA02-281, UFLA02-293, and UFLA03-18 was assessed using fungal strains CML1846 (*Rhizoctonia solani*), Lv134 race 65 (*Colletotrichum lindemuthianum*), race 63-63 (*Pseudocercospora griseola*), and UFLA 44 (*Sclerotinia sclerotiorum*), responsible for damping-off, anthracnose, angular leaf spot, and white mold diseases in common beans, respectively.

For *Rhizoctonia solani*, *Colletotrichum lindemuthianum*, and *Sclerotinia sclerotiorum*, a 5 mm diameter disc of each fungal culture was placed at the center of Petri dishes (9 cm diameter). Each fungal inoculum was pre-cultured on Potato Dextrose Agar (PDA) for seven days. The endophytic strains were cultured in liquid medium 79 (Fred & Waksman, 1928) under agitation at 110 rpm and 28 °C for three days. The culture medium used in this assay was bean exudate (Ferreira et al., 2020). In the plates, 20 µL of the bacterial inoculant were pipetted at four points around the fungal culture. Evaluation was based on measuring the fungal colony diameter (DF) in cm and the size of the inhibition halo when formed by the bacteria.

The inoculum of *Pseudocercospora griseola* was pre-cultured in tubes with an inclined PDA medium for ten days. After this period, 5 mL of autoclaved distilled water was added and agitated to obtain a spore suspension. An aliquot of 50 µL of this suspension was spread on a PDA medium using a Drigalski loop. Then, 20 µL of bacterial inoculants were pipetted at four points in different quadrants of the Petri dish.

Biocontrol trials of diseases in common bean plants by strains of the genus *Pseudomonas*

Greenhouse tests were conducted for the four diseases from June 2022 to January 2023. For damping-off, anthracnose, and angular leaf spot trials, 500 mL plastic cups and two

common bean cultivars, ‘BRS Notável’ and ‘Pérola’, were used. ‘BRS Notável’, a semi-early cycle cultivar with semi-erect architecture, is susceptible to angular leaf spot and damping-off and moderately resistant to anthracnose. ‘Pérola’, a typical cycle cultivar with semi-prostrate architecture, is susceptible to anthracnose, white mold, and damping-off and moderately susceptible to angular leaf spot. Treatments included inoculation with strains UFLA02-281, UFLA02-293, and UFLA03-18, negative control with no bacteria, and a control inoculated with CIAT899 (*R. tropici*).

Only the ‘Pérola’ cultivar was used for the white mold trial, and the experiment was conducted in 2L pots. Treatments included inoculation with strains UFLA02-281, UFLA02-293, UFLA03-18, CIAT899, and negative control with no disease control, along with two nitrogen sources, mineral N in the form of urea, and inoculation with strain UFLA02-127 (*Rhizobium tropici*) for biological nitrogen fixation. The experimental design was completely randomized, with three repetitions for each treatment. Bacterial inoculants of strains UFLA02-281, UFLA02-293, UFLA03-18, CIAT899, and UFLA02-127 were prepared in a liquid medium 79 for three days. Inoculation of endophytic strains was performed at the time of sowing.

A soil mixture (pH = 5.7; P = 0.11 mg dm^-3; K = 10.48 mg dm^-3; Al = 0.0 cmolc dm^-3; Ca = 0.16 cmolc dm^-3; Mg = 0.08 cmolc dm^-3; Na = 3.00 mg dm^-3; SB = 0.27 cmolc dm^-3; t = 0.27 cmolc dm^-3; T = 1.57 cmolc dm^-3; MO = 0.28 dag/kg; P-Rem = 7.20 mg/L; V = 17.0%; Clayey texture; clay = 49 dag/kg; silt = 37 dag/kg; sand = 14 dag/kg) collected on the UFLA campus (21°13'49.3"S 44°58'02.3"W) and Tropstrato HT substrate (composition: pine bark, vermiculite, pg mix 14.16.18, potassium nitrate, simple superphosphate, and peat) in a 2:1 ratio (2 for soil and 1 for substrate) was used.

The pathogenic fungal strain inoculated, its preparation, application, and disease index evaluation differed for each disease assessed.

For damping-off (*Rhizoctonia solani*), the strain used was CML 1846. The fungus was pre-grown on BDA medium for seven days, and then four 5 mm discs of the fungus were transferred to 100 grams of autoclaved rice husks with 40 mL of distilled water. After ten days, this material was air-dried in paper bags in the oven (48 h, 60-65°C), and the dried material was crushed. Twenty-four hours before planting, 50 mg of this dried material per kg of soil was placed in the soil. The plants were kept in the greenhouse at 16°C at night and an average of 24°C during the day at 70% relative humidity. The final evaluation was performed on the 15th day after sowing, assessing germination rate, dry mass, plant height, and disease index, according to Noronha et al. (1995).

For anthracnose, the strain used was LV134 race 65 (*Colletotrichum lindemuthianum*). This fungus grew on PDA plates for ten days. A portion of the fungus mycelium was placed in test tubes containing autoclaved pods for 1 hour. This material was incubated for 8-12 days for spore multiplication. Spore scraping was then performed with a blade and distilled water. The Neubauer chamber calibrated the inoculum concentration to 1.2 x 10^6 spores per mL^{-1}. The spores were inoculated on plants ten days after emergence by spraying until run-off. Inoculated plants were kept in a humid chamber (95%) for 72 hours and then maintained in a greenhouse with 80% relative humidity and around 24 °C. Evaluation was performed ten days after pathogen inoculation using the scale proposed by Rava et al. (1996).

The *Pseudocercospora griseola* race 63-63 inoculum was prepared in PDA tubes. After ten days, the mycelial mass in the tubes was suspended with distilled water and filtered with gauze. The inoculum concentration to be applied was 2×10^4 spores per mL^{-1}, determined with the Neubauer chamber. Ten days after sowing, the spore suspension was inoculated on plants by spraying on both sides of the leaves until run-off. After inoculation, the plants were kept in a greenhouse with 80% relative humidity and a temperature of around 16°C at night and 24 °C on average during the day. Twelve days after inoculation, disease severity was assessed using the scale proposed by Librelon et al. (2016).

The UFLA 44 isolate of *Sclerotinia sclerotiorum* was used for the white mold experiment. A disc of mycelium, grown on PDA at 23 °C for seven days, was removed from the colonized medium using plastic micropipette tips. The straw test method proposed by Petzoldt and Dickson (1996) was used for inoculation. Inoculation was performed 28 days after sowing. The pots remained in the greenhouse at 16°C at night and 25°C during the day, with 70% relative humidity. Seven days after pathogen inoculation, disease severity was assessed using a diagrammatic scale from 1 to 9, proposed by Singh et al. (2014).

Data from *in vivo* trials were subjected to normality tests and analysis of variance of means using the Tukey test with a significance level of 5% in the R software (R Core Team, 2020). Disease indexes for each disease were transformed into percentages according to their respective diagrammatic scales.

Results

Genomic characterization of strains of the genus *Pseudomonas*

The complete genomes of strains UFLA02-281, UFLA02-293, and UFLA03-18 were assembled, and their information is presented in Table 1. In the results obtained from checkM, strain UFLA02-281 exhibited 100% completeness and a very low contamination of 0.2. Strain

UFLA02-293 showed a completeness of 99.63% and a considered low contamination of 2.11, while strain UFLA03-18 displayed 100% completeness with a very low contamination of 0.77. Therefore, all three genomes were classified as of high quality. Following genome assembly and quality checks, they were deposited and made available on NCBI with respective GENBANK accession codes (Table 1).

Table 1. General information on the genomes of nodule endophytic strains of the Genus *Pseudomonas*.

Assembly Statistics	**Strains**		
	UFLA02-281	UFLA02-293	UFLA03-18
Size (bp)	5,924,688	6,878,115	6144495
G+C content (%)	64.4	59.96	60.6
N50	58776	27149	28562
L50	33	84	67
Number of Subsystems	377	390	372
Number of Coding Sequences	5473	6703	5905
Predicted proteins	5267	6565	5756
CDS	5,471	6,697	5,900
tRNA	62	52	59
rRNA	3	5	4
GENBANK	JASJFR000000000	JASJFS000000000	JASJFQ000000000

Identification and Taxonomic Classification of the *Pseudomonas* Strains

The UFLA02-281/02-293/03-18 strains, compared by Average Nucleotide Identity with the genomes of 368 type strains of *Pseudomonas* species available on NCBI, were classified as belonging to different species among themselves (Table 2).

Table 2. ANI and dDDH values obtained from the comparison of strains UFLA02-281, UFLA02-293 and UFLA03-18 with type strains of the species to which they were most closely related from the genus *Pseudomonas*.

Strains	**UFLA02-281**	**Pseudomonas xanthosomatis COR54^T**	**UFLA02-293**	**Pseudomonas cremoris WS 5106^T**	**UFLA03-18**	**Pseudomonas bananamidigenes BW11P2^T**
Genome length (bp)	5,924,688	5,987,104	6,878,115	7,220,038	6,144,495	5,971,527
GC content (%)	64.39	64.19	59.96	60.05	60.6	60.62
Nº. proteins	5267	5157	6565	6556	5756	5428

ANI (%)	99.06	96.84	97.77
dDDH (%)	90.2	84.8	91.0
C+G difference (%)	0.21	0.09	0.02

Functional annotation of genes

UFLA02-281 analysed by RASTk has 2997 protein-coding sequences (CDS) distributed across 21 functional subsystems. In KOALABlast (KEGG), 2192 CDS were annotated, covering 27 subsystems. For the UFLA02-293 strain, 3748 CDS were annotated in RASTk and 2679 in KOALABlast. In the case of the UFLA03-18 strain, 3202 CDS were annotated in RASTk and 2368 in KOALABlast, distributed across functional subsystems. Tables with annotated genes in the genomes of strains UFLA02-281/02-293 and 03-18 can be found in the supplementary material (Supplementary Tables S1-S8), including the function of each gene, COG and KO numbers, and whether they were found in the genomes of all three studied strains. Table 3 summarizes the main functions of biological control, plant growth promotion, and essential annotated genes.

Table 3. Summary of the main categories and genes related to plant growth promotion and biological control.

Gene Category	Gene	Function	UFLA02-281	UFLA02-293	UFLA03-18
Plant Growth Promotion	*accD*	Involved in the production of plant growth-promoting substances such as 1-aminocyclopropane-1-carboxylate (ACC) deaminase, indole-3-pyruvic acid (IPyA), and phenazines.	x	x	x
	trpA		x	x	x
	trpC		x	x	x
	speB		x		x
Nitrogen Fixation	*nifU*	Associated with nitrogen fixation, contributing to increased nitrogen availability for plants.	x	x	x
	fixA		x	x	x
	fixB		x	x	x
Phosphate Solubilization	*pst*	Participate in the solubilization of phosphate, enhancing phosphorus availability for plants.	x	x	x
	pho		x	x	x
Siderophore Production	*pvd*	Involved in the synthesis of siderophores, facilitating iron uptake and promoting plant growth.	x	x	x
	pch		x	x	x
	fpv		x	x	x
	efeO			x	x
	efeB			x	x
	pel		x	x	

Biofilm Formation	*psl*	Genes related to the formation of biofilms, which can contribute to plant root colonization.	x	x	x
	ldcA		x	x	x
Antimicrobial Production	*Phz*	Involved in the synthesis of antimicrobial compounds, such as phenazines, hydrogen cyanide, chitinases and toxins.	x	x	x
	hcn		x		x
	toxA				
	PhzF		x	x	x
	chiA				x
	chiB			x	
Quorum Sensing	*wecB*	Regulate the quorum sensing system, influencing biofilm formation and secondary metabolite production.			x
	kinB		x	x	x
	lasR			x	
	dacA		x	x	x
	ribA		x		x

Each X represents that the gene was annotated in the strain's genome

Clusters of genes involved in secondary metabolite synthesis

The results generated by antiSMASH identified 13 coding regions for secondary metabolites in the genome of the UFLA02-281 strain, 14 in the UFLA02-293 strain, and 16 in the UFLA03-18 strain. The detailed results for each strain obtained through antiSMASH can be found in the supplementary material (Supplementary Tables S9-S11), and a summary of the metabolites is presented in Table 4.

Among the identified metabolites, hydrogen cyanide was found in the UFLA02-281 and UFLA03-18 strains, showing 100% similarity to the same metabolite identified in *Pseudomonas fluorescens* (BGC0002345), known for its antimicrobial function. The redox cofactor lankacidin C was identified in the genomes of all three strains, each with 13% similarity to the compound phaseolotoxin (BGC0000919) identified in the species *Pseudomonas syringae*.

The UFLA02-281 genome identified NRPS with 75% similarity to pseudodesmin A (BGC0002518) and an NRP-metallophore with 58% similarity to azotobactin D (BGC0002433).). The UFLA02-293 genome revealed the metabolite pyoverdine SMX-1 with the highest similarity at 29% (BGC0002693). For the metabolites identified in the UFLA03-18 genome, an NRPS was classified with 100% similarity to anikasin (BGC000150), previously identified in *P. fluorescens*.

Table 4. Summary of secondary metabolites noted by antismash in the genome of strains of the genus *Pseudomonas*.

Type	Most similar known cluster	UFLA02-281	UFLA02-293	UFLA03-18
hydrogen-cyanide	hydrogen-cyanide	+		+
NAGGN	s/i	+	+	+
NRPS	anikasin			+
redox-cofactor	lankacidin C	+	+	+
NRPS,RiPP-like	chitinimide			+
betalactone	fengycin		+	+
RiPP-like	s/i	+	+++	++++
arylpolyene	APE Vf	+	+	+
arylpolyene	APE Ec		+	
NRPS	pyoverdine SMX-1		+	+
NRPS	Pf-5 pyoverdine	++	+	++
NRPS-like	fragin		+	+
ranthipeptide	Pf-5 pyoverdine			+
NRPS	putisolvin III/IV/V	+		
NRPS	viscosinamide A/pseudodesmin A	+		
NRP-metallophore	pacifibactin	+		
NRP-metallophore	azotobactin D	+		
RiPP-like	FR901228	+		
NRPS	s/i	+	+	
butyrolactone	s/i		+	
RiPP-like	lipopolysaccharide		+	

Each + represents a metabolite annotated by antiSMASH.

Comparative analysis between genomes of the *Pseudomonas* genus

The complete genome sequences of strains UFLA02-281/02-293 and 03-18 were compared with type strains of species widely used in the biological control of plant diseases, namely ATCC13525^T (*Pseudomonas fluorescens*), ATCC9446^T (*P. chlororaphis*), and NBRC102199^T (*P. oryzihabitans*), to identify shared orthologs. Gene families evolve through

speciation processes, giving rise to orthologs. As depicted in Fig 6, 2.316 core gene clusters are present in *Pseudomonas* strains. These orthologous protein-coding gene clusters exhibit a relatively high conservation across Pseudomonas.

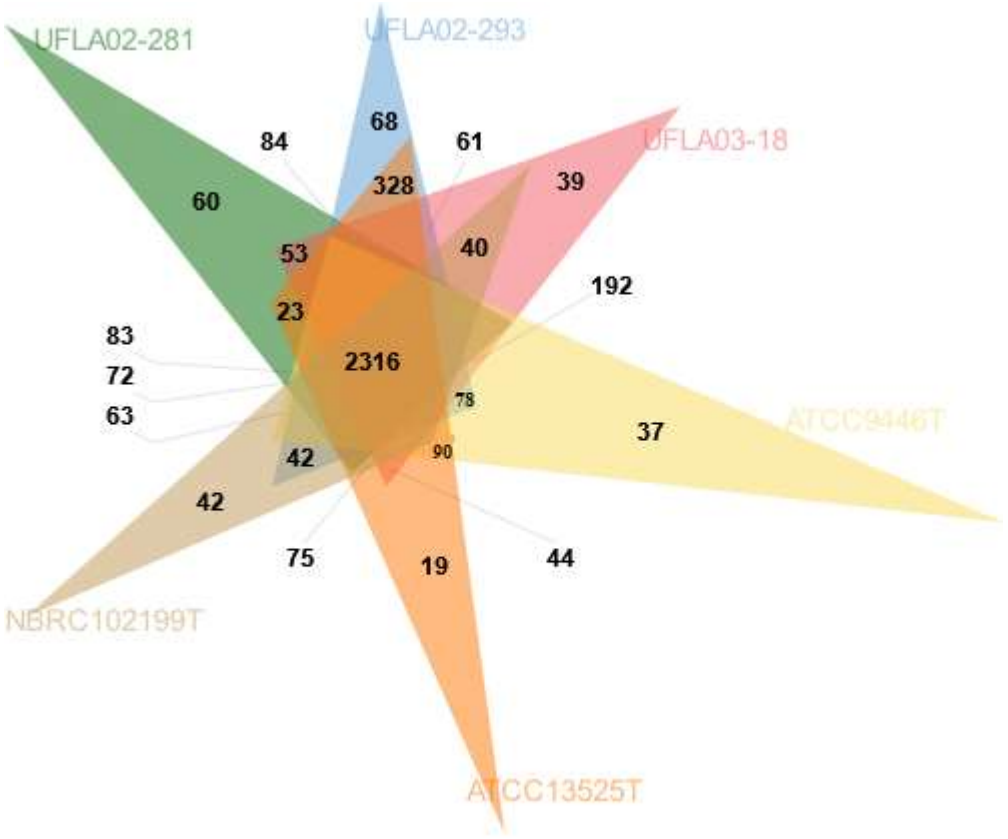

Fig 1. Orthologous clusters found in species of the genus *Pseudomonas*. Image generated by Orthovenn 3. Identificar as espécies destas 3 tipos

In total, 5.206 gene clusters were identified for strain UFLA02-293, 4.297 for strain UFLA02-281, and 4.804 for strain UFLA03-18. Strain UFLA02-293 exhibited 68 unique clusters and shares 5.332 genes with strain ATCC13525^{T}, featuring 328 unique clusters. Strain UFLA03-18 possesses 39 exclusive clusters and shares 5.196 genes with strain ATCC9446^{T} and 192 unique clusters. Finally, strain UFLA02-281 also showed 60 exclusive clusters and shares comparable quantities of genes with strains ATCC13525^{T}, ATCC9446^{T}, and NBRC102199^{T}, numbering 5.027, 5.087, and 5.076, respectively. The highest number of shared gene clusters is observed with strain ATCC9446^{T}, amounting to 83 clusters.

