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AVALIAÇÃO DE ISOLADOS BACTERIANOS DE *Bacillus* PARA PROMOÇÃO DE CRESCIMENTO VEGETAL EM DUAS CULTIVARES DE ALGODÃO

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Tese apresentada à Faculdade de Ciências Farmacêuticas, Alimentos e Nutrição da Universidade Federal de Mato Grosso do Sul, como parte das exigências do Programa de Pós-Graduação em Biotecnologia e Biodiversidade, para obtenção do título de *Doctor Scientiae*
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RESUMO

VIANA, Thianny Fernanda Carrelo, Universidade Federal de Mato Grosso do Sul, Julho de 2024. **AVALIAÇÃO DE ISOLADOS BACTERIANOS DE *Bacillus* PARA PROMOÇÃO DE CRESCIMENTO VEGETAL EM TRÊS CULTIVARES DE ALGODÃO.** Orientadora: Gecele Matos Paggi. Coorientadora: Marivaine da Silva Brasil Mansur Cardoso.

A busca e o entendimento das funções de rizobactérias promotoras de crescimento vegetal e com potencial para biocontrole tem se tornado cada vez mais frequente devido à necessidade de uma agricultura mais sustentável, com menor uso de insumos químicos e que geram maior produtividade. O algodão é uma cultura que necessita de um aporte de nutrientes como nitrogênio, fósforo e potássio que precisam estar disponibilizados no solo para auxiliar diretamente na qualidade da fibra do algodão. Além disso, há necessidade do uso de defensivos químicos para controle de doenças encontradas nesta cultura. A cotonicultura é de grande importância econômica para o país, porém o custo da sua produção é bastante alto. O presente trabalho tem como objetivo isolar bactérias, principalmente do gênero *Bacillus*, de algodoeiro e avaliar os parâmetros de promoção de crescimento vegetal dos isolados encontrados, assim como observar o potencial dos isolados em inibir o crescimento de fungos fitopatogênicos de algodão. Amostras de raízes de algodão foram utilizadas para o isolamento das bactérias. O método de isolamento utilizado foi o de pasteurização, que seleciona isolados produtores de endósporos. Os isolados bacterianos foram incubados em meio Ágar Nutriente, no qual foram repicadas e purificadas para posterior estocagem e avaliações das características de promoção de crescimento vegetal. Dos 54 isolados bacterianos isolados, 46 foram sequenciados, sendo 43 *Bacillus* testados para a solubilização de fosfato de cálcio, a produção de AIA (ácido indol-3-acético), a atividade da CMCase (carboximetilcelulase) e a produção de sideróforos. Os isolados bacterianos obtidos foram submetidos a testes de confronto *in vitro* para verificar o potencial de inibição de crescimento de fitopatógenos de algodão. Os halos ao redor das colônias bacterianas foram medidos e então as taxas de inibição, em porcentagem, foram calculadas. Dos testes bioquímicos para promoção de crescimento vegetal, foram selecionados isolados bacterianos que apresentaram resultados significativos para o ensaio *in vivo* em casa de vegetação com as cultivares de algodão FM 985 e TMG 47. Os tratamentos foram conduzidos usando 1 mL (10^8 UFC/mL) de cultura bacteriana e um inoculante comercial como controle,

sendo que para cada tratamento foram utilizadas nove repetições, observadas por 110 dias. Neste experimento, as plantas foram avaliadas quanto aos parâmetros físicos, fitoquímicos e de macronutrientes. Considerando os parâmetros morfológicos, houve uma relação positiva entre as cultivares e os tratamentos feitos com bactérias, sendo observado um aumento da altura, massa fresca da parte aérea, massa seca de frutos e raízes, volume e comprimento da raiz e número de folhas. Com relação aos parâmetros fitoquímicos, observou-se melhorias nas concentrações de açúcares totais, ascorbato peroxidase, compostos fenólicos, aminoácidos livres, açúcar total, clorofila A, açúcares redutores, flavonoides, catalase e clorofila B. Foi observado que os isolados tiveram potencial como biocontroladores das doenças estudadas e mostraram resultados significativos na inibição de crescimento dos fungos patogênicos *Corynespora cassiicola* e *Fusarium oxysporum* f. sp. *vasinfectum*, com taxas de até 71,89% de inibição do crescimento dos fungos. Através dos resultados obtidos, foi possível concluir que os isolados bacterianos possuem características de promoção de crescimento vegetal, destacando resultado significante no desenvolvimento de raízes, proporcionado pelo isolado P6T32B7, que além deste destaque também obteve resultados significantes nos testes feitos na planta. Este isolado tem potencial para que futuramente sejam realizados testes em campo e possível formulação de produtos.