***In vitro* assay results**

The results of *Pseudomonas* strains *in vitro* varied according to the fungal strain to be controlled. Strain UFLA02-281 almost wholly inhibited the phytopathogenic fungi LV134 race

65 (*Colletotrichum lindemuthianum*) and race 63-63 (*Pseudocercospora griseola*), with no inhibition observed for the advancement of the fungus CML 1846 (*Rhizoctonia solani*) and UFLA 44 (*Sclerotinia sclerotiorum*). Strain UFLA03-18 exhibited better control of the fungus LV134 race 65 (*C. lindemuthianum*), while strain UFLA02-293 showed less reduction in the advancement of fungi compared to the other strains *in vitro* (Fig. 2).

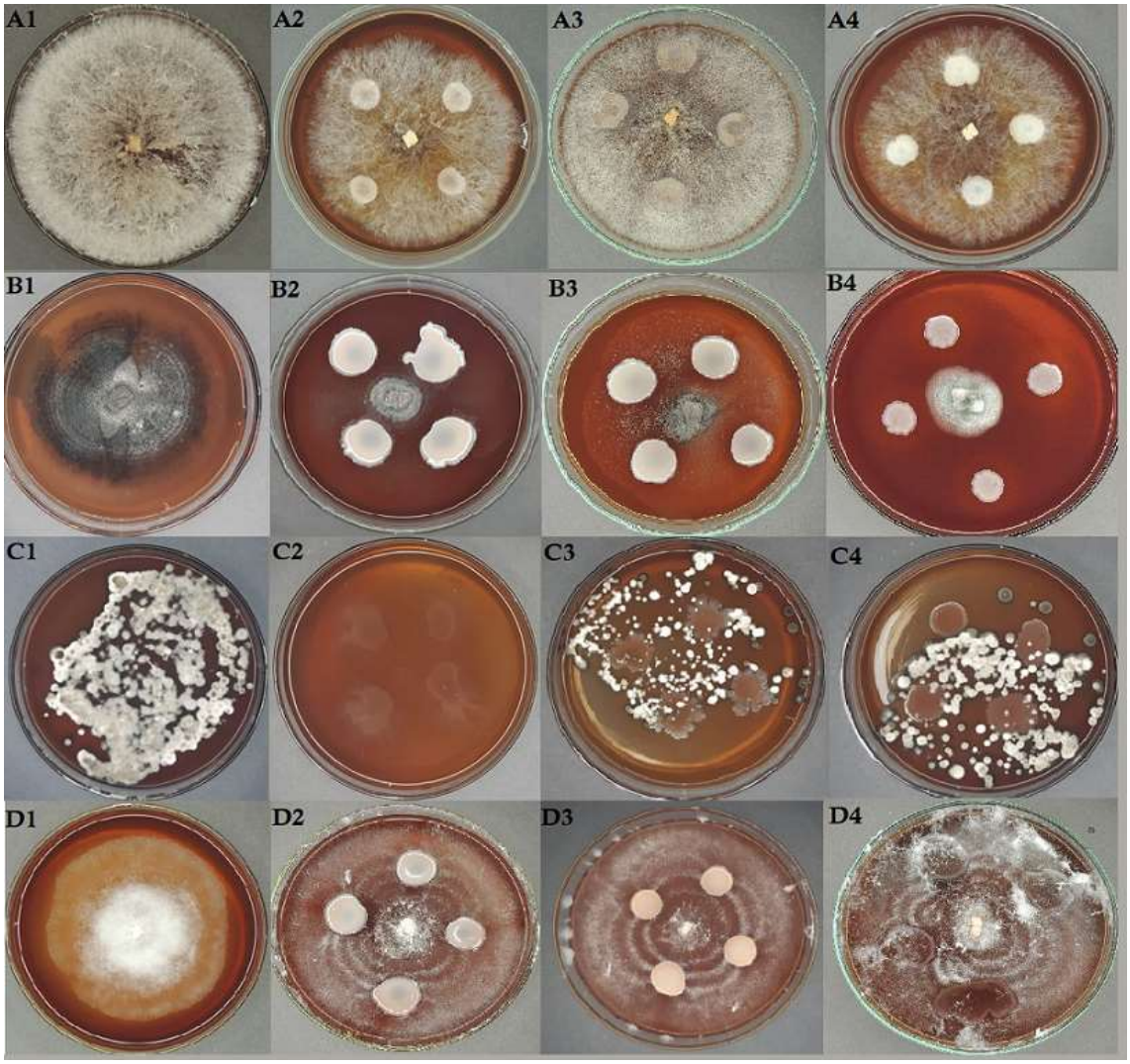

Fig 2. Antifungal activity of strains UFLA02-281/02-293/03-18 against fungi compared to controls without the strain. (A1) Control CML1846 (*Rhizoctonia solani*). (A2) Inhibition UFLA02-281 x CML1846. (A3) Inhibition UFLA02-293 x CML1846. (A4) Inhibition UFLA03-18 x CML1846. (B1) Control LV134 race 65 (*Colletotrichum lindemuthianum*). (B2) Inhibition UFLA02-281 x LV134 race 65. (B3) Inhibition UFLA02-293 x LV134 race 65. (B4) Inhibition UFLA03-18 x LV134 race 65. (C1) Control race 63-63 (*Pseudocercospora griseola*). (C2) Inhibition UFLA02-281 x race 63-63. (C3) Inhibition UFLA02-293 x race 63-63. (C4) Inhibition UFLA03-18 x race 63-63. (D1) Control UFLA 44 (*Sclerotinia sclerotiorum*). (D2) Inhibition UFLA02-281 x UFLA44. (D3) Inhibition UFLA02-293 x UFLA44. (D4) Inhibition UFLA03-18 x UFLA44.

In vivo assay results

In the damping-off control assay, strains UFLA02-281 and UFLA03-18 ensured the highest plant germination rate. Plants inoculated with strain UFLA03-18 exhibited the most significant growth; however, the highest shoot and root dry weight average was observed for strain UFLA02-281. Regarding the disease index, plants inoculated with strains UFLA02-281 and UFLA02-293 achieved the lowest indices of 2 and 1,67, respectively (Table 5) on a scale ranging from 0-5. For the 'Pérola' cultivar, the lowest disease index was recorded for strain UFLA02-281, followed by strain UFLA02-293 with a DI of 2,67 while the control exhibited a DI of 4,67, almost reaching the maximum of the scale.

Table 5. Effect of inoculating strains UFLA02-281, UFLA02-293, and UFLA03-18 on common bean plants grown in soil infested with *Rhizoctonia solani*, assessing their impact on growth promotion and mitigation of damping-off symptoms during the initial 15-day development period after planting.

Treatments	BRS NOTÁVEL								PÉROLA							
	GR		SDW		RDW		DI		GR^{ns}	SDW		RDW		DI		
	%		-----g plant^{1}-----				(0-5)		%	-----g plant^{1}-----				(0-5)		
UFLA02-281	86,7	a	0,195	a	0,107	a	2	A	73,3	0,176	a	0,105	a	3	ABC	
UFLA02-293	60,0	ab	0,135	b	0,068	bc	2,67	AB	66,7	0,087	b	0,050	b	1,67	A	
UFLA03-18	80,0	ab	0,093	bc	0,041	d	4	BC	80,0	0,099	b	0,051	b	3,67	BC	
Control CIAT899	73,3	ab	0,130	bc	0,075	b	3,33	ABC	73,3	0,093	b	0,049	b	2,67	AB	
Control	46,7	b	0,090	c	0,051	cd	4,67	C	53,3	0,073	b	0,046	b	4,67	C	

GR – germination rate; SDW – shoot dry weight; RDW – roots dry weigth; DI – disease index. ^{ns} not significant; Means followed by different letters indicate statistically significant differences, as determined by Tukey's test at a 5% significance level.

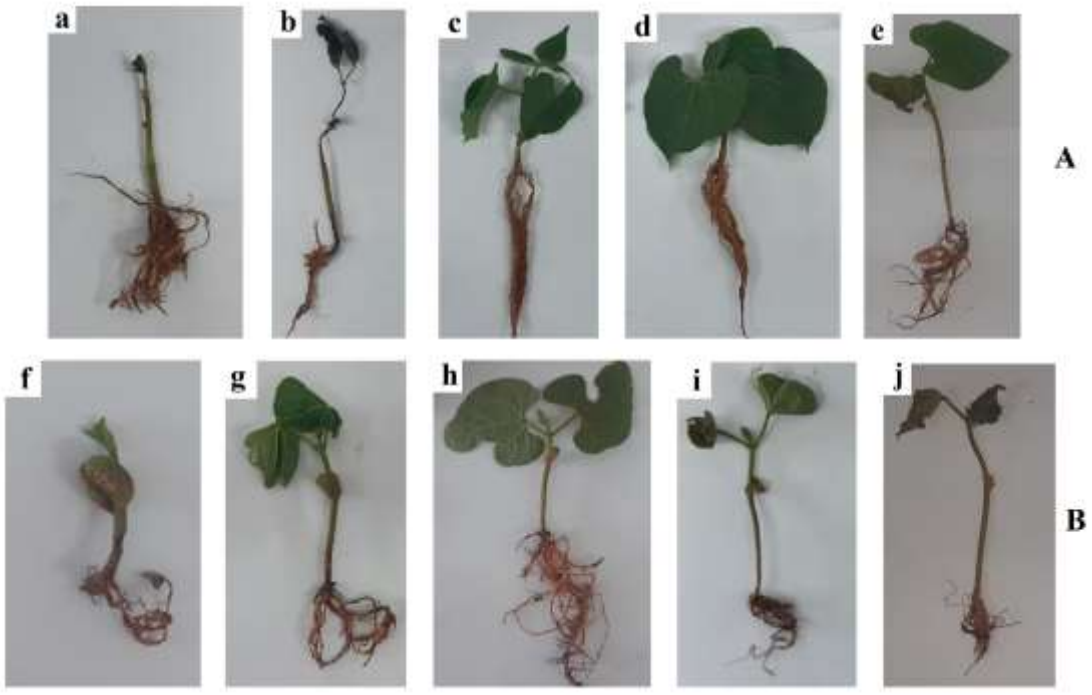

Fig 3. Control of damping of in bean plants inoculated with *Pseudomonas* strains UFLA02-281/02-293/03-18. **A**- BRS Notável cultivar; **a)** Control; **b)** Control CIAT899; **c)** UFLA02-281; **d)** UFLA02-293; **e)** UFLA03-18; **B**- Pérola cultivar; **f)** Control; **g)** Control CIAT899; **h)** UFLA02-281; **i)** UFLA02-293; **j)** UFLA03-18; Photographs of common bean plants and their symptoms 15 days after planting.

In the anthracnose assay, the dry weight of the shoot and roots showed no statistically significant differences. The 'BRS Notável' cultivar is moderately resistant to anthracnose; nevertheless, the control showed a disease index (DI) of almost 4 grade, while the DI values for treatments where *Pseudomonas* strains were inoculated ranged from 1,33 to 1,67, not passing grade 2 (Table 6). For the 'Pérola' cultivar, susceptible to anthracnose, the control's DI was 6,17, with strains UFLA02-293 and UFLA03-18 presenting the lowest DI averages, at 2,83 and 2,33, respectively, on a rating scale that ranged from 1 to 9.

Table 6. Results of inoculation of strains UFLA02-281, UFLA02-293, and UFLA03-18 in common bean plants sprayed with *Colletotrichum lindemuthianum* assessing their impact on growth promotion and mitigation of anthracnose symptoms during the initial 22-day development period after planting.

Treatments	BRS NOTÁVEL				PÉROLA			
	SDW^{ns}	RDW^{ns}	DI		SDW^{ns}	RDW^{ns}	DI	
	---g plant^{1}---		(1-9)		---g plant^{1}---		(1-9)	
UFLA02-281	0,467	0,211	1,33	A	0,306	0,151	3,67	AB
UFLA02-293	0,586	0,243	1,50	A	0,390	0,173	2,83	A
UFLA03-18	0,582	0,229	1,67	A	0,466	0,258	2,33	A
Control CIAT899	0,418	0,162	1,83	A	0,416	0,173	5,50	AB
Control	0,462	0,182	3,83	B	0,464	0,194	6,17	B

SDW – shoot dry weight; RDW – roots dry weigth; DI – disease index. ^{ns} not significant; Means followed by different letters indicate statistically significant differences, as determined by Tukey's test at a 5% significance level.

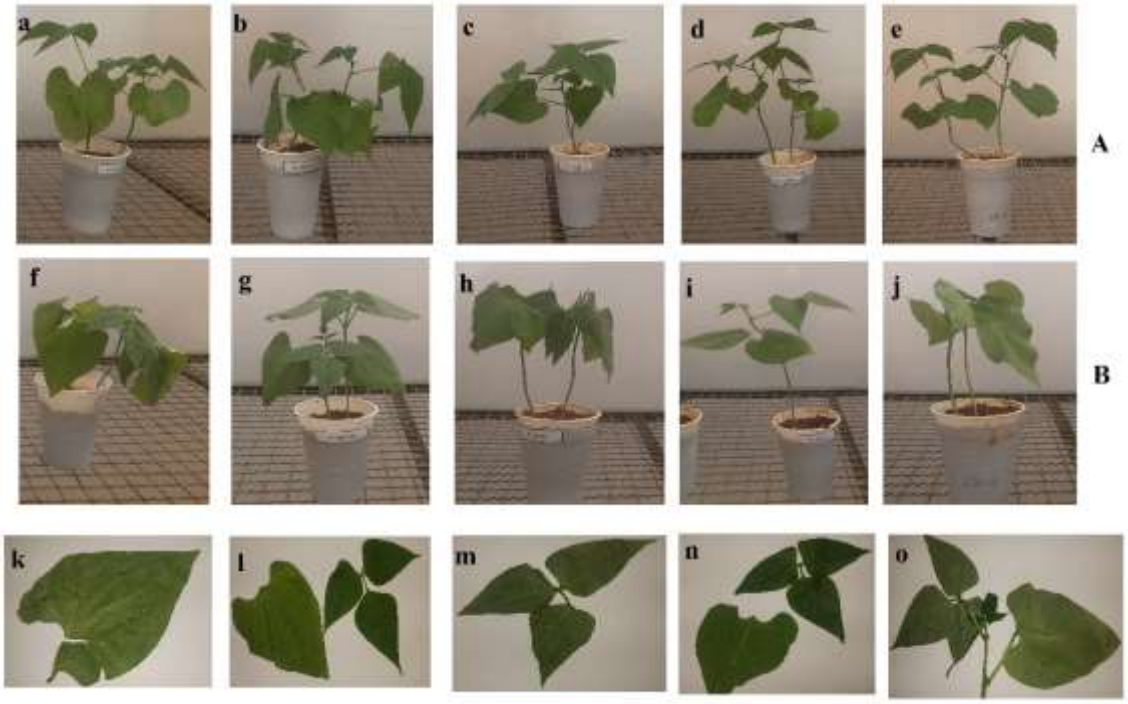

Fig 4. Control of anthracnose in bean plants inoculated with *Pseudomonas* strains UFLA02-281/02-293/03-18. **A**-BRS Notável cultivar; **a)** Control; **b)** Control CIAT899; **c)** UFLA02-281; **d)** UFLA02-293; **e)** UFLA03-18; **B**-Pérola cultivar; **f)** Control; **g)** Control CIAT899; **h)** UFLA02-281; **i)** UFLA02-293; **j)** UFLA03-18; **k)** focus on plant leaf control; **l)** focus on plant leaf control CIAT899; **m)** focus on plant leaf inoculated with UFLA02-281; **n)** focus on plant leaf inoculated with UFLA02-293; **o)** focus on plant leaf inoculated with UFLA03-18. Photographs of common bean plants and their symptoms 22 days after planting.

In the angular leaf spot assay, there was no significant effect on the dry weight of the shoot in the two cultivars used. Concerning root dry weight, for the cultivar 'BRS Notável,' the strain UFLA03-18 obtained the highest dry mass of roots, while for the cultivar 'Pérola,' there was no statistical difference in the treatments used. No statistical difference was found between the weight of dry shoot in the two cultivars. Regarding the disease index (DI) for both cultivars, the control had a DI of 4,67, and the treatments inoculated with *Pseudomonas* strains obtained a DI ranging from 1,67 to 2,17, on a rating scale that ranged from 1-9 (Table 7).

Table 7. Results of the inoculation of strains UFLA02-281, UFLA02-293, and UFLA03-18 in common bean plants sprayed with *Pseudocercospora griseola*, evaluating their impact on promoting growth and symptoms of angular leaf spot in the bean plant during the initial development period of 23 days after planting.

Treatments	BRS NOTÁVEL					PEROLA			
	SDW^{ns}	RDW		DI		SDW ^{ns}	RDW ^{ns}	ID	
	----g plant^{1}----			(1-9)		----g plant^{1}----		(1-9)	
UFLA02-281	0,523	0,159	b	1,67	A	0,544	0,169	2,50	AB
UFLA02-293	0,426	0,171	b	2,50	A	0,506	0,148	2	A
UFLA03-18	0,356	0,220	a	2,67	A	0,701	0,211	2,17	AB
Control CIAT899	0,565	0,169	b	2,83	A	0,561	0,172	3,33	B

Control	0,474	0,134	b	4,67	B	0,465	0,185	4,67	C

SDW – shoot dry weight; RDW – roots dry weigth; DI – disease index. [ns] not significant; Means followed by different letters indicate statistically significant differences, as determined by Tukey's test at a 5% significance level.

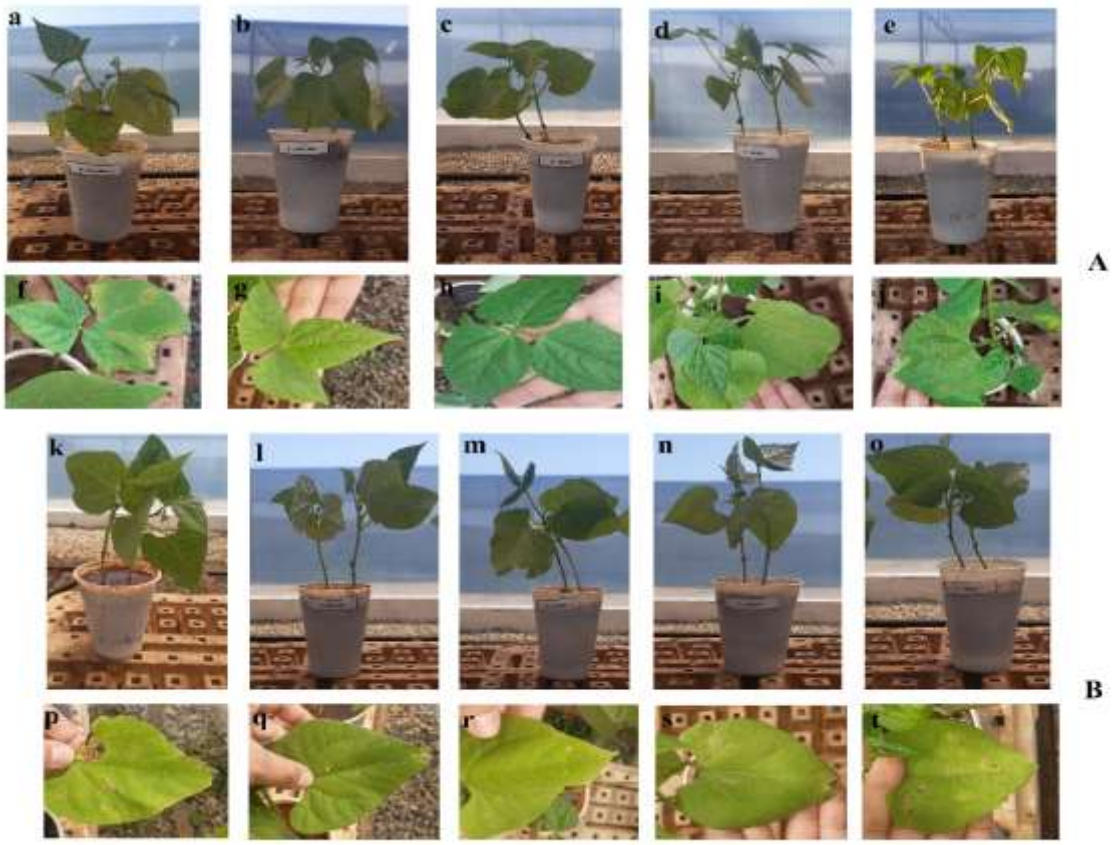

Fig 5. Control of angular leaf spot in bean plants inoculated with *Pseudomonas* strains UFLA02-281/02-293/03-18. **A**- BRS Notável cultivar; **a)** Control; **b)** Control CIAT899; **c)** UFLA02-281; **d)** UFLA02-293; **e)** UFLA03-18; **f)** focus on plant leaf control; **g)** focus on plant leaf control CIAT899; **h)** focus on plant leaf inoculated with UFLA02-281; **i)** focus on plant leaf inoculated with UFLA02-293; **j)** focus on plant leaf inoculated with UFLA03-18. **B**- Pérola cultivar; **k)** Control; **l)** Control CIAT899; **m)** UFLA02-281; **n)** UFLA02-293; **o)** UFLA03-18; **p)** focus on plant leaf control; **q)** focus on plant leaf control CIAT899; **r)** focus on plant leaf inoculated with UFLA02-281; **s)** focus on plant leaf inoculated with UFLA02-293; **t)** focus on plant leaf inoculated with UFLA03-18. Photographs of common bean plants and their symptoms 23 days after planting.

In the white mold assay, where only the 'Pérola' cultivar was used, the average root dry weight did not differ according to the Tukey test. Concerning shoot dry weight, the highest averages were in the UFLA02-281 + UFLA02-127 and UFLA03-18 + N treatments. Regarding the disease index (DI), the controls without inoculating *Pseudomonas* strains (CONTROL - UFLA02-127 and CONTROL - N) ranged between 2,33 and 2,5. The lowest disease indices were found in the treatments UFLA02-281 + UFLA02-127, UFLA02-293 + UFLA02-127, and UFLA03-18 + UFLA02-127, with respective DIs of 2,0, 2,67 and 1,83 (Table 8).

Table 8. Results of inoculation of strains UFLA02-281, UFLA02-293, and UFLA03-18 in common beans inoculated with *Sclerotinia sclerotiorum* using the straw method, evaluating growth promotion and control of white mold symptoms 35 days after planting in the Pérola cultivar

Treatments	SDW		RDW [ns]	DI	
	-----g plant[1]-----			(1-9)	
UFLA02-281 + UFLA02-127	1,588	ab	0,887	2,00	A
UFLA02-281 + N	1,384	bcd	0,789	2,67	AB
UFLA02-293 + UFLA02-127	1,299	cd	0,771	1,83	A
UFLA02-293 + N	1,478	abc	0,745	2,83	AB
UFLA03-18 + UFLA02-127	1,441	abc	0,758	1,67	A
UFLA03-18 + N	1,62	a	0,926	2,67	AB
CONTROL CIAT899	1,397	abc	0,747	2,33	AB
CONTROL - UFLA02-127	1,163	d	0,701	3,33	B
CONTROL - N	1,287	cd	0,774	3,5	B

SDW – shoot dry weight; RDW – roots dry weigth; DI – disease index. [ns] not significant; Means followed by different letters indicate statistically significant differences, as determined by Tukey's test at a 5% significance level.

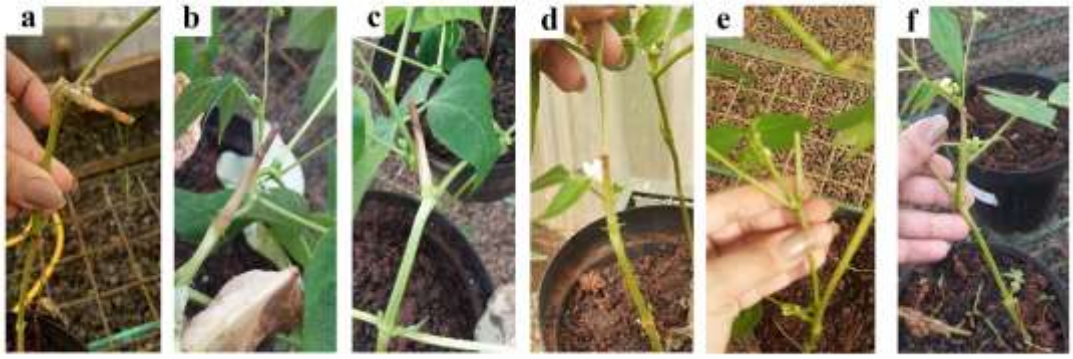

Fig 6. Control of white mold in bean plants inoculated with *Pseudomonas* strains UFLA02-281/02-293/03-18. **a)** Control N; **b)** Control UFLA02-127; **c)** Control CIAT899; **d)** UFLA02-281 + UFLA02-127; **e)** UFLA02-293 + N; **f)** UFLA03-18 + UFLA02-127. Photographs of bean plants of the Perola cultivar and their symptoms 35 days after planting.

Discussion

The three strains evaluated were identified as belonging to three species within the genus *Pseudomonas*, that were recently described by Hofmann et al. (2021) and Girard et al. (2022). Strain UFLA02-293 belongs to the species *P. cremoris* (WS 5106^T), with its type strain isolated from concentrated skim milk (Hofmann et al., 2021). The strain COR54^T (*P. xanthosomatis*) was isolated from the roots of red cocoyam (*Xanthosoma sagittifolium*), and strain BW11P2^T (*P. bananamidigenes*) from the rhizosphere of banana plants. Previously, all these type strains were classified as *Pseudomonas putida* (Girad et al., 2022). Strains studied in this work were

isolated from common bean nodules (UFLA02-281 and UFLA02-293) and cowpea nodules (UFLA03-18). The standard Average Nucleotide Identity (ANI) cutoff for strains to be considered the same species is >96% (Kim et al., 2014; Yoon et al., 2017; Ciufo et al., 2018), and for digital DNA-DNA hybridization (dDDH), the cutoff is >70% (Goris et al., 2007; Meier-Kolthoff et al., 2014; Li & Whitman, 2015), and the results obtained in this study met these criteria.

The strains UFLA02-281/02-293 and 03-18 were previously tested for *in vitro* control of *Rhizoctonia solani* using the same culture medium (bean exudates + agar), and all three strains showed inhibition halos against the fungus (Ferreira et al., 2020). However, in the tests conducted in this study, strain UFLA03-18 did not inhibit this fungus's advancement or form an inhibition halo. In vitro tests are valuable for observing bacterial behavior in controlled conditions with isolated factors. Nonetheless, examining microbial interactions in a more complex ecosystem, such as soil, is crucial (Shehata et al., 2016; Silva et al., 2019).

This study demonstrates that inoculation of the three strains reduced the symptoms of the diseases evaluated and improved plant development. Regarding *in vivo* results, strains UFLA02-281/02-293 and 03-18 were already tested for growth promotion and damping-off control (*Rhizoctonia solani*) in common bean and fava bean crops (Ferreira et al. 2020, 2023). For other diseases (anthracnose, angular leaf spot, and white mold), in vitro and in vivo tests were conducted with inoculated strains for the first time.

In the genomes of the three strains, the genes *trpA* and *trpC*, involved in tryptophan production, were annotated. Tryptophan assists in increased auxin production, which, in turn, can stimulate various physiological processes in plants, such as cell elongation, root development, and overall plant growth (Chieb & Gachomo, 2023; Olatunji et al., 2017). Another essential gene for plant growth promotion, *accD*, was annotated in UFLA02-281/02-293 and 03-18 genomes. This gene expresses the ACC enzyme complex that catalyzes the carboxylation of acetyl-CoA to form malonyl-CoA, an essential precursor in fatty acid synthesis. Fatty acids are fundamental components of the bacterial cell membrane, and this function is essential for promoting plant growth (Glick, 2014; Duca et al., 2014).

Regarding nitrogen fixation, only the genes *nifU*, *fixA*, and *fixB* were identified in the genomes of the three strains studied. Essential genes such as *nifH* and *nifD* were not identified in the genomic sequences of the bacteria (Ado & Santos, 2020; Fani et al., 2000; Zehr & Turner, 2001), and no *nod* genes were annotated. While not all *Pseudomonas* strains fix nitrogen for

plants, species with identified genes and expression of this activity have been documented in the literature, such as *P. protegens* Pf5 (Setten et al., 2013), *P. azotifigens* 6H33b^T (Hatayaman et al., 2005), *P. putida* K1 (Mirza et al., 2006), *P. koreensis* CY4, and *P. entomophila* CN11 (Li et al., 2017).

The genes belonging to the *pst* (*pstB*, *pstA*, *pstC*, and *pstS*) and *pho* (*phoU*, *phoH*, *phoD*, *phoP*, *phoQ*, *phoB*, and *phoR*) groups were found in the genomes of the three strains, with *phoD* being the only gene not recorded for UFLA02-281 (table suplementary S6). These genes play a crucial role in phosphate metabolism, as they are involved in the phosphate solubilization pathway, increasing phosphorus availability in plants (Giles et al., 2014; Huang & Cui, 2023). Other important genes for phosphorus availability, including *ppx* and *ppk1*, contributing to specific phosphate transport, were also identified in the genomes of the three strains. In *Pseudomonas* species, including *P. aeruginosa* (Blus-Kadosh et al., 2013; Lee et al., 2014) and P. *fluorescens* (Monds et al., 2006; Zhang et al., 2007), these genes play roles in phosphorus metabolism, as described above.

Regarding biological control, biofilm production can assist in protecting microorganisms, and the ability to colonize competitive sites can contribute to pathogen reduction. The gene *kinB* identified in the genomes of the three strains UFLA02-281/02-293/03-18 encodes a Kinase B protein generally associated with signal transduction and cellular process regulation. In some *Pseudomonas* species, this protein may play a role in biofilm formation and quorum sensing, essential for microbial population growth and competition for colonization sites (Mukherjee et al., 2019; Damron et al., 2012). The genes *chiA* and *chiC* were annotated in the genome of strain UFLA03-18, and the gene chiB (Supplementary Table S3, Table 8) was annotated in the genome of UFLA02-293. These genes expressing hydrolytic enzymes play an essential role in suppressing plant pathogens. Among several species exhibiting chitinolytic activity, *P. fluorescens* (Nielsen & Sørensen, 1999; Suganthi et al., 2015) and *P. aeruginosa* (Gupta et al., 2006; Chen et al., 2015) produce chitinases and are considered promising biocontrol agents.

A desirable characteristic in bacteria used in biocontrol is the ability to synthesize secondary metabolites with antimicrobial properties. Secondary metabolites are naturally produced substances that are not important as a source of energy or reserves (Mishra & Arora, 2018). The genes *hcnC*, *hcnB*, and *hcnA* were annotated in the genomes of UFLA02-281 and UFLA03-18, expressing the production of hydrogen cyanide, a metabolite that inhibits the growth of various pathogenic fungi. This secondary metabolite was also identified in the antiSMASH analysis (Table 8) in these strains, with 100% similarity to the same metabolite

found in strains of *P. fluorescens*, an important species for biological control. The gene *phzF* was annotated in the genomes of the three strains, expressing the production of phenazines. This metabolite can act in direct antagonism or induce systemic resistance in plants (Ahemad & Kibret, 2014). In strains UFLA02-281 and UFLA02-293, two pyoverdine siderophores were identified in antiSMASH, while in strain UFLA03-18, four were identified. They provide a competitive advantage for the strain since biologically available iron is limited (Hartney et al., 2013; Drehe et al., 2018; Timofeeva et al., 2022). The efficacy of the biocontrol activity of siderophore-producing bacteria depends strictly on factors such as the number of siderophores they produce (Gu et al., 2020; Kramer et al., 2020; Deb & Tatung, 2023).

Conclusions

According to the ANI and dDDH values obtained from genome analyses, strain UFLA02-281 belongs to the species *Pseudomonas xanthosomatis*, strain UFLA02-293 was identified as *Pseudomonas cremoris*, and strain UFLA03-18 as *Pseudomonas bananamidigenes*.

In vivo assays showed that *Pseudomonas* strains UFLA02-281/02-293 and 03-18 have the potential as biocontrol agents for fungal diseases and biofertilizers in common beans. Identifying genes and gene clusters in their genomes further highlights their genetic potential and capabilities. However, it is essential to note that although the results are promising, field studies are needed to establish their effectiveness as biological control agents or biofertilizers.

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Author contributions RMC: Conceptualization, methodology, investigation, formal analysis, visualization and writing the manuscript draft. DCM: methodology, investigation and review. RAL: investigation. FMSM: Conceptualization, methodology, investigation, supervision and manuscript edition and review. All authors read and approved the fnal manuscript.

Data availability All data analyzed during this study are included in this article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary material

Table S1. Genes involved in colonization

Pathway	Gene	Function/Definition	KO	COG	UFLA02-281	UFLA02-293	UFLA03-18
Biofilm formation and quorum sensing	*wecB*	UDP-N-acetylglucosamine 2-epimerase (EC 5.1.3.14)	K01791	COG0381	-	-	+
	kinB	Sporulation kinase B (EC 2.7.13.3)	K07697	COG0642	+	+	+
	dacA	D-alanyl-D-alanine carboxypeptidase (EC 3.4.16.4)	K07258	COG1686	+	+	+
	ldcA	Muramoyltetrapeptide carboxypeptidase (EC 3.4.17.13)	K01297	COG1619	+	+	+
	ribA	GTP cyclohydrolase II (EC 3.5.4.25)	K01497	COG0807	+	+	+
	pel	Pectate lyase [EC:4.2.2.2]	K01728	-	+	+	-
	pslB	Polysaccharide biosynthesis protein PslH	K21001	COG0438	+	+	+
	pslH	Mannose-1-phosphate guanylyltransferase / mannose-6-phosphate isomerase [EC:2.7.7.13 5.3.1.8]	K16011	COG0836	+	+	-
	pslA	Polysaccharide biosynthesis protein PslA	K20997	COG2148	-	+	-
	pelB	Polysaccharide biosynthesis protein PelB	K21007	-	-	+	-
	pelC	Polysaccharide biosynthesis protein PelC	K21008	-	-	+	-
	pelD	Polysaccharide biosynthesis protein PelD	K21009	-	-	+	-
	pelE	Polysaccharide biosynthesis protein PelE	K21010	-	-	+	-
	pelF	Polysaccharide biosynthesis protein PelF	K21011	COG0438	-	+	-
	pelG	Polysaccharide biosynthesis protein PelG	K21012	COG4267	-	+	-
	pelA	Polysaccharide biosynthesis protein PelA	K21006	COG3868	-	+	-

	lasR	LuxR family transcriptional regulator, quorum-sensing system regulator LasR	K18304	COG2771	-	+	-
Rhizosphere colonization	*xerD*	Integrase/recombinase	K04763	COG4974	+	+	+
	xerC	Integrase/recombinase (XerC/CodV family)	K03733	COG4973	+	+	+
Motility and Chemotaxis	*cheW*	Positive regulator of CheA protein activity (CheW)	K03408	COG0835	+	+	+
	fliM	Flagellar motor switch protein FliM	K02416	COG1868	+	+	+
	fliN	Flagellar motor switch protein FliN	K02417	COG1886	+	+	+
	fliG	Flagellar motor switch protein FliG	K02410	COG1536	+	+	+
	cheB	Chemotaxis response regulator protein-glutamate methylesterase CheB (EC 3.1.1.61)	K03412	COG2201	+	+	+
	cheR	Chemotaxis protein methyltransferase CheR (EC 2.1.1.80)	K00575	COG1352	+	+	+
	cheD	Chemotaxis protein CheD	K03411	COG1871	-	+	-
Lipopolysaccharide biosynthesis	*lapA*	Lipopolysaccharide assembly protein A	K08992	COG3771	+	-	+
	gtrB	B-glycosyltransferase, glycosyltransferase family 2 protein	K20534	COG0463	-	+	+
Colanic acid biosynthesis	*wzb*	Low molecular weight protein tyrosine phosphatase (EC 3.1.3.48)	K25307	COG0394	+	+	+
	gmd	GDP-mannose 4,6-dehydratase (EC 4.2.1.47)	K01711	COG1089	+	+	+
	fcl	GDP-L-fucose synthetase (EC 1.1.1.271)	K02377	COG0451	-	+	-
	manC	Sugar-phosphate guanylyltransferase / Sugar-phosephate isomerase	K00971	COG0662	-	+	-