Palavras-chave: AIA, clorofila, macronutrientes, raízes, Rizobactérias.

ABSTRACT

VIANA, Thianny Fernanda Carrelo, Federal University of Mato Grosso do Sul, July 2024. **EVALUATION OF BACTERIAL ISOLATES OF *Bacillus* FOR PROMOTING PLANT GROWTH IN THREE COTTON CULTIVARS.** Advisor: Gecele Matos Paggi. Co-supervisor: Marivaine da Silva Brasil Mansur Cardoso.

The search for and understanding of the functions of rhizobacteria that promote plant growth and have the potential for biocontrol has become increasingly frequent due to the need for more sustainable agriculture, with less use of chemical inputs and which generates greater productivity. Cotton is a crop that requires a supply of nutrients including nitrogen, phosphorus, and potassium that must be available in the soil for better cotton fiber quality. Furthermore, there is a need to use chemical pesticides to control diseases found in this crop. Although cotton farming is of great economic importance for the country, its production cost is quite high. The present work aims to isolate bacteria of the genus *Bacillus* from cotton and evaluate the plant growth promotion parameters by the isolates found in the species, as well as observe the potential of the isolates to inhibit the growth of phytopathogenic cotton fungi. Cotton root samples were used to isolate the bacteria. The isolation method used was pasteurization, which selects endospore-producing isolates. The bacterial isolates were incubated in Nutrient Agar medium, in which they were subcultured and purified for subsequent storage and evaluation of plant growth promotion characteristics. 54 bacterial isolates were obtained, and 46 were sequencing, these 43 of which were *Bacillus* tested for calcium phosphate solubilization, IAA (indole-3-acetic acid) production, CMCase (carboxymethylcellulase) activity, and siderophore production. The bacterial isolates obtained were subjected to in vitro confrontation tests to verify their potential to inhibit the growth of cotton phytopathogens. The halos around the bacterial colonies were measured and then the inhibition rates, in percentage, were calculated. From the biochemical tests to promote plant growth, bacterial isolates were selected that showed better performance for the in vivo test in a greenhouse with the cotton cultivars FM 985 and TMG 47. The treatments were carried out using 1 mL (10^8 CFU/mL) of bacterial strain culture and a commercial inoculant as control, with nine replicates used for each treatment, observed for 110 days. In the experiment, the plants were evaluated for morphological, phytochemical, and macronutrient parameters. Considering the morphological parameters, there was a positive relationship between

the cultivars and the treatments made with bacteria, with an increase in height, fresh mass of the aerial part, dry mass of fruits and roots, volume and length of the root, and number of leaves. Regarding phytochemical parameters, improvements were observed in the concentrations of total sugars, ascorbate peroxidase, phenolic compounds, free amino acids, total sugar, chlorophyll A, reducing sugars, flavonoids, catalase, and chlorophyll B. The bacterial isolates showed potential in inhibiting the growth of the pathogenic fungi *Corynespora cassiicola* and *Fusarium oxysporum* f. sp. *vasinfectum*, with rates of up to 71.89% inhibition of fungal growth. Through the results obtained, it was possible to conclude that the isolates have characteristics that promote plant growth, highlighting the P6T32B7 isolate that obtained the best results in tests carried out on the plant, showing potential for future tests to be carried out in the field. This isolate has the potential for future field testing and possible product formulation.

Keywords: AIA, chlorophyll, macronutrients, roots, Rhizobacteria.

INTRODUÇÃO GERAL

1 O algodão: *Gossypium hirsutum* L.

1.1 Caracterização vegetal, aspectos históricos e comerciais

As espécies de algodão são dicotiledôneas, pertencentes à família Malvaceae e ao gênero *Gossypium* spp., que compreende mais de 50 espécies distribuídas em cinco continentes (Barros et al., 2020). As quatro espécies cultivadas consistem em duas diploides (*G. arboreum* L. e *G. herbaceum* L.) e duas tetraploides (*G. barbadense* L. e *G. hirsutum*), sendo que *G. hirsutum* raça *latifolium* Hutch. tem a maior participação, representando cerca de 95% da produção mundial (Tlatlaa et al., 2023). Esta espécie apresenta flores que variam da coloração roxa à branca, semente com aspecto oval comumente chamada de "*boll*".