Table S2. Genes involved in the synthesis of resistance inducers

Resistance Inductors	**Type of resistance**	**Gene**	**Function/Definition**	**KO**	**COG**	**UFLA02-281**	**UFLA02-293**	**UFLA03-18**

2, 3-Butanediol	ISR	*butA*	2,3-butanediol dehydrogenase, S-alcohol forming, (R)-acetoin-specific (EC 1.1.1.4) / Acetoin (diacetyl) reductase (EC 1.1.1.304)	K03366	COG1028	-	+	-
	ISR	*budC*	2,3-butanediol dehydrogenase, S-alcohol forming, (S)-acetoin-specific (EC 1.1.1.76)	K03366	COG1028	-	+	-
	ISR	*butB*	2,3-butanediol dehydrogenase, R-alcohol forming, (R)- and (S)-acetoin-specific (EC 1.1.1.4)	K00004	COG1063	-	+	-
	ISR	*ilvN*	Acetolactate synthase small subunit (EC 2.2.1.6)	K01653	COG0440	+	+	-
Acetoin	ISR	*alsD*	Alpha-acetolactate decarboxylase (EC 4.1.1.5)	K01575	COG3527			
	ISR	*mmuM*	Homocysteine S-methyltransferase (EC 2.1.1.10)	K00547	COG2040	+	+	+
Methanethiol	ISR	*metN*	Methionine ABC transporter ATP-binding protein	K02071	COG1135	+	+	+
	ISR	*mtnE*	Glutamine-dependent 2-keto-4-methylthiobutyrate transaminase	K08969	COG0436	+	+	+
	ISR	*metE*	Methionine synthase II (cobalamin-independent)	K00549	COG0620	+	+	+
	ISR	*lytB*	4-hydroxy-3-methylbut-2-enyl diphosphate reductase (EC 1.17.7.4)	K03527	COG0761	+	+	+
Isoprene	ISR	*gcpE*	(E)-4-hydroxy-3-methylbut-2-enyl-diphosphate synthase (flavodoxin) (EC 1.17.7.3)	K03526	COG0821	+	+	+
	ISR	*ispF*	2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (EC 4.6.1.12)	K01770	COG0245	+	+	+
	ISR	*ispE*	Small acid-soluble spore protein SspF	K00919	COG1947	+	+	+
Peptidoglycan	MTI	*dacA*	Diadenylate cyclase spyDAC	K18672	COG1624	+	+	+
Flagellin	MTI	*flgL*	Flagellar hook-associated protein FlgL	K02397	COG1344	+	+	+
EF-Tu	MTI	*tuf*	Translation elongation factor Tu	K02358	COG0050	+	+	+
Response to subsequent pathogen attacks.	PDR	*ptxR*	Transcriptional regulator PtxR, LysR family	K05596	COG0583	-	-	+

IRS – Induction of systemic resistance; MTI- microbe triggered immunity; PDR - Priming for Defense Responses

Table S3. Genes involved in the production of bacteriocins, antibacterial peptides synthesized by ribosomes and antibacterial compounds.

Pathway	**Gene**	**Function/Definition**	**KO**	**COG**	**UFLA02-281**	**UFLA02-293**	**UFLA03-18**
	pfeR, pirR	Two-component response regulator PfeR, enterobactin	K19610	COG0745	+	+	-

Bacitracin stress response	*gmhB*	D-glycero-beta-D-manno-heptose-1,7-bisphosphate 7-phosphatase (EC 3.1.3.82); Histidinol-phosphatase (EC 3.1.3.15)	K03273	COG0241	+	+	+
Tolerance to colicin E2	*creA*	Conserved uncharacterized protein CreA	K05805	COG3045	+	+	+
	creB	Two-component response regulator CreB	K07663	COG0745	-	+	-
	creC	Two-component response regulator CreC	K07641	COG0642	-	+	-
	creD	Inner membrane protein CreD	K06143	COG4452	+	+	+
Resistance to fluoroquinolones	*gyrA*	DNA gyrase subunit A (EC 5.99.1.3)	K02469	COG0188	+	+	+
	gyrB	DNA gyrase subunit B (EC 5.99.1.3)	K02470	COG0187	+	+	+
Resistance to antibiotics and toxic compounds	*ampC*	Beta-lactamase class C and other penicillin binding proteins	K01467	COG1680	+	+	+
	blI	Metal-dependent hydrolases of the beta-lactamase superfamily I	K07576	COG1236	+	-	-
	ampG	Beta-lactamase induction signal transducer AmpG	K08218	COG0477	+	+	+
Protein synthesis (LSU ribosomal proteins)	*infC*	Translation initiation fator IF-3	K02520	COG0290	+	+	+
	rpmI	LSU ribosomal protein L35p	K02916	COG0291	+	+	+
	rplT	LSU ribosomal protein L20p	K02887	COG0292	+	+	+
Protein synthesis (SSU ribosomal proteins)	*rpsL*	SSU ribosomal protein S12p (S23e)	K02950	COG0048	+	+	+
	rpsG	SSU ribosomal protein S7p (S5e)	K02992	COG0049	+	+	+
	fusA	Translation elongation factor G	K02355	COG0480	+	+	+
	tuf	Translation elongation factor Tu	K02358	COG0050	+	+	+
Detoxification	*frmB*	S-formylglutathione hydrolase (EC 3.1.2.12)	K01070	COG0627	+	+	+
	frmA	S-(hydroxymethyl)glutathione dehydrogenase (EC 1.1.1.284)	K00121	COG1062	+	+	+
	iciA	Transcriptional regulator, LysR family, in formaldehyde detoxification operon	K05596	COG0583	+	+	+

Hydrogen cyanide	*hcnC*	Hydrogen cyanide synthase HcnC [EC:1.4.99.5]	K10816	COG0665	+	-	+
	hcnB	Hydrogen cyanide synthase HcnB [EC:1.4.99.5]	K10815	COG0446	+	-	+
	hcnA	Hydrogen cyanide synthase HcnA [EC:1.4.99.5]	K10814	COG0446	+	-	+
Phenazines	*PhzF*	Phenazine biosynthesis protein PhzF like	K07506	COG2207	+	+	+
Hydrolytic enzymes - Chitinases	*chiA*	Bifunctional chitinase/lysozyme [EC:3.2.1.14 3.2.1.17]	K13381	-	-	-	+
	chiC	Putative chitinase	K03791	COG3179	-	-	+
	chiB	Basic endochitinase B [EC:3.2.1.14]	K20547	-	-	+	-

Table S4. Genes involved in potassium metabolism, iron acquisition and siderophore production.

Pathway	Gene	Function/Definition	KO	COG	UFLA02-281	UFLA02-293	UFLA03-18
Potassium metabolism	*kdpA*	Potassium-transporting ATPase A chain (EC 3.6.3.12) (TC 3.A.3.7.1)	K01546	COG2060	+	+	+
	kdpB	Potassium-transporting ATPase B chain (EC 3.6.3.12) (TC 3.A.3.7.1)	K01547	COG2216	+	+	+
	kdpC	Potassium-transporting ATPase C chain (EC 3.6.3.12) (TC 3.A.3.7.1)	K01548	COG2156	+	+	+
	ybiO	Potassium efflux system KefA protein	K22044	COG0668	+	+	+
	mscL	Large-conductance mechanosensitive channel	K03282	COG1970	+	+	+
	kup	KUP system potassium uptake protein	K03549	COG3158	+	+	+
	slyD	FKBP-type peptidyl-prolyl cis-trans isomerase SlyD [EC:5.2.1.8]	K03775	COG1047	+	+	+
Siderophores and acquisition of iron	*efeB*	Ferrous iron transport peroxidase EfeB	K16301	COG2837	-	+	+
	efeU	Ferrous iron transport permease EfeU	K07243	COG0672	+	+	+
	efeO	Ferrous iron transport periplasmic protein EfeO, contains peptidase-M75 domain and (frequently) cupredoxin-like domain	K07224	COG2822	-	+	+

	pvdE	Putative pyoverdin transport system ATP-binding/permease protein	K06160	COG4615	+	+	+
	pvdA	L-ornithine N5-monooxygenase [EC:1.14.13.195 1.14.13.196]	K10531	COG3486	+	+	+
	pvdQ	Acyl-homoserine-lactone acylase [EC:3.5.1.97]	K07116	COG2366	+	+	+
	pch	Voltage-gated potassium channel	K10716	COG1226	+	+	+
	pchP	Phosphorylcholine phosphatase	K21830	-	+	+	+
	pchB	Isochorismate pyruvate lyase [EC:4.2.99.21]	K04782	COG1605	-	+	-
	fpv	Outer-membrane receptor for ferric coprogen and ferric-rhodotorulic acid	K16088	COG4773	+	+	+
Pyoverdine synthesis	*pvdE*	Putative pyoverdin transport system ATP-binding/permease protein	K06160	COG4615	+	+	+
	ribF;	Riboflavin kinase / FMN adenylyltransferase [EC:2.7.1.26 2.7.7.2]	K11753	COG0196	+	+	+
	pvdA,	SIDA; L-ornithine N5-monooxygenase [EC:1.14.13.195 1.14.13.196]	K10531	COG3486	+	+	+
	ectB, dat;	Pyoverdin biosynthesis protein PvdH, L-2,4-diaminobutyrate:2-oxoglutarate aminotransferase (EC 2.6.1.76)	K00836	COG4992	+	+	+
	znuC	ABC transporter in pyoverdin gene cluster, ATP-binding component	K09817	COG1121	+	+	+
	fhuE, fpvA, fptA	Outer membrane ferripyoverdine receptor TC.FEV.OM1	K16088	COG4773	+	+	+
	pvdQ	Acyl-homoserine-lactone acylase [EC:3.5.1.97]	K07116	COG2366	+	+	+
Resistance to chromium compounds	*cutC*	Copper homeostasis protein CutE	K03820	COG3142	+	+	+
	tlyC	Magnesium and cobalt efflux protein CorC	K03699	COG1253	+	+	+
	chrB	Chromate resistance protein ChrB	K07213	COG2608	+	-	-
	chrA	Chromate transport protein ChrA	K07240	COG2059	+	+	+

Table S5. Genes involved in the production of phytohormones and volatile organic compounds

Pathway	Gene	Function/Definition	KO	COG	UFLA02-281	UFLA02-293	UFLA03-18
4-hydroxybenzoate (PBH)	*ubiA*	Menaquinone via futalosine polyprenyltransferase	K03179	COG0382	+	+	+
	ubiD	UbiD family decarboxylase associated with menaquinone via futalosine	K16239	COG0043	+	+	+
Hydrogen sulfide (H2S)	*cysC*	Adenylylsulfate kinase (EC 2.7.1.25)	K00860	COG0529	+	+	+
	cysE	Serine acetyltransferase (EC 2.3.1.30)	K00640	COG1045	+	+	+
	mccB	Cystathionine gamma-lyase (EC 4.4.1.1)	K17217	COG0626	+	+	+
	mccA	Cystathionine beta-synthase (EC 4.2.1.22)	K17216	COG0031	-	+	+
	met3	Sulfate adenylyltransferase (EC 2.7.7.4)	K00958	COG2046	+	+	+
	cysH	Phosphoadenylyl-sulfate reductase [thioredoxin] (EC 1.8.4.8)	K00390	COG0175	+	+	+
Indole-3-acetic acid (IAA) and Indole production	*trpC*	Indole-3-glycerol phosphate synthase (EC 4.1.1.48)	K01609	COG0134	+	+	+
	trpE	Anthranilate synthase, aminase component (EC 4.1.3.27)	K01657	COG0147	+	+	+
	ald	Alanine dehydrogenase (EC 1.4.1.1)	K00259	COG0686	+	+	+
	aspA	Aspartate ammonia-lyase (EC 4.3.1.1)	K01744	COG1027	+	+	+
Auxin Biosynthesis	*trpA*	Tryptophan synthase alpha chain (EC 4.2.1.20)	K01695	COG0159	+	+	+
	trpB	Tryptophan synthase beta chain (EC 4.2.1.20)	K01696	COG0133	+	+	+
	trpF	Phosphoribosylanthranilate isomerase (EC 5.3.1.24)	K01817	COG0135	+	+	+
	trpD	Anthranilate phosphoribosyltransferase (EC 2.4.2.18)	K00766	COG0547	+	+	+
Polyamine/ethylene modulation	*speE*	Spermidine synthase (EC 2.5.1.16)	K00797	COG0421	-	+	+
	arcC	Carbamate kinase (EC 2.7.2.2)	K00926	COG0549	+	+	+
	ydcU	Spermidine Putrescine ABC transporter permease component PotB (TC 3.A.1.11.1)	K02054	COG1176	+	+	+
	speB	Agmatinase (EC 3.5.3.11)	K01480	COG0010	+	-	+

	afuC	Putrescine transport ATP-binding protein PotA (TC 3.A.1.11.1)	K02010	COG3842	+	+	+
	aguA	Agmatine deiminase (EC 3.5.3.12)	K10536	COG2957	+	+	+
	accD	Acetyl-CoA carboxylase carboxyl transferase subunit beta [EC:6.4.1.2 2.1.3.15]	K01963	COG0777	+	+	+

Table S6. Genes involved in phosphorus metabolism

Pathway	Gene	Function/Definition	KO	COG	UFLA02-281	UFLA02-293	UFLA03-18
Phosphonates	*gnd*	6-phosphogluconate dehydrogenase, decarboxylating (EC 1.1.1.44)	K00033	COG0362	+	+	+
	gntK	Gluconokinase (EC 2.7.1.12)	K25031	COG1070	+	+	+
	ppc	Phosphoenolpyruvate carboxylase (EC 4.1.1.31)	K01595	COG2352	+	+	+
	gltB	Glutamate synthase [NADPH] large chain (EC 1.4.1.13)	K00265	COG0070	+	+	+
	acnA	Aconitate hydratase (EC 4.2.1.3)	K01681	COG1048	+	+	+
	sucD	Succinyl-CoA ligase [ADP-forming] alpha chain (EC 6.2.1.5)	K01902	COG0074	+	+	+
	sucC	Succinyl-CoA ligase [ADP-forming] beta chain (EC 6.2.1.5)	K01903	COG0045	+	+	+
	sdhA	Succinate dehydrogenase flavoprotein subunit (EC 1.3.5.1)	K00239	COG1053	+	+	+
	sdhB	Succinate dehydrogenase iron-sulfur protein (EC 1.3.5.1)	K00240	COG0479	+	+	+
	fumC	Fumarate hydratase class II (EC 4.2.1.2)	K01679	COG0114	+	+	+
	pdhA	Pyruvate dehydrogenase E1 component alpha subunit (EC 1.2.4.1)	K00161	COG1071	+	+	+
	poxB	Pyruvate dehydrogenase (quinone) (EC 1.2.5.1)	K00156	COG0028	+	+	+
	metG	Methionyl-tRNA synthetase (EC 6.1.1.10)	K01874	COG0143	+	+	+
	phnE	Pyridoxamine 5'-phosphate oxidase (EC 1.4.3.5)	K02042	COG3639	+	+	+

	phnC	Phosphonate transport system ATP-binding protein [EC:7.3.2.2]	K02041	COG3638	+	+	+
	phnD	Phosphonate transport system substrate-binding protein	K02044	COG3221	+	+	+
	phnB	PhnB protein; putative DNA binding 3-demethylubiquinone-9 3-methyltransferase domain protein	K04750	COG2764	+	+	+
	phnA	Protein PhnA	K06193	COG2824	+	+	+
	phnP	Phosphoribosyl 1,2-cyclic phosphate phosphodiesterase [EC:3.1.4.55]	K06167	COG1235	-	+	-
	phnX	Phosphonoacetaldehyde hydrolase(EC:3.11.1.1)	K05306	-	+	+	+
Specific phosphate transport	*pstB*	Phosphate ABC transporter, ATP-binding protein PstB (TC 3.A.1.7.1)	K02036	COG1117	+	+	+
	pstA	Phosphate ABC transporter, permease protein PstA (TC 3.A.1.7.1)	K02038	COG0581	+	+	+
	pstC	Phosphate ABC transporter, permease protein PstC (TC 3.A.1.7.1)	K02037	COG0573	+	+	+
	pstS	Phosphate ABC transporter, substrate-binding protein PstS (TC 3.A.1.7.1)	K02040	COG0226	+	+	+
Phosphate metabolism	*ppx*	Exopolyphosphatase (EC 3.6.1.11)	K01524	COG0248	+	+	+
	ppk1	Polyphosphate kinase (EC 2.7.4.1)	K00937	COG0855	+	+	+
	phoU	Phosphate transport system regulatory protein PhoU	K02039	COG0704	+	+	+
	phoH	Phosphate starvation-inducible protein PhoH, predicted ATPase	K06217	COG1702	+	+	+
	phoD	Alkaline phosphatase D [EC:3.1.3.1]	K01113	COG3540	-	+	+
	phoP	Two-component system, OmpR family, response regulator PhoP	K07660	COG0745	+	+	+
	phoQ	Two-component system, OmpR family, sensor histidine kinase PhoQ [EC:2.7.13.3]	K07637	COG0642	+	+	+
	phoB	Two-component system, OmpR family, phosphate regulon response regulator PhoB	K07657	COG0745	+	+	+

	phoR	Two-component system, OmpR family, phosphate regulon sensor histidine kinase PhoR [EC:2.7.13.3]	K07636	COG0642	+	+	+

Table S7. Genes involved in the production of organic acids.

Pathway	Gene	Function/Definition	KO	COG	UFLA02-281	UFLA02-293	UFLA03-18
Acetic Acid	*adhE*	Acetaldehyde dehydrogenase (EC 1.2.1.10) / Alcohol dehydrogenase (EC 1.1.1.1)	K04072	COG1012	-	-	+
	adh2	Long-chain-alcohol dehydrogenase (EC 1.1.1.192)	K19955	COG1979	-	+	-
Citric Acid	*gltA*	Citrate synthase (si) (EC 2.3.3.1)	K01647	COG0372	+	+	+
	icd	Isocitrate dehydrogenase [NADP] (EC 1.1.1.42)	K00031	COG0538	+	+	+
Glycolic Acid	*glyA*	Glycine hydroxymethyltransferase [EC:2.1.2.1]	K00600	COG0112	+	+	+
	gph	Phosphoglycolate phosphatase (EC 3.1.3.18)	K01091	COG0546	+	+	+
	allD	Ureidoglycolate dehydrogenase (EC 1.1.1.154)	K00073	COG2055	+	+	+
Latic Acid	*ldh*	L-lactate dehydrogenase (EC 1.1.1.27)	K00016	COG0039	+	+	+
Malic Acid	*fumC*	Fumarate hydratase class II (EC 4.2.1.2)	K01679	COG0114	+	+	+
Oxalic Acid	*maeA*	Oxaloacetate-decarboxylating [EC:1.1.1.38]	K00027	COG0281	+	+	+
Gluconic Acid	*gcd*	Quinoprotein glucose dehydrogenase [EC:1.1.5.2]	K00117	COG4993	+	+	+
Cyanic acid	*cyaA*	Adenylate cyclase, class 1 [EC:4.6.1.1]	K05851	COG3072	+	+	+

Table S8. Genes involved in nitrogen metabolism

Pathway	Gene	Function/Definition	KO	COG	UFLA02-281	UFLA02-293	UFLA03-18
Nitrogen Metabolism	*cynS*	Cyanate hydratase (EC 4.2.1.104)	K01725	COG1513	+	-	+

	glnA	Glutamine synthetase type I (EC 6.3.1.2)	K01915	COG0174	+	+	+
	gltD	Glutamate synthase [NADPH] small chain (EC 1.4.1.13)	K00266	COG0493	+	+	+
	amt	Ammonium transporter	K03320	COG0004	+	+	+
	gltB	Glutamate synthase [NADPH] large chain (EC 1.4.1.13)	K00265	COG0070	+	+	+
Nitrate and nitrite ammonification	*nirD*	Nitrate and nitrite ammonification	K00363	COG2146	+	+	-
	nirB	Nitrate and nitrite ammonification	K00362	COG1251	+	+	-
Denitrifying reductase gene clusters, Nitrate and nitrite ammonification	*narI*	Nitrate reductase gamma subunit [EC:1.7.5.1 1.7.99.-]	K00374	COG2181	-	+	-
Fixação	*nifU*	Nitrogen fixation protein NifU and related proteins	K04488	COG0822	+	+	+
	fixB	Electron transfer flavoprotein alpha subunit	K03522	COG2025	+	+	+
	fixA	Electron transfer flavoprotein beta subunit	K03521	COG2086	+	+	+
Ammonia assimilation, Glutamine, Glutamate, Aspartate and Asparagine Biosynthesis, Glutamine synthetases	*glnQ*	ABC transporter, ATP-binding protein (cluster 3, basic aa/glutamine/opines)	K10038	COG1126	+	+	+
	glnH	Glutamine ABC transporter, substrate-binding protein GlnH / Glutamine ABC transporter, permease protein GlnP	K10036	COG0834	+	+	+
	glnP	Glutamine transport system permease protein	K10037	COG0765	+	+	+
	glnA	Glutamine synthetase type I (EC 6.3.1.2)	K01915	COG0174	+	+	-
	glnL	Two-component system, response regulator GlnL	K07719	-	+	+	-
	glnK	Two-component system, sensor histidine kinase GlnK [EC:2.7.13.3]	K07717	COG0642	+	+	+
	glnP	Glutamine ABC transporter, permease protein GlnP	K10040	COG0765	+	+	+
	glnS	Glutaminyl-tRNA synthetase (EC 6.1.1.18)	K01886	COG0008	+	+	+

Nitrilase	*nit*	Plant-induced nitrilase (EC 3.5.5.1), hydrolyses beta-cyano-L-alanine	K01501	COG0388	+	+	+
	reg	Transcriptional regulator in custer with plant-induced nitrilase	K01501	COG0388	+	-	-

Table S9. Secondary metabolites were noted in the genome of the UFLA02-281 strain by antiSMASH.

Region	Type	From	To	Most similar known cluster		Similarity
Region 1.1	hydrogen-cyanide	110,3	123,2	hydrogen cyanide	Other	100%
Region 5.1	redox-cofactor	80,71	102,9	lankacidin C	NRP+Polyketide	13%
Region 9.1	arylpolyene	27,27	70,87	APE Vf	Other	35%
Region 43.1	NRPS	10,85	48,72	putisolvin III/putisolvin VI/putisolvin V	NRP	55%
Region 44.1	NRPS	1	44,74	Pf-5 pyoverdine	NRP	11%
Region 47.1	NRPS	1	42,02	Pf-5 pyoverdine	NRP	4%
Region 59.1	RiPP-like	24,97	34,99			
Region 89.1	NAGGN	1	11,55			
Region 91.1	NRPS	1	21,95	viscosinamide A/pseudodesmin A	NRP	75%
Region 96.1	NRPS,NRP-metallophore	1	19,44	pacifibactin	NRP+Polyketide	13%
Region 105.1	NRP-metallophore,NRPS	1	16,51	azotobactin D	NRP	58%
Region 107.1	RiPP-like	8,91	16,15	FR901228	NRP:Cyclic depsipeptide+Polyketide:Modular type I polyketide	9%
Region 128.1	NRPS	1	10,28			

Table S10. Secondary metabolites were noted in the genome of the UFLA02-293 strain by antiSMASH.

Region	Type	From	To	Most similar known cluster		Similarity
Region 10.1	redox-cofactor	1	18,73	lankacidin C	NRP+Polyketide	13%
Region 17.1	betalactone	30,03	51,24	fengycin	NRP	13%
Region 21.1	NRPS	1	48,69	Pf-5 pyoverdine	NRP	9%
Region 26.1	butyrolactone	3,471	14,51			
Region 30.1	NAGGN	23,58	38,27			
Region 31.1	RiPP-like	14,63	25,45			
Region 36.1	RiPP-like	11,6	22,48			
Region 38.1	NRPS,NRP-metallophore	1	39,4	pyoverdine SMX-1	NRP	29%
Region 47.1	hydrogen-cyanide	1	10,78			
Region 97.1	RiPP-like	6,669	17,51			
Region 102.1	RiPP-like	406	12,6	lipopolysaccharide	Saccharide:Lipopolysaccharide	5%
Region 124.1	NRPS-like	1	18,75	fragin	NRP	25%
Region 138.1	arylpolyene	1	16,83	APE Ec	Other	26%
Region 194.1	arylpolyene	1	12,06	APE Vf	Other	15%

Table S11. Secondary metabolites were noted in the genome of the UFLA03-18 strain by antiSMASH.

Region	Type	From	To	Most similar known cluster		Similarity
Region 2.1	NAGGN	74,85	89,616			
Region 4.1	hydrogen-cyanide	2,931	15,852	hydrogen cyanide	Other	100%
Region 9.1	NRPS	1	61,334	anikasin	NRP	100%
Region 20.1	redox-cofactor	35,24	50,735	lankacidin C	NRP+Polyketide	13%
Region 25.1	NRPS,RiPP-like	1	45,18	chitinimide A,B,C,D,E,F,G	NRP+Polyketide	58%
Region 27.1	betalactone	14,1	37,294	fengycin	NRP	13%
Region 33.1	RiPP-like	16,18	27,227			
Region 54.1	arylpolyene	4,069	33,128	APE Vf	Other	35%
Region 78.1	NRPS	1	25,732	pyoverdine SMX-1	NRP	19%

Region 80.1	NRPS	1	25,382	Pf-5 pyoverdine	NRP	11%
Region 116.1	RiPP-like	12,24	19,809			
Region 159.1	NRPS	1	13,718	Pf-5 pyoverdine	NRP	7%
Region 163.1	NRPS-like	1	13,456	fragin	NRP	37%
Region 230.1	RiPP-like	1	6,792			
Region 232.1	RiPP-like	733	6,702			
Region 267.1	ranthipeptide	1	4,878	Pf-5 pyoverdine	NRP	3%

ARTIGO 3- Co-inoculation of endophytic rhizobacterial strains with the endophytic fungus *Induratia coffeana* controls symptoms of fungal diseases and promotes growth in common beans

Co-inoculation of endophytic rhizobacterial strains with the endophytic fungus *Induratia coffeana* controls symptoms of fungal diseases and promotes growth in common beans

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Abstract

Co-inoculation of different beneficial microorganisms often results in a synergistic effect enhancing plant growth. This study aimed to assess the effect of co-inoculations with seven strains of different bacterial genera (*Pseudomonas*, *Paenibacillus*, and *Rhizobium*) and one strain of endophytic fungus of the genus *Induratia* on the growth promotion and disease control of common bean. *In vitro* tests were conducted to determine compatibility between bacteria and fungi. At the same time, *in vivo* greenhouse trials evaluated disease control from co-inoculations on anthracnose, angular leaf spot, white mold, and damping off. The strains UFLA02-281/02-293/03-10 and 03-18 were also evaluated for the production of volatile organic compounds (VOCs). All strains evaluated were compatible with CML4019 of the *Induratia* genus, except the UFLA03-10 strain. Co-inoculation of the UFLA02-281 strain with the CML4019 provided the lowest disease rates in the four trials in at least one evaluated bean cultivar. Co-inoculations reduced disease symptoms by over 80%. Co-inoculation of rhizobia with CML4019 reduced disease symptoms in plants, mainly with CIAT899. UFLA03-10 produced the highest quantity (11) of volatile organic compounds identified by chromatography. Overall, co-inoculation with fungus CML4019 and the endophytic bacteria used in this study positively affected plant growth and efficiently reduced disease symptoms in the early development stage of beans.

Keywords: damping off, white mold, anthracnose, angular leaf spot, volatile organic compounds, plant growth promotion rhizobacteria.

Highlights

- *In vitro* test showed incompatibility between UFLA03-10 and CML4019.
- Co-inoculation of bacteria and fungus reduced bean disease, fostering plant growth.
- Co-inoculation of UFLA02-281 and CML4019 controlled better the four diseases.
- UFLA03-10 produced more volatile organic compounds than other strains.

1. Introduction

The cultivation of common beans (*Phaseolus vulgaris* L.) in Brazil is of great economic and social importance, as it is the most consumed plant protein by the population (Resende et al., 2018). This species is predominantly cultivated by family farmers who are in need of more economically and environmentally sustainable production systems. One of the most common problems in bean cultivation that reduces productivity is the occurrence of diseases caused by fungi. Among the most relevant fungal species we can mention: *Colletotrichum lindemuthianum*, which causes anthracnose (Padder et al., 2017); *Sclerotinia sclerotiorum*, which causes white mold (Ferreira et al., 2019); *Pseudocercospora griseola*, which causes angular leaf spot (Nay et al., 2019); and *Rhizoctonia solani*, which can cause root rot and, in extreme cases, plant damping-off in beans (Ajayi-Oyetunde & Bradley, 2018). These fungi can affect diverse crops, with their inoculums persisting in soil and plant debris, posing a significant challenge to their control (Miklas et al., 2013; Torres et al., 2016).

The primary methods for controlling these diseases in the field include the application of chemical fungicides to reduce inoculum, seed treatment and disinfection, disease-resistant cultivars, and crop rotation. However, the biological imbalance resulting from the use of chemical pesticides alters nutrient and organic matter cycles, eliminates beneficial organisms, and reduces biodiversity, necessitating the development and introduction of disease management alternatives (Bending et al., 2007; Shahid & Khan, 2022).

Endophytic microorganisms as growth promoters and biological control agents are becoming a viable alternative to chemical pesticides (Sharma et al., 2018). The relationship between plants and endophytes produces crucial bioactive compounds to increase plant vitality, strengthen environmental resilience, and promote sustainable ecosystems within host organisms. Furthermore, the fundamental role of endophytes goes beyond mere bioactivity; they actively contribute to nutrient cycling, bioremediation, and biodegradation processes, promoting environmental balance. Endophytes exhibit multifaceted protective mechanisms, shielding crops from pathogens and mitigating abiotic stresses (Kummar et al., 2020; Boro et al., 2022). Moreover, their production of growth-regulating substances facilitates better nutrient assimilation, increasing biomass and yields. This intricate interaction underscores the fundamental role of endophytes in promoting plant health, environmental sustainability, and agricultural productivity.

Endophytic bacteria of various genera and species demonstrate the ability to colonize plants and promote growth through hormone production, pathogen suppression, N2 fixation, and phosphate solubilization, among other mechanisms (Hussein et al., 2018; Sabaté et al., 2018; Sendi et al., 2020). Strains such as UFLA 03-10 (*Paenibacillus* sp.) (Marra et al., 2012); UFLA03-18 (*Pseudomonas* sp.) (Oliveira-Longatti et al., 2014); UFLA 02-281 and UFLA02-293 (*Pseudomonas* sp.) (Ferreira et al., 2018) have been tested in conjunction with CIAT899 (*Rhizobium tropici*) for the biological control of damping-off in common beans (Ferreira et al., 2020) and fava beans (Ferreira et al., 2023) caused by *Rhizoctonia solani*, showing a reduction in disease symptoms in plants inoculated with them.

In vitro tests were conducted to verify the antagonism of endophytic strains UFLA03-10/02-281/02-293 and 03-18 against strains of the phytopathogenic fungi *Colletotrichum lindemuthianum*, *Sclerotinia sclerotiorum*, *Pseudocercospora griseola*, and *Rhizoctonia solani*. The inoculation of strain UFLA03-10 (*Paenibacillus peoriae*) (Cardoso et al., 2024, unpublished) and strains of the genus *Pseudomonas* UFLA02-281 *(P. xanthosomatis*), UFLA02-293 (*P. cremoris*), and UFLA03-18 (*P. bananamidigenes*) in common bean plants (Cardoso et al., 2024, unpublished) was also performed to evaluate the reduction in symptoms of white mold, angular leaf spot, anthracnose, and damping-off in common bean. The *in vitro* results showed a significant inhibition of these fungi, and the inoculation of these strains reduced disease symptoms by more than 50% in common bean .

Endophytic fungi can trigger numerous plant mechanisms, protecting against biotic and abiotic disturbances (Fontana et al., 2021). Mota et al. (2021) demonstrated that endophytic fungi of the genus *Induratia* promote growth and control angular leaf spots, white mold, and anthracnose in beans. Among the fungal strains used, CML4019 (*Induratia coffeana*) showed the best control over the studied diseases.

Combining endophytic fungi with endophytic bacteria in agriculture, known as co-inoculation, offers several benefits. The synergistic effect promotes plant growth, nutrient absorption, and disease resistance. The cooperation between these microorganisms can lead to a more sustainable and productive agricultural system (Bandara et al., 2006; Adeleke et al., 2022).

For biological control strategies, some species of endophytic bacteria (Marzouk et al., 2021; Audrain et al., 2015) and endophytic fungi (Roy & Banerjee, 2019; Morath et al., 2012) can produce antifungal metabolites through their secondary metabolism pathways. Among

these compounds, volatile organic compounds (VOCs) are noteworthy, as their production allows endophytes to respond, adapt to environmental changes, and interact with other microorganisms. VOCs are studied for their antifungal activities to control diseases in the field and post-harvest (Elkahoui et al., 2015; Chaouachi et al., 2021; Montes-Osuna et al., 2022).

The objective of this study was to evaluate *in vitro* and *in vivo* (common bean) the interaction of endophytic bacteria, previously reported as plant growth promoters and antagonists to *Rhizoctonia solani*, *Colletotrichum lindemuthianum*, *Sclerotinia sclerotiorum*, and *Pseudocercospora griseola*, in co-inoculation with the fungus CML4019, previously reported as an antagonist to *Colletotrichum lindemuthianum*, *Sclerotinia sclerotiorum*, and *Pseudocercospora griseola*, for promoting plant growth and biological control of four diseases, and to verify the relationship of this control with VOCs production.

2. Material and methods

2.1. Origin and cultural characteristics of the microorganisms used

The strains of endophytic nodule bacteria used belong to the Collection of Plant Growth-Promoting Bacteria at the the laboratory of Biology, Microbiology, and Soil Biological Processes at the Soil Science department in the Federal University of Lavras, selected from previous studies conducted in the department (Table 1).

Table 1. Comprehensive data about bacterial strains exhibiting assessed growth-promoting attributes.

Strain	GT	pH	Color	Species	Origin – State and trap culture	Characteristics evaluated	Reference
UFLA 03-10	3-4	Acid	Beige	*Paenibacilus peoriae*	MG *V. unguiculata*	Biological control of *Rhizoctonia solani* and growth promotion in common and fava beans.	Ferreira et al., 2020, 2023
						Growth promotion and phosphate solubilization for *Oryza sativa* plants	Costa et al., 2015
						Efficient in solubilizing phosphate from biochar-based phosphate fertilizers	Leite et al., 2020
						Functional genomic analysis and biological control of anthracnose, ring spot, damping-off and white mold in common beans.	Cardoso et al., 2024
						Phosphate Solubilization and production of organic acids	Marra et al., 2012; 2019

UFLA 02-293	3-4	Acid	Yellow	*Pseudomonas cremoris*	AM *P. vulgaris*	Biological control of *Rhizoctonia solani* and growth promotion in common and fava beans	Ferreira et al., 2020, 2023
						Functional genomic analysis and biological control of anthracnose, ring spot, damping-off and white mold in common beans.	Cardoso et al., 2024
UFLA 03-18	3-4	Acid	Yellow	*Pseudomonas bananamidigenes*	AM *V. unguiculata*	Biological control of *Rhizoctonia solani* and growth promotion in common and fava beans	Oliveira-Longatti et al., 2014
						Functional genomic analysis and biological control of anthracnose, ring spot, damping-off and white mold in common beans.	Cardoso et al., 2024
UFLA 02-281	3-4	Acid	Yellow	*Pseudomonas xanthosomatis*	AM *P. vulgaris*	Biological control of *Rhizoctonia solani* and growth promotion in common and fava beans	Marra et al., 2012
						Functional genomic analysis and biological control of anthracnose, ring spot, damping-off and white mold in common beans.	Cardoso et al., 2024
UFLA 02-100	2-3	Acid	Yellow	*Rhizobium*	RO *P. vulgaris*	Biological N fixation in common beans.	Nogueira, 2005; Soares et al., 2006
						Acid tolerance increases the yield and profitability of common beans in tropical soils.	Oliveira et al., 2017
UFLA 02-127	2-3	Acid	Yellow	*Rhizobium*	RO *P. vulgaris*	Biological N fixation in common beans.	Nogueira, 2005; Soares et al., 2006
						Acid tolerance increases the yield and profitability of common beans in tropical soils.	Oliveira et al., 2017
CIAT899	1-3	Acid	Yellow	*Rhizobium tropici*	Cali-Colômbia *P. vulgaris*	Rhizobia is registered as an inoculant for common beans for biological nitrogen fixation.	