O algodoeiro é uma cultura de grande importância para o mercado agrícola mundial (CONAB, 2023). Historicamente, o uso do algodão pelo homem possui evidências em diferentes regiões do mundo. Estudos arqueológicos revelaram que algumas civilizações antigas já utilizavam o algodão como matéria-prima por volta de 4.500 a.C na América do Sul (Tlatlaa et al., 2023). No Brasil, a espécie *Gossypium barbadense* já era cultivada pelos indígenas antes da chegada dos portugueses em 1500, e é bem provável que a espécie *G. hirsutum* var. *Marie Galante* (Watt) Hutch também tenha sido introduzido antes no nordeste do país, vindo da América Central (Arriel et al., 2023). Entretanto, a espécie endêmica silvestre do nordeste brasileiro, *G. mustelinum*, nunca foi relatada como utilizada nos cultivos (Barroso et al., 2021).

No século XVI, o algodão arbóreo (*G. barbadense*) já vinha sendo cultivado comercialmente no nordeste do Brasil, especialmente no Maranhão e na Bahia, como atividade complementar dos agricultores (Freire, 1998). Com a Revolução Industrial na Inglaterra e seu incentivo para que o Brasil produzisse algodão bruto, ocorreu a transformação do algodão em uma cultura de exportação entre 1812 e 1821. Esses eventos contribuíram para um ciclo econômico no país que estimulou o início do processo de industrialização (Arriel et al., 2023). No final do século XVIII, o Maranhão destacou-se como o maior produtor e exportador de algodão no Brasil, levando à instalação da primeira fábrica em 1855 (Amaral, 1958). Em 1916, foi relatado que o

algodoeiro cultivado no Nordeste era predominantemente o herbáceo *G. hirsutum* (Torrend, 1916). Após a redução da produção de algodão pelos Estados Unidos devido à Guerra de Secessão no final do século XIX, houve um aumento na demanda por algodão para abastecer a indústria têxtil inglesa, o que impulsionou a instalação de vários moinhos nas regiões de cultivo (Menezes et al., 2010; Arriel et al., 2023).

Com a expansão da cultura do algodoeiro no Brasil, ele passou a contribuir de maneira significativa para a produção agrícola mundial e no último século, foi um importante exportador de fibra de algodão (Barros et al., 2020). Atualmente, a cultura do algodão ocupa uma posição relevante no mercado agrícola brasileiro, uma vez que possui versatilidade no agronegócio, pois toda a planta é aproveitada, desde a sua fibra na indústria manufatureira até o caroço utilizado para alimentação bovina (Alves et al., 2023). Na safra 2022/2023, o Brasil produziu 3,150 milhões de toneladas de algodão, representando um aumento de 23,3% em relação à safra de 2021/2022 (CONAB, 2023). A área plantada no país totalizou 1,663 milhões de hectares, um crescimento de 4% em relação ao ano anterior (CONAB, 2023). O cultivo de algodão concentra-se principalmente na região do Cerrado, com os estados de Mato Grosso e Bahia respondendo por cerca de 90% da área cultivada (CONAB, 2024).

As plantas cultivadas têm como produto, a partir dos frutos, a pluma que é usada principalmente na indústria têxtil (Kumar et al., 2021). Comparada às fibras sintéticas e artificiais, a fibra do algodão é considerada a mais importante no mundo (Ferreira et al., 2022). Na safra de 2021/2022, foram exportadas cerca de 1,7 milhões de toneladas de pluma para a Ásia, e na safra de 2023/2024, o Brasil superou 2,6 milhões de toneladas destinadas à exportação (ABRAPA, 2023).