GT – growth time in days; RO – Rondônia, AM – Amazonas; MG – Minas Gerais

The fungal strains used in this study, CML 4019 (*Induratia coffeana*) and CML 4015 (*Induratia* sp.) (Mota et al., 2021), were isolated from samples of fresh leaves and healthy stems of coffee plants (*Coffea arabica*) and are preserved in the Lavras Mycological Collection (CML) at the Department of Phytopathology, Federal University of Lavras, Brazil. The endophytic fungi were grown on potato dextrose agar (PDA) medium and incubated at 25°C for ten days. The phytopathogenic fungi belong to the Plant Disease Resistance Laboratory Collection at the Federal University of Lavras. *Colletotrichum lindemuthianum, Sclerotinia*

sclerotiorum, *Pseudocercospora griseola*, and *Rhizoctonia solani* were activated on PDA medium at temperatures of 24°C, 23°C, 24°C, and 25°C, respectively. General information about the fungi used is provided in Table 2.

Table 2. General information on the fungi used in biocontrol trials of bean diseases.

Strain	Species	Origin	Host	Classification
CML4019	*Induratia coffeana*	MG	*Coffea arabica*	Endophytic
CML1846	*Rhizoctonia solani*	-	*P. vulgaris*	Damping-off
LV134 – Race 65	*C. lindemuthianum*	MG	*P. vulgaris*	Anthracnose
Race 63-63	*P. griseola*	MG	*P. vulgaris*	Angular leaf spot
UFLA44	*S. sclerotiorum*	MG	*P. vulgaris*	White mold

MG – Minas Gerais

2.2. In vitro compatibility between beneficial endophytic bacterial and fungal strains

In this study, the fungal strain CML4019 (*Induratia coffeana*), which demonstrated superior efficacy in controlling anthracnose, white mold, and angular leaf spot in common beans (Mota et al., 2021), was evaluated for its compatibility with the endophytic bacteria UFLA 02-281, UFLA 02-293, UFLA 03-18, UFLA 03-10, and rhizobia strains CIAT899, UFLA 02-100, and UFLA 02-127.

The endophytic bacteria and rhizobia were grown in liquid culture medium 79 under agitation at 110 rpm and 28°C for three days. The fungal culture of the two endophytic strains was previously grown on a PDA medium for 12 days. The culture medium used in the compatibility test was PDA (Potato Dextrose Agar) (16 g L-1), which was autoclaved for 30 minutes and poured into Petri dishes. In the plates, 5mm of the grown fungal culture was placed in the center, and then 20 µL of the bacterial inoculant was pipetted at four points around the fungal culture. Evaluation was performed by measuring the diameter of the fungal colony (DF) in cm after 12 days of plate setup. Utilizing the fungal colony diameters, the percentage of inhibition (IP%) of the fungus was calculated using Ghildiyal and Pandey's formula (2008): IP (%) = (MD – md / MD) * 100 (where MD = control mean; md = mean of the fungal colony diameter confronted with bacteria). Inhibition greater than 80% was considered as no

compatibility between the bacterium and the fungus, between 20-80% as partial compatibility, and inhibition less than 20% as total compatibility between the microorganisms.

2.3 Bioessays

Greenhouse tests for the four diseases were conducted from June 2022 to January 2023. For the assays, a soil mixture was used (pH = 5.7; P = 0.11 mg dm-³; K = 10.48 mg dm-³; Al = 0.0 cmolc dm-³; Ca = 0.16 cmolc dm-³; Mg = 0.08 cmolc dm-³; Na = 3.00 mg dm-³; SB = 0.27 cmolc dm-³; t = 0.27 cmolc dm-³; T = 1.57 cmolc dm-³; MO = 0.28 dag/kg; P-Rem = 7.20 mg/L; V = 17.0%; Clayey texture; clay = 49 dag/kg; silt = 37 dag/kg; sand = 14 dag/kg) collected on the UFLA campus (21°13'49.3"S 44°58'02.3"W) and Tropstrato HT substrate (composition: pine bark, vermiculite, pg mix 14.16.18, potassium nitrate, superphosphate, and peat), in a 2:1 ratio, with two parts soil to 1 part substrate.

For the anthracnose, angular leaf spot, and damping-off assays, 500 mL plastic cups and two common bean cultivars, BRS Notável and Pérola, were used. The BRS Notável cultivar is semi-early maturing, semi-erect, moderately resistant to anthracnose, and susceptible to angular leaf spot and damping-off. The Pérola cultivar is a regular cycle, semi-prostrate architecture, moderately susceptible to angular leaf spot and susceptible to anthracnose, angular leaf spot, and white mold. The treatments evaluated in this study included co-inoculations of the endophytic fungus CML4019 with the endophytic bacterial strains UFLA02-281, UFLA02-293, UFLA03-10, and UFLA03-18, as well as the rhizobia strains UFLA02-100, UFLA02-127, and CIAT899. Control groups consisted of bacteria, fungus, and an absolute control with no microorganism inoculation.

Only the Pérola cultivar was used for the white mold assay, and the experiment was conducted in 2L pots. Treatments included co-inoculations of the fungus CML4019 with the strains UFLA02-281, UFLA02-293, UFLA03-10, UFLA03-18, UFLA02-100, UFLA02-127, and CIAT899, with two nitrogen sources: mineral N in the form of urea, and the inoculation of the strain UFLA 02-127 (*Rhizobium* sp.) for biological nitrogen fixation, in the absence of rhizobia inoculation in the treatment (UFLA02-100 or CIAT899).

Control groups for this assay included endophytic bacteria inoculated with the rhizobia UFLA02-127 or with nitrogen fertilization, the inoculation of all three rhizobia strains (UFLA02-100, UFLA02-127, and CIAT899), the inoculation of only the fungus CML4019 with nitrogen, and an absolute control with no endophytic microorganisms inoculated, only

nitrogen fertilization. The design of the four assays was randomized entirely, with three replicates of each treatment.

The bacterial inoculants were prepared in medium 79 containing (g L-1): K2HPO4, 0.5; MgSO4.7H2O, 0.2; NaCl, 0.1; mannitol, 10.0, and yeast extract 0.4; pH 6.8–7.0 (Fred & Waksman 1928), for three days. Inoculation of the endophytic strains was done at sowing time, using 1 mL per seed, with five seeds planted in each cup/pot. The endophytic fungus CML4019 was grown in liquid PD (potato-dextrose) medium at 25°C, rotating at 110 rpm for ten days. In 0.5 L of the medium, five pieces of 5mm of the fungus grown later in plates were placed. 1 mL of the mycelial mass formed in the medium, which was crushed, was used at sowing time.

Each phytopathogenic fungal strain inoculated, its preparation, application, and disease index evaluation differed for each disease.

For damping-off (*Rhizoctonia solani*), strain CML 1846 was used. The fungus grew previously on PDA medium for seven days, then four discs of 5 mm from the fungus were transferred to 100 grams of rice husk previously autoclaved with 40 mL of distilled water. After ten days, this material was dried in paper bags in the oven (48 h, 60-65°C), and the dried material was ground. Twenty-four hours before planting, 50 mg of this dried material per kg of soil was placed in the soil. The plants were kept in the greenhouse at 16°C at night and 24°C during the day, at 70% relative humidity. The final evaluation was performed on the 15th day after sowing. For this assay, germination rate, dry weight, and plant height were evaluated, and the disease index was assessed according to Noronha et al. (1995).

For anthracnose, strain LV134 race 65 (*Colletotrichum lindemuthianum*) was used. This fungus grew on PDA plates for ten days, after which a portion of the fungal mycelium was placed in pods inserted into test tubes and previously autoclaved for 1 hour. This material was incubated for 8-12 days for spore multiplication. The spores were then scraped with a blade and distilled water. The Neubauer chamber calibrated the inoculum concentration to 1.2×10^6 spores per mL^{-1}. The spores were inoculated on the plants ten days after emergence by spraying until runoff on the plants. The inoculated plants were kept in a humid chamber (95%) for 72 hours and then maintained in a greenhouse with 80% relative humidity and around 24°C. Evaluation was performed ten days after pathogen inoculation using the scale proposed by Rava et al. (1996).

Pseudocercospora griseola race 63-63 inoculum preparation was prepared in PDA tubes. After ten days, the mycelial mass in the tubes was suspended with distilled water and

filtered with gauze. The inoculum concentration to be applied was 2×10^4 spores per mL^{-1}, determined using the Neubauer chamber. Ten days after sowing, the spore suspension was inoculated on the plants by spraying on both sides of the leaves until runoff. After inoculation, the plants were kept in a greenhouse with 80% relative humidity and a temperature of around 16°C at night and 24°C during the day. Twelve days after inoculation, disease severity was evaluated using the scale proposed by Librelon et al. (2016).

For the white mold experiment, isolate UFLA 44 of *S. sclerotiorum* was used. Discs of mycelium, grown on PDA at 23°C for seven days, were removed from the colonized medium using plastic micropipette tips. The straw test method proposed by Petzoldt and Dickson (1996) was adapted for inoculation. Inoculation was performed 28 days after sowing. The pots remained in the greenhouse at 16°C at night and 25°C during the day, with 70% relative humidity. Seven days after pathogen inoculation, disease severity was assessed using a diagrammatic scale from 1 to 9, proposed by Singh et al. (2014).

2.4. Identification of volatile organic compounds (VOCs) of bacterial strains

The strains used for this test were UFLA02-281/02-293-03-10 and 03-18. The bacteria were grown in liquid medium 79 for three days for this test. Chromatography vials were prepared containing medium 79 (2 mL-1) inoculated with 50 µl of the bacterial culture four days before analysis. Five replicates of each bacterium and the controls (UFLA02-127 and medium without inoculation) were prepared to analyze volatile organic compounds identified by gas chromatography-mass spectrometry (GC-MS). The parameters for GC-MS analysis were identical to those described in previous works by Gomes et al. (2020).

2.5 Statistical analysis

The data from the *in vitro* and *in vivo* assays were subjected to normality testing using R software (R Core Team, 2020). Analysis of the variance of the means was performed in SisVAR using the Scott-Knott test at a significance level of 5% (Ferreira, 2011).

3 Results

3.1 In vitro compatibility test of endophytic bacteria strains with endophytic fungi

The endophytic bacterial strains used in this assay were compatible with fungi of the genus *Induratia* CML4019 (*Induratia coffeana*) (Table 3), except strain UFLA03-10, which

inhibited the growth of the tested fungi (Figures 1 and 2). Despite this result, the co-inoculation of strain UFLA03-10 with fungus CML4019 was used in the greenhouse assays.

Table 3. *In vitro* compatibility between nodule endophytic strains and the fungal strain CML4019 (*Induratia coffeana*)

	CML4019			
	CD (cm)		**IP (%)**	**COMP**
UFLA 02-281	2,90	b	28,69	partial
UFLA 02-293	3,23	b	20,49	partial
UFLA 03-10	0,00	a	100	no
UFLA 03-18	3,00	b	26,23	partial
CIAT 899	3,87	c	4,92	full
UFLA 02-127	4,00	c	1,64	full
UFLA 02-100	4,03	c	0,82	full
CONTROL	4,07	c		

CD – colony diameter; IP inhibition percentage; COMP – compatibility.

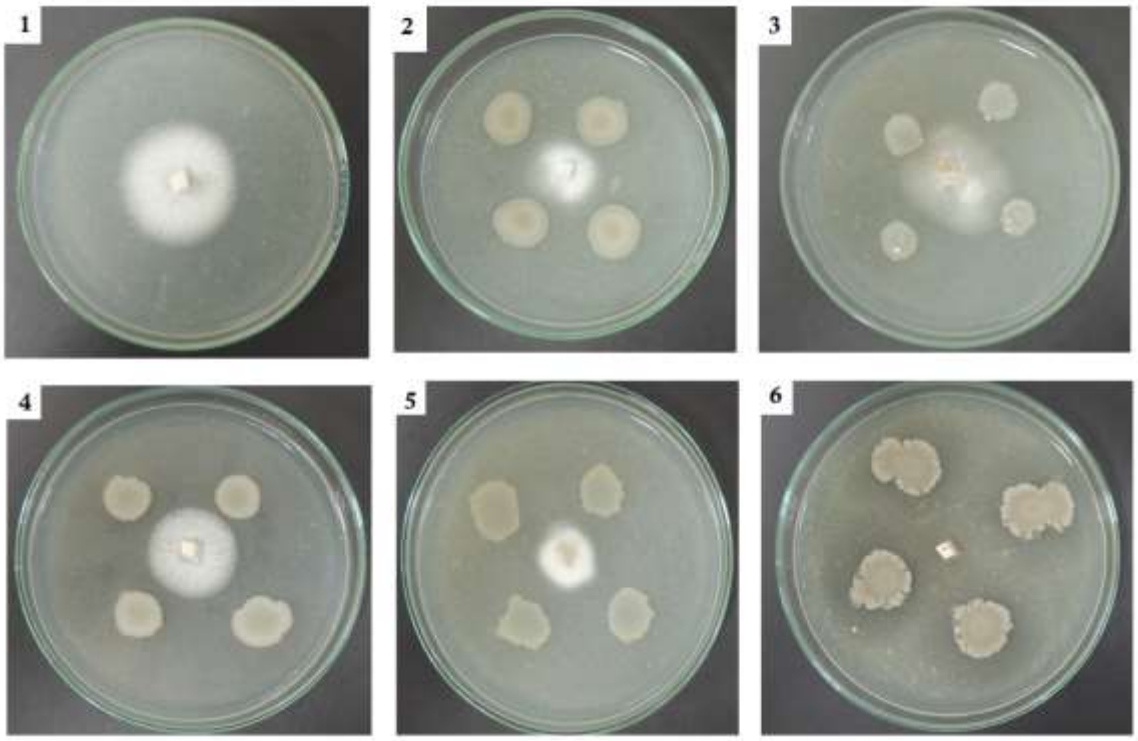

Fig. 1. Result of the compatibility test between strain CML4019 (*Induratia coffeana*) and endophytic bacterial strains. 1- Control CML4019; 2- UFLA03-18 x CML4019; 3- UFLA02-127 x CML4019; 4 – UFLA02-293 x CML4019. 5- UFLA02-281 x CML4019; 6- 03-10 x CML4019.

3.4 Bioassays results

For the anthracnose assay, there was no difference between the treatment and control means for shoot dry weight (SDW) of both cultivars and root dry weight (RDW) for the Pérola cultivar. For the BRS Notável cultivar, the means of disease index (DI) were lower, with the control group, which had no endophytic microorganisms inoculated, showing the lowest DI. Due to its moderate resistance to anthracnose, this cultivar. For the Pérola cultivar, the Scott-Knott test separated the DI means into four groups. The controls of UFLA02-100, UFLA02-127, CIAT899, and the absolute control had the highest DI, while the treatments with lower DI means and better disease control results were UFLA02-281 + CML 4019, UFLA03-18 + CML 4019, UFLA03-10 Control, UFLA03-18 Control, and CML 4019 Control. The results of the means of the assay are shown in Table 4 (Fig 3).

Table 4. Result of the co-inoculation trial of endophytic strains with the fungus CML4019 in common bean, evaluating growth promotion and control over the symptoms of anthracnose disease in the initial development phase of the bean plant 23 days after sowing.

TREATMENTS	BRS NOTÁVEL					PÉROLA				
	SDW^{ns}	RDW		DI		SDW^{ns}	RDW^{ns}		DI	
	--- g plant^{1}---			(1-9)		--- g plant^{1}---			(1-9)	
UFLA02-281 + CML 4019	0,525	0,223	a	1,83	a	0,425	0,154		2,17	a
UFLA02-293 + CML 4019	0,586	0,243	a	1,33	a	0,424	0,173		2,83	b
UFLA03-10 + CML 4019	0,496	0,245	a	1,67	a	0,384	0,178		3,33	c
UFLA03-18 + CML 4019	0,566	0,253	a	1,67	a	0,400	0,192		2,00	a
UFLA02-100 + CML4019	0,525	0,325	a	2,33	a	0,443	0,168		4,00	c
UFLA02-127 + CML 4019	0,556	0,213	b	2,00	a	0,474	0,203		2,67	b
CIAT 899 + CML 4019	0,425	0,232	a	1,33	a	0,550	0,214		2,83	b
Control UFLA02-281	0,501	0,221	a	1,33	a	0,306	0,151		3,67	c
Control UFLA02-293	0,454	0,185	b	1,50	a	0,277	0,159		2,83	b
Control UFLA03-10	0,661	0,265	a	1,50	a	0,525	0,251		2,00	a
Control UFLA03-18	0,582	0,229	a	1,67	a	0,343	0,235		2,33	a
Control UFLA02-100	0,511	0,188	b	2,33	a	0,357	0,117		5,50	d
Control UFLA02-127	0,455	0,184	b	2,16	a	0,332	0,152		5,00	d
Control CIAT 899	0,352	0,162	b	1,83	a	0,382	0,173		5,50	d
Control CML 4019	0,455	0,186	b	1,33	a	0,421	0,162		1,83	a
CONTROL ABS	0,462	0,182	b	3,83	b	0,313	0,160		6,17	d

SDW – shoot dry weight; RDW – roots dry weigth; DI – disease index. ^{ns} not significant; Means followed by different letters indicate statistically significant differences, as determined by the Scott-Knott test at a 5% significance level.

Fig 2. Effect of co-inoculation treatments in common bean *in vivo* tests 23 days after planting. **A)** Cultivar 'BRS Notável' inoculated with phytopathogen *C. lindemuthianum* race 65. **a-** UFLA02-281 + CML4019. **b-** UFLA02-293 + CML4019. **c-** UFLA03-18 + CML4019. **d-** UFLA03-10 + CML4019. **e-** CIAT899 + CML4019. **f-** UFLA02-100 + CML4019. **g-** UFLA02-127 + CML4019. **h1-** Control CML4019. **h2-** Control. **B)** Cultivar 'Perola' inoculated with phytopathogen *C. lindemuthianum* race 65. **i-** UFLA02-281 + CML4019. **j-** UFLA02-293 + CML4019. **k-** UFLA03-18 + CML4019. **l-** UFLA03-10 + CML4019. **m-** CIAT899 + CML4019. **n-** UFLA02-100 + CML4019. **o-** UFLA02-127 + CML4019. **p1**- Control CML4019. **p2-** Control.

In the angular leaf spot control assay, there was no significant difference in the mean height and SDW for the Pérola cultivar and SDW in the BRS Notável cultivar. Regarding the DI means, for the Pérola cultivar, the Scott-Knott test separated the means into three groups. The highest DI and the lowest control were observed in the absolute control, with a DI of grade 4,67. The controls with the rhizobia Control UFLA02-100, Control UFLA02-127, and Control CIAT 899 showed DI means of rage 3-4. The best means, i.e., lower DI values, were found in the controls with endophytic bacteria, fungus, and co-inoculations (Table 5) since the common bean cultivar Pérola is moderately susceptible to angular leaf spot.

For the BRS Notável cultivar, there were more variations among the treatments with the co-inoculations and controls. The lowest DI means, indicating the best disease control results, were found in the co-inoculations UFLA02-281 + CML 4019, UFLA02-293 + CML 4019, UFLA03-18 + CML 4019, and UFLA02-100 + CML4019, as well as in the UFLA03-10 Control. The highest DI mean was observed in the absolute control without the inoculation of endophytic microorganisms, with a DI mean of grade 4,67 (Fig 4).

Table 5. Result of the co-inoculation test of endophytic strains with the endophytic fungus CML4019 in common bean, evaluating growth promotion and control over the symptoms of angular spot disease in the initial development phase of the bean plant, 22 days after sowing.

TREATMENTS	BRS NOTÁVEL					PEROLA				
	SDW^{ns}	**RDW**		**DI**		**SDW^{ns}**	**RDW**		**DI**	
	---g plant^{-1}---			**(1-9)**		**---g plant^{-1}---**			**(1-9)**	
UFLA02-281 + CML 4019	0,429	0,171	b	1,33	a	0,628	0,192	b	2,67	a

UFLA02-293 + CML 4019	0,545	0,187	b	1,83	a	0,524	0,133	c	1,83	a
UFLA03-10 + CML 4019	0,529	0,171	b	1,33	a	0,565	0,173	b	2,83	a
UFLA03-18 + CML 4019	0,435	0,144	b	2,33	b	0,581	0,310	a	2,00	a
UFLA02-100 + CML4019	0,574	0,189	b	2,67	b	0,621	0,181	b	2,33	a
UFLA02-127 + CML 4019	0,623	0,197	b	2,00	a	0,478	0,174	b	2,17	a
CIAT 899 + CML 4019	0,582	0,184	b	2,33	b	0,609	0,174	b	2,00	a
Control UFLA02-281	0,523	0,159	b	1,67	a	0,544	0,169	b	2,5	a
Control UFLA02-293	0,426	0,171	b	2,50	b	0,506	0,148	c	2,00	a
Control UFLA03-10	0,665	0,252	a	2,17	b	0,499	0,149	c	2,33	a
Control UFLA03-18	0,356	0,220	a	2,67	b	0,701	0,211	b	2,17	a
Control UFLA02-100	0,544	0,172	b	3,17	c	0,607	0,198	b	3,83	b
Control UFLA02-127	0,449	0,177	b	3,33	c	0,675	0,203	b	3,67	b
Control CIAT 899	0,565	0,169	b	2,83	b	0,561	0,172	b	3,33	b
Control CML 4019	0,397	0,143	b	2,33	b	0,562	0,128	c	2,00	a
CONTROL ABS	0,474	0,134	b	4,67	d	0,465	0,185	b	4,67	c

SDW – shoot dry weight; RDW – roots dry weigth; DI – disease index. ^{ns} not significant; Means followed by different letters indicate statistically significant differences, as determined by the Scott-Knott test at a 5% significance level.

Fig 3. Effect of co-inoculation treatments in common bean *in vivo* tests 22 days after planting. **A)** Cultivar 'BRS Notável' inoculated with phytopathogen *P. griseola* race 63-63. **a-** UFLA02-281 + CML4019. **b-** UFLA02-293 + CML4019. **c-** UFLA03-18 + CML4019. **d-** UFLA03-10 + CML4019. **e-** CIAT899 + CML4019. **f-** UFLA02-100 + CML4019. **g-** UFLA02-127 + CML4019. **h1-** Control CML4019. **h2-** Control. **B)** Cultivar 'Perola' inoculated with phytopathogen *C. lindemuthianum* race 65. **i-** UFLA02-281 + CML4019. **j-** UFLA02-293 + CML4019. **k-** UFLA03-18 + CML4019. **l-** UFLA03-10 + CML4019. **m-** CIAT899 + CML4019. **n-** UFLA02-100 + CML4019. **o-** UFLA02-127 + CML4019. **p1**- Control CML4019. **p2**- Control.

For the damping-off assay, all the indices evaluated showed differences in means according to the Scott-Knott test (Table 6). The UFLA03-10 Control was the only one with a seed germination rate of 100%. Regarding the shoot dry weight and root dry weight, the co-inoculation of the strain UFLA02-281 with the fungus CML4019 presented the best means for both bean cultivars, along with the UFLA03-10 Control and the UFLA02-281 Control. Regarding the DI means, for the BRS Notável cultivar, the test separated the results into two

groups. The co-inoculations UFLA02-281 + CML 4019, UFLA03-10 + CML 4019, and CIAT 899 + CML 4019, as well as the UFLA02-281 Control, UFLA03-10 Control, UFLA02-293 Control, and CML 4019 Control, showed the best results with the lowest DI means.

For the Pérola cultivar, the test separated the results into three groups. The absolute control without inoculation showed a DI mean of grade 5, indicating no disease control. The best DI results for the Pérola cultivar were found in the co-inoculations UFLA02-293 + CML 4019, UFLA03-10 + CML 4019, and CIAT 899 + CML 4019, and in the controls: UFLA02-293 Control, UFLA03-10 Control, CIAT 899 Control, and CML 4019 Control, with the lowest DI mean found in the Control UFLA03-10 at grade 1,33 (Fig 5).

Table 6. Result of the co-inoculation test of endophytic strains with the endophytic fungus CML4019 in common bean, evaluating growth promotion and control over the symptoms of damping-off caused by *Rhizoctonia solani* in the initial development phase of the bean plant, 15 days after sowing.

TREATMENTS	BRS NOTÁVEL								PEROLA							
	GR		SDW		RDW		DI		GR		SDW		RDW		DI	
	%		---g plant^{-1}---				(0-5)		%		---g plant^{-1}---				(0-5)	
UFLA02-281 + CML 4019	80	B	0,206	a	0,114	a	1,67	a	73,3	b	0,194	A	0,109	A	3,00	b
UFLA02-293 + CML 4019	73,3	B	0,174	b	0,087	b	3,67	b	60,0	c	0,118	C	0,079	B	2,00	a
UFLA03-10 + CML 4019	100	A	0,157	c	0,068	c	2,33	a	100,0	a	0,135	C	0,084	B	1,67	a
UFLA03-18 + CML 4019	86,7	A	0,119	c	0,052	d	3,67	b	80,0	b	0,119	C	0,065	C	3,00	b
UFLA02-100 + CML4019	46,6	C	0,142	c	0,100	b	3,67	b	46,7	d	0,130	C	0,069	C	3,67	b
UFLA02-127 + CML 4019	53,3	C	0,123	c	0,099	b	3,67	b	60,0	c	0,148	B	0,086	B	3,00	b
CIAT 899 + CML 4019	73,3	B	0,133	c	0,089	b	2,33	a	80,0	b	0,142	B	0,081	B	2,33	a
Control UFLA02-281	86,6	A	0,195	a	0,107	a	2,00	a	73,3	b	0,176	A	0,105	A	3,00	b
Control UFLA02-293	60	C	0,135	c	0,068	c	2,67	a	66,7	c	0,087	D	0,051	D	1,67	a
Control UFLA03-10	100	A	0,216	a	0,117	a	1,67	a	100,0	a	0,187	A	0,108	A	1,33	a
Control UFLA03-18	80	B	0,093	e	0,041	d	4,00	b	80,0	b	0,099	D	0,050	D	3,67	b
Control UFLA02-100	40	C	0,138	c	0,077	c	4,00	b	40,0	d	0,087	D	0,052	D	3,67	b
Control UFLA02-127	46,6	C	0,112	d	0,071	c	4,00	b	40,0	d	0,127	C	0,062	C	3,67	b
Control CIAT 899	73,3	B	0,130	c	0,075	c	3,33	b	73,3	b	0,093	D	0,049	D	2,67	a
Control CML 4019	73,3	B	0,137	c	0,090	b	2.67	a	86,7	b	0,113	C	0,076	B	2,33	a
CONTROL ABS	47	C	0,090	e	0,051	d	4,67	b	53,3	c	0,073	D	0,046	D	4,67	c

GR – germination rate; SDW – shoot dry weight; RDW – roots dry weigth; DI – disease index. ^{ns} not significant; Means followed by different letters indicate statistically significant differences, as determined by the Scott-Knott test at a 5% significance level.

Fig 4. Effect of co-inoculation treatments in common bean *in vivo* tests 15 days after planting. **A)** Cultivar 'BRS Notável' inoculated with phytopathogen *R. solani* CML1846. **a-** UFLA02-281 + CML4019. **b-** UFLA02-293 + CML4019. **c-** UFLA03-18 + CML4019. **d-** UFLA03-10 + CML4019. **e-** CIAT899 + CML4019. **f-** UFLA02-100 + CML4019. **g-** UFLA02-127 + CML4019. **h-** Control CML4019. **i-** Control. **B)** Cultivar 'Perola' inoculated with phytopathogen *R. solani* CML1846. **j-** UFLA02-281 + CML4019. **k-** UFLA02-293 + CML4019. **l-** UFLA03-18 + CML4019. **m-** UFLA03-10 + CML4019. **n-** CIAT899 + CML4019. **o-** UFLA02-100 + CML4019. **p-** UFLA02-127 + CML4019. **q-** Control CML4019. **r-** Control.

In the white mold assay, the results for plant development indices varied considerably regarding the co-inoculations and controls used, with the highest RDW from the UFLA03-10 + CML4019 + UFLA02-127 co-inoculation (Table 7). Regarding the DI, the best mean values were found in the co-inoculations UFLA02-281 + CML4019 + UFLA02-127, UFLA 03-18 + CML4019 + N, and CML4019 + CIAT899, as well as in the controls: UFLA02-293 + UFLA02-127 Control, UFLA03-10 + UFLA02-127 Control, UFLA03-10 + N Control, and UFLA03-18 + UFLA02-127 Control. The lowest disease controls for white mold were the mean values of the Control UFLA02-127 and Control N (Fig 6).

Table 7. Result of the co-inoculation test of endophytic strains with the endophytic fungus CML4019 in common bean plants of the Pérola cultivar, evaluating growth promotion and control over white mold symptoms in the initial development phase of the bean plant, 35 days after sowing.

TREATMENTS	SDW		RDW		DI	
	---g plant^{-1}---				(1-9)	
UFLA02-281 + CML4019 + UFLA02-127	0,148	a	0,877	b	1,50	a
UFLA02-281 + CML4019 + N	1,541	a	0,765	c	2,17	b
UFLA02-293 + CML4019 + UFLA02-127	1,568	a	0,962	b	2,33	b
UFLA02-293 + CML4019 + N	1,418	b	0,760	c	2,00	b
UFLA03-10 + CML4019 + UFLA02-127	1,346	b	1,158	a	3,00	c
UFLA03-10 + CML4019 + N	1,300	b	0,932	b	2,33	b
UFLA03-18+ CML4019 + UFLA 02-127	1,408	b	0,912	b	2,33	b
UFLA 03-18 + CML4019 + N	1,551	a	0,762	c	1,83	a
Control UFLA02-281 + UFLA02-127	1,588	a	0,887	b	2,00	b

Control UFLA02-281 + N	1,384	b	0,789	c	2,67	c
Control UFLA02-293 + UFLA02-127	1,299	b	0,771	c	1,83	a
Control UFLA02-293 + N	1,478	a	0,745	c	2,83	c
Control UFLA03-10 + UFLA02-127	1,482	a	0,774	c	1,67	a
Control UFLA03-10 + N	1,575	a	0,925	b	1,50	a
Control UFLA03-18 + UFLA02-127	1,441	a	0,758	c	1,67	a
Control UFLA03-18 + N	1,620	a	0,926	b	2,67	c
CML4019 + UFLA02-100	1,325	b	0,784	c	2,33	b
CML4019 + UFLA02-127	1,442	a	0,916	b	2,17	b
CML4019 + CIAT899	1,560	a	0,894	b	1,83	a
CONTROL - CML4019 + N	1,276	b	0,759	c	2,50	c
CONTROL - 02-100	1,342	b	0,690	c	2,67	c
CONTROL - 02-127	1,163	b	0,701	c	3,33	d
CONTROL - CIAT 899	1,397	b	0,747	c	2,33	b
CONTROL - N	1,287	b	0,774	c	3,50	d

N- nitrogen; SDW – shoot dry weight; RDW – roots dry weigth; DI – disease index. ^{ns} not significant; Means followed by different letters indicate statistically significant differences, as determined by the Scott-Knott test at a 5% significance level.

Fig 5. Effect of co-inoculation treatments in common bean *in vivo* tests cultivar 'Perola' inoculated with phytopathogen UFLA 44 of *S. sclerotiourum* 35 days after planting. **a-** UFLA02-281 + CML4019 + UFLA02-127. **b-** UFLA02-293 + CML4019 + N. **c-** UFLA03-18 + CML4019 + N. **d-** UFLA03-10 + CML4019 + 02-127. **e-** CIAT899 + CML4019. **f-** UFLA02-100 + CML4019. **g-** UFLA02-127 + CML4019. **h-** Control CML4019. **i-** Control.

3.5 Identification of VOCs produced by nodule endophytic bacteria

Gas chromatography allowed for the identification of VOCs produced by the bacterial strains. Strain UFLA03-10 exhibited the highest quantity of identified compounds. Seven compounds were identified in the analysis, with acetoin and 2,3-butanediol showing the most intense peaks (Table 8). Regarding the other strains, UFLA03-18 was found to have three VOCs: dimethyl sulfide, 2-nonanone, 2-undecanone, and 2-dodecanone. Strain UFLA02-281 exhibited 2-nonanone, and strain UFLA02-293 showed only 1-undecane. The specifications and potential use in biological disease control are detailed in Table S5 of the supplementary material.

Table 8. Volatile organic compounds identified in nodule endophytic strains by gas chromatography.

Name	CAS	03-10	02-281	02-293	03-18	CN

1-butanol	71 - 36 - 3	x				x
3-hydroxy-2-butanone (acetoin)	513 - 86 - 0	xxx				
Dimethyl sulfide	624 - 92 - 0				x	
Isobutyric acid	79 - 31 - 2	x				
2,3-butanediol	513 - 85 - 9	xx				
2,3-pentanedione	600-14-6	x				
3-methylbutanoic acid	503 - 74 - 2	x				
2-methylbutanoic acid	116 - 53 - 0	x				
Dihydro-2-methyl-3(2H)-thiophenone	13679 - 85 - 1	x				
1-Undecene	821 - 95 - 4			x		
2-nonanone	821-55-6		x		x	
2-undecanone	112 - 12 - 9				x	
2-dodecanone	693 - 54 - 9				x	x

CAS- Chemical Abstracts Service. Each x means that the peak is not very intense, and xxx that the peak is very intense. CN- controle negativo UFLA02-127.

From the chromatograms (Supplementary Material, Fig S1-S5) obtained for the samples, the total ion chromatograms (TIC) were extracted, grouped into a matrix, and aligned, followed by PCA. PCA of all samples yielded a scores plot in which PC1 explained 63.72% of the data and PC2 16.27%. It is observed that sample UFLA03-10 separates from the others, as these samples clustered in the negative region of PC1 and positive region of PC2, while the others grouped in the positive region of PC1 and negative region of PC2 (FIg 7). The PCA indicates that sample UFLA03-10 differs significantly from the other samples, as it produced almost no compounds, and all samples were very close to the control and negative control in the PCA, indicating that the compounds present in the culture medium are also present.

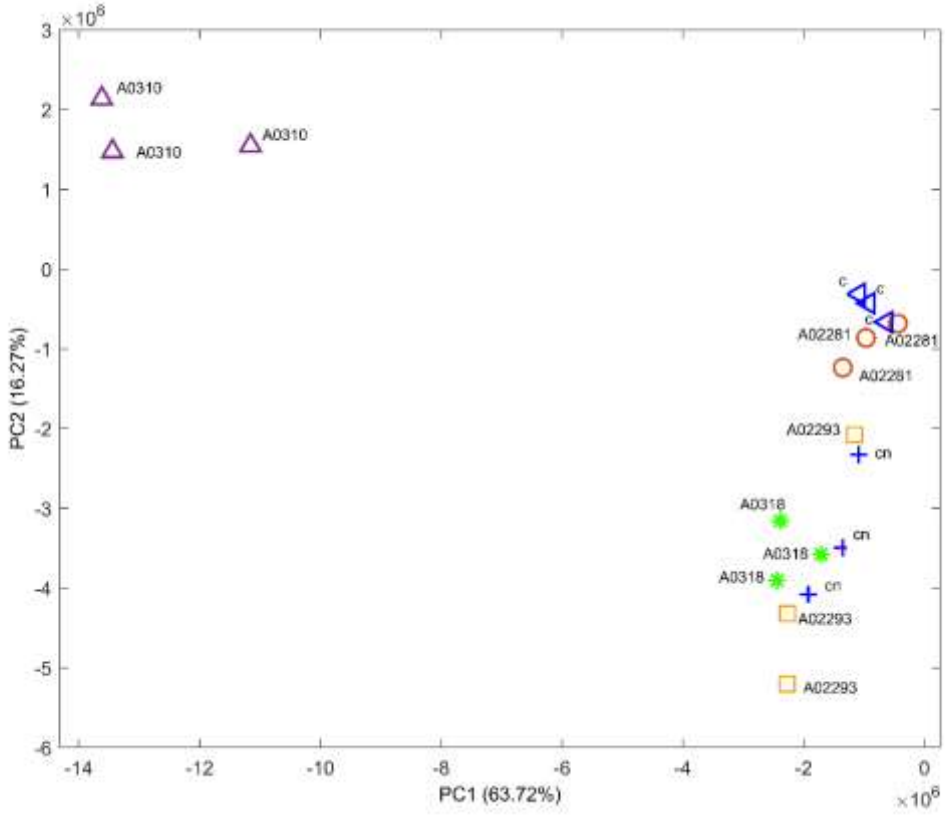

Fig. 6. Principal Component Analysis (PCA) was performed on the chromatograms obtained for the endophytic bacterial strains from nodules, with the total ion chromatograms (TIC) being extracted, grouped into a matrix, and aligned.

4. Discussion

The environment is crucial for microbial interactions. *In vitro* tests can be interesting to observe the behavior of bacteria under more controlled conditions with isolated factors. The methodology and culture medium used can influence the results, so we must keep other types of testing (De Boer et al., 2007; Sivananthan et al., 2013). On the other hand, in soil, many interactions are happening simultaneously. Strain UFLA03-10 was incompatible with fungus CML4019 *in vitro*, however it showed promising results *in vivo* assays when co-inoculated with CML4019, especially regarding disease symptom control in plants.

Co-inoculation with endophytic microorganisms is the primary strategy for combining the benefits that microorganisms associated with plants can generate. However, there must be compatibility and complementarity between them. Once selected endophytic microorganisms start competing with each other, there is an energy loss, and the functions are not performed with a focus on plants (Bogas et al., 2024; Dudeja et al., 2012).

Fungus CML4019 in the assays by Mota et al. (2021) showed *in vitro* control over the fungi causing diseases in common bean *S. sclerotiorum*, *C. lindemuthianum*, and *P. griseola*. Bioassays with bean plants presented the lowest means in disease indexes, mainly for anthracnose. Besides the potential to control diseases, strain CML4019 can be used as a growth-promoting microorganism, as its inoculation in bean plants increased the number of pods per plant to more than 10 (Hayashibara et al., 2022).

Co-inoculation has excellent potential to increase the yield of legume crops with lower production costs than using chemicals. In their work, Sánchez and colleagues (2014) obtained results where the co-inoculation of *Rhizobium pisi* with *Pseudomonas monteilii* produced higher yields and numbers of nodules than inoculated and non-inoculated control plants, demonstrating the potential use of mixed inoculants to improve everyday bean productivity.

In the co-inoculation with rhizobia for nitrogen biological fixation, in the white mold assay, the results showed an affinity between endophytic microorganisms, obtaining dry matter and disease index results equal to or better than nitrogen fertilization. The inoculation of endophytic bacteria UFLA03-10/03-18/02-281/02-293 with strain CIAT899 (*Rhizobium tropici*) has already been performed in other works with positive interaction between their co-inoculation (Ferreira et al., 2020; 2023).

Co-inoculation of rhizobia with strain CML4019 also provided low disease indices for the two cultivars tested. They showed compatibility between the two microorganisms since *Rhizobium*'s effect is nitrogen biological fixation, and CML4019's effect is biological control, combining two essential abilities for promoting plant growth. In contrast, controls inoculated only with *Rhizobium* presented disease indices for the four diseases closer to uninoculated controls, except for the CIAT899 strain control, which presented a low disease index (25.93%) in the white mold assay and cultivar Pérola (53.33%) in the damping-off assay.

Among co-inoculations of the fungus CML4019 with nodular endophytic bacteria strains UFLA03-10/03-18/02-281/02-293, co-inoculation between UFLA02-281 and CML4019 presented the best overall result, with more than 80% reduction in symptoms in tests on at least one of the cultivars tested, highlighting that in the in vitro test the compatibility between the microorganisms was partial. These results are significant as this is the first time that fungi of the genus Induratia have been inoculated with endophytic bacterial strains from nodules. The current production system highlights the use of co-inoculations, as the dynamics between different microorganisms can generate synergism and improve the efficiency of inoculations in the way they are carried out. With greater diversity, we can obtain products ensuring essential functions within the agricultural system (Poveda & Eugui, 2022).

The identified VOCs (Volatile organic compounds), mainly in strain UFLA03-10, can justify microbial activity. However, the lack of these compounds in *Pseudomonas* strains used in this work may determine that the compounds produced by them are not volatile, being of other chemical classifications such as antibiotics and extracellular compounds (Cardozo et al., 2013; Van Der Voort et al., 2015).

Among the identified VOCs, 3-hydroxy-2-butanone (acetoin) identified in UFLA03-10, is widely studied as a probiotic, with its antimicrobial activity caused by competitive exclusion, stimulation of the host's immune modulation defense system, and production of organic acids or hydrogen peroxide that reduce pH, production of antimicrobials such as bacteriocins, antioxidants, production of a signaling molecule that triggers a change in gene expression, also resulting in systemic resistance induction (Lu et al., 2016; Petrov & Petrova, 2021; Monika et al., 2021).

The identification of VOCs for fungal strain CML4019 was performed in previous works, with 21 different types of VOCs identified, classified chemically as alcohols, esters, terpenes, acids, and ketones (Guimaraes et al., 2021)—VOCs produced by *Induratia* spp. strains completely suppressed the growth of the phytopathogenic fungi *R. solani, Cercospora coffeicola*, and *Phoma* sp. They showed a fungicidal effect against *Aspergillus ochraceus* in coffee beans and *Fusarium verticillioides* in corn seeds (Monteiro et al., 2017).

In comparison with the volatile compounds identified in the bacterial strains, only 2-methylbutanoic acid identified in strain UFLA03-10 and 2-nonanone identified in strains UFLA02-281 and UFLA03-18 were similar to those found in strain CML 4019. These two types of volatile compounds can have antimicrobial properties, contributing to the defense of organisms that produce them. The compound 2-nonanone can also be involved in plant defense, acting as a chemical barrier against attacks by pests and pathogens and contributing to natural biological control in agricultural systems (Debassi et al., 2024).

Co-inoculating bacteria with the fungi used in this study proved to be an effective strategy for controlling several important plant diseases, including damping-off, anthracnose, angular leaf spot, and white mold, during the early developmental stages of common bean plants. The results are particularly promising as they demonstrate that the fungal strain CML4019 (*Induratia coffeana*) successfully establishes a symbiotic relationship within the common bean, working synergistically with endophytic bacteria to suppress these diseases. This highlights the potential of microbial interactions in providing more robust protection to plants.

The findings further underscore the importance of using multiple microorganisms in agricultural settings, particularly when they can coexist without adverse effects. This cooperative interaction between fungi and bacteria creates a protective shield for the plant, enhancing its natural defenses without causing harm or competition between the inoculated species.

Credit authorship contribution statement

Raysa Marques Cardoso: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft, Visualization. **Fatima Maria de Souza Moreira:** Conceptualization, Supervision, Methodology, Investigation, Writing - review & editing. **Patrícia Gomes Cardoso:** Conceptualization, Supervision, Writing - review & editing. **Leticia Fagundes:** Investigation and formal analysis. **Marcio Pozzobon Pedroso:** Investigation, formal analysis and review.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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SUPPLEMENTARY MATERIAL

Table S1. List of VOCs identified in UFLA02-281, 02-293, 03-10 and 03-18 with their specification and possible function in the biological control of plant diseases.

Compound Name	Function	Biological Control in Plant Diseases
1-butanol	Solvent, industrial applications	May inhibit the growth of pathogenic fungi and bacteria. Can contribute to disease suppression in plants by promoting the growth of beneficial microorganisms.
3-hydroxy-2-butanone (acetoin)	Flavoring agent, precursor to diacetyl	Can act as a signaling molecule in plants, inducing systemic resistance against pathogens. May enhance plant defenses and contribute to disease resistance.
Dimethyl sulfide	Flavor compound, natural product of microbial metabolism	May have antimicrobial properties and can influence the rhizosphere microbiome, contributing to disease suppression.
Isobutyric acid	Food flavoring, fragrance	Can be involved in the production of volatile organic compounds (VOCs) with antimicrobial properties, contributing to the biological control of plant diseases.
2,3-butanediol	Flavor enhancer, food additive	May have antimicrobial properties and can contribute to the suppression of soil-borne pathogens.
2,3-pentanedione	Flavoring agent, precursor to diacetyl	May have antimicrobial effects and could play a role in inducing systemic resistance in plants.
3-methylbutanoic acid	Fruity and cheesy flavor in food	May participate in the production of VOCs with antifungal properties, contributing to the biological control of plant diseases.
2-methylbutanoic acid	Sweat-like odor, found in some cheeses	May contribute to the production of VOCs with antimicrobial properties, potentially aiding in the suppression of plant pathogens.
Dihydro-2-methyl-3(2H)-thiophenone	Aroma compound, used in flavor and fragrance industry	The release of certain thiophenones by bacteria may have antimicrobial effects, contributing to disease control in plants.

1-Undecene	Used in flavor and fragrance applications	May exhibit antimicrobial properties and contribute to the inhibition of pathogenic microorganisms in the plant environment.
2-nonanone	Fragrance ingredient, solvent	May have antifungal properties, potentially assisting in the control of fungal diseases in plants.
2-undecanone	Fragrance compound	Can contribute to the production of VOCs with antimicrobial properties, potentially participating in the biological control of plant diseases.
2-dodecanone	Fragrance ingredient, flavoring agent	May have antimicrobial effects, potentially contributing to the suppression of plant pathogens.

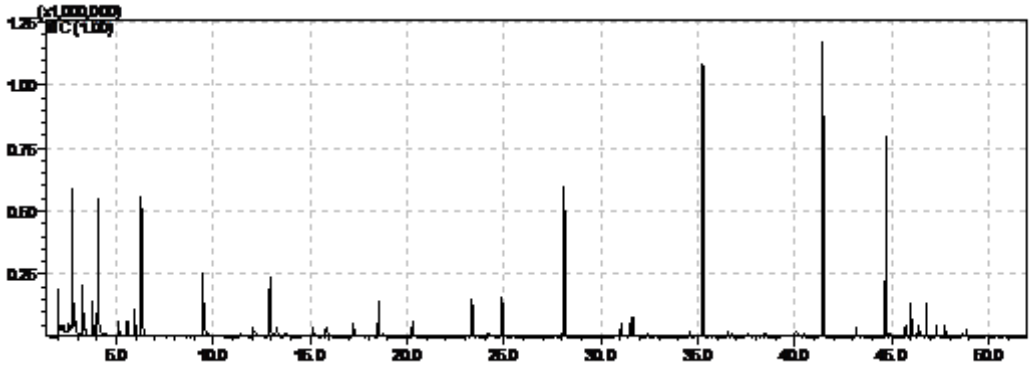

Fig S1. Chromatogram of control volatile compounds.

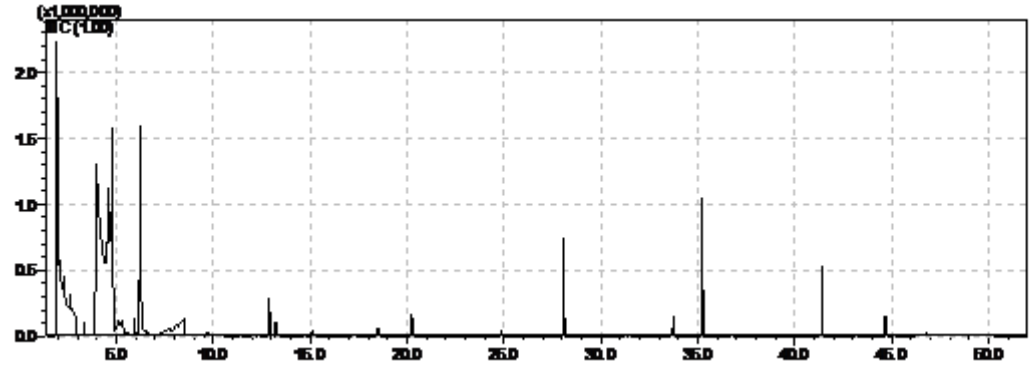

Fig S2. Chromatogram of volatile compounds of strain UFLA03-10

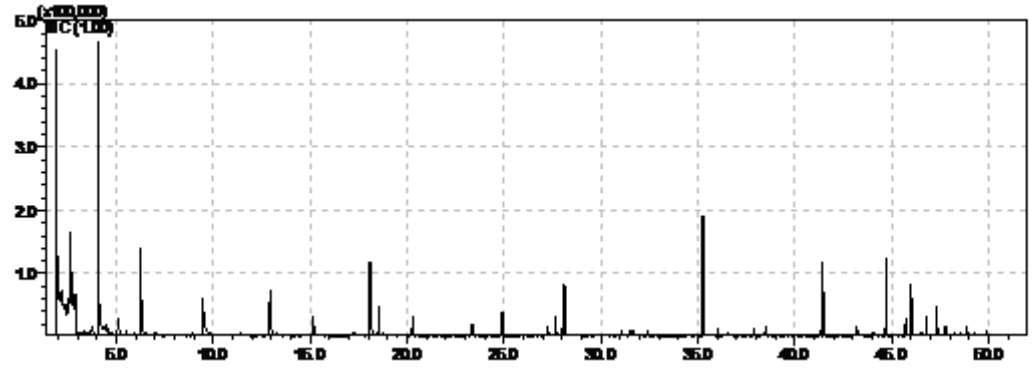

Fig S3. Chromatogram of volatile compounds of strain UFLA02-281

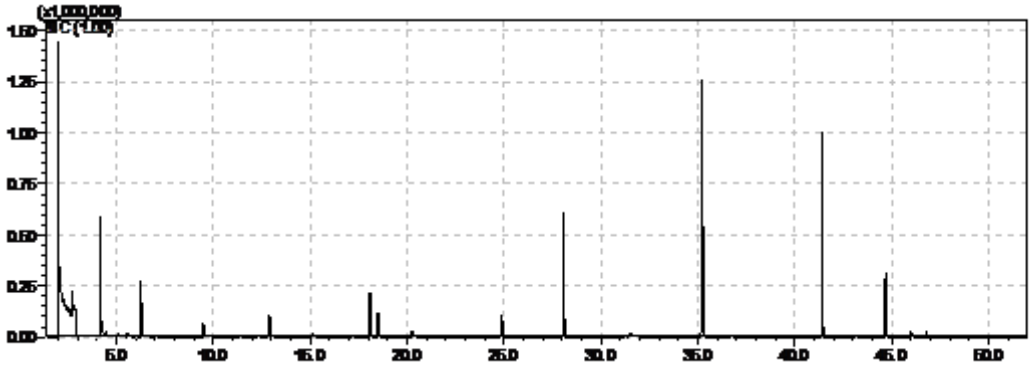

Fig S4. Chromatogram of volatile compounds of strain UFLA02-291

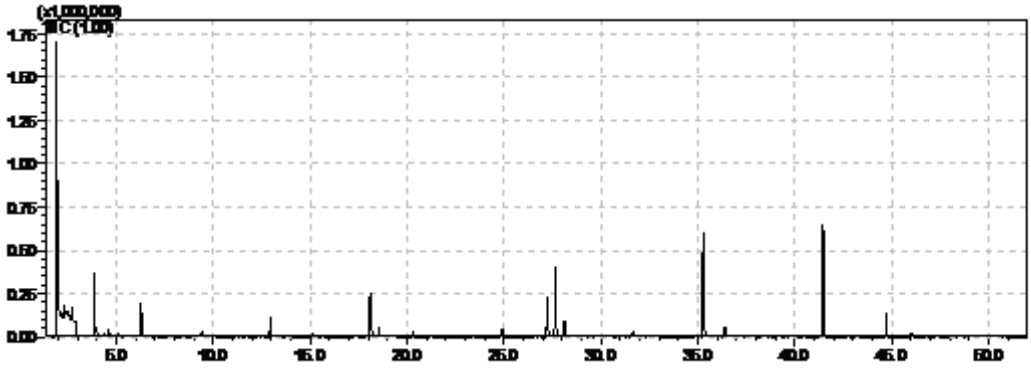

Fig S5. Chromatogram of volatile compounds of strain UFLA03-18

CONSIDERAÇÕES FINAIS

As estirpes endofíticas de nódulos estudadas nesse trabalho pertencem a espécies já descritas, UFLA03-10 (*Paenibacillus peoriae*), UFLA02-281 (*Pseudomonas xanthosomatis*), UFLA02-293 (*Pseudomonas cremoris*) e UFLA03-18 (*Pseudomonas bananamidigenes*). Além da identificação a avaliação genômica também identificou genes e agrupamentos de genes de interesse para a promoção de crescimento em plantas e controle biológico de fitopatógenos. Ao identificar e caracterizar estes genes, podemos desenvolver estratégias para aumentar os efeitos benéficos das bactérias endofíticas nas plantas, levando à melhoria da produtividade das culturas.

A inoculação das estirpes UFLA02-281, UFLA02-293, UFLA03-10 e UFLA03-18 e a co-inoculação com o fungo endofítico CML 4019 (*Induratia coffeana*) proporcionou melhores índices de promoção de crescimento e redução dos sintomas das doenças antracnose, mancha angular, tombamento e mofo branco nas cultivares de feijão utilizadas. No geral, a inoculação e co-inoculação de microrganismos endofíticos representam estratégias promissoras para a agricultura sustentável, oferecendo múltiplos benefícios. Mesmo assim estudos posteriores em campo devem ser desenvolvidos para comprovar a utilização desses microrganismos.

Dadas as preocupações crescentes sobre perdas de colheitas devido a ameaças patogénicas, uma eventual utilização destes microrganismos como bioinoculantes representa uma promessa significativa para a agricultura sustentável. Essa abordagem poderia proporcionar uma alternativa ecológica aos fungicidas químicos, reduzindo a dependência de fatores de produção químicos e, ao mesmo tempo, promovendo produções mais saudáveis e mais resilientes. Ao aproveitar as capacidades naturais dos microrganismos, os agricultores poderiam proteger melhor culturas vitais, como o feijão comum, garantindo tanto a estabilidade do rendimento como a produtividade agrícola a longo prazo.