Atualmente, o Mato Grosso é o principal produtor de pluma, com 2,2 milhões de toneladas colhidas, respondendo por 68,8% da produção nacional, seguido pela Bahia e pelo Mato Grosso do Sul (ABRAPA,2023). Em termos de produção, o país ocupa a 3ª posição no ranking mundial, atrás da China e da Índia. Quanto às exportações, o Brasil é o segundo maior fornecedor, com participação de 25% do total, valor próximo ao dos Estados Unidos, que é o líder mundial. Essa importância no mercado global se reflete na economia brasileira, e as vendas externas movimentam cerca de US$3,3 bilhões por ano (Neves et al., 2024).

Além do uso da pluma para a tecelagem de tecidos, a cultura do algodão pode

também oferecer a semente (caroço) que pode ser utilizada na fabricação de ração para alimentação animal (Soares Severino et al., 2019; Xu & Zhang, 2022). O caroço do algodão tem alta concentração de óleo, por isso, a maior parte do caroço é destinado à produção de biodiesel. A casca do algodão, o principal subproduto da cadeia produtiva, é usado como ração em dietas para ruminantes (Eiras et al., 2016). Já a torta de algodão, outro subproduto, começou a ser também usado como concentrado proteico na dieta de ruminantes, principalmente em países tropicais (Getu et al., 2020; Tipu et al., 2021).

Portanto, o algodão tem grande importância para o Brasil, sendo utilizado como um insumo de destaque tanto no âmbito social quanto econômico, pois gera desenvolvimento no local de sua produção devido a cadeia produtiva complexa e de alto valor que possui. O algodão é considerado uma *commodity*, pois a migração da produção de áreas habitualmente produtoras para a região do Cerrado brasileiro permitiu que o país se tornasse um exportador da pluma (Ferreira et al., 2022). A cotonicultura, que é a produção da fibra de algodão, é uma das atividades agrícolas mais importantes do mundo, sendo uma prática comum em mais de cem países, e o Brasil tem ficado nos últimos cinco anos no mesmo nível que outros países produtores, como China, Índia, EUA e Paquistão, sendo que esta cultura ocupa todos os anos cerca de 35 milhões de hectares de área plantada no mundo todo (ABRAPA, 2021; Usda, 2018).

1.2 Uso de agroquímicos na cultura do algodão

A cultura do algodoeiro no Brasil enfrenta grandes problemas devido ao clima tropical, que favorece o crescimento de muitos microrganismos fitopatogênicos, causando doenças relacionadas a fatores críticos que limitam a produtividade dessa cultura (Alcantara et al., 2023). Além disso, para alcançar uma alta produtividade, ainda há a exigência do uso de insumos e técnicas de manipulação do solo que podem gerar danos ambientais (Yang et al., 2020). Como por exemplo, técnicas de cultivo convencionais que deixam o solo nu e mais suscetível à erosão (Acosta-Martinez et al., 2023), o uso desequilibrado de nutrientes e fertilizantes que além de serem prejudiciais à saúde do solo, podem afetar o rendimento da cultura do algodão (Kumari et al., 2023).

O algodoeiro assim como outras plantas cultiváveis sofrem pressão com relação ao aparecimento de doenças e pragas, mas por ser uma planta cultivada, há a disponibilidade de variedades que possuem genes de resistência a determinados tipos de pragas (Kumar et al., 2021). Uma das primeiras variedades de algodão transgênico se

destaca o algodão Bt, que apresenta um gene da bactéria *Bacillus thurigiensis*, com potencial de produzir uma proteína inseticida, que promove resistência à praga lagarta-do-algodoeiro (Khan et al., 2023; Razzaq et al.,2023). Também pode-se destacar o uso do controle biológico, que está recebendo cada vez mais atenção como meio alternativo de controle de doenças, tanto pré como no pós- colheita (Collinge et al., 2022). O uso de bactérias do gênero *Bacillus* tem se destacado por produzirem composto voláteis que podem controlar o crescimento de *Verticillium dahliae*, uma praga com grande importância econômica e considerada o "câncer do algodão" (Zhang et al., 2023). O gênero também tem se apresentado um grande aliado no combate às doenças de origem fúngica no algodão causadas pelo gênero *Fusarium* (Abdelmoteleb et al., 2022).

Para a produção do algodão há uma exigência da planta quanto à disponibilização dos nutrientes, como por exemplo a presença de nitrogênio, fósforo e potássio no solo, que pode interferir diretamente na produção e na qualidade da fibra (dos Dias & Santos, 2023). Visando diminuir o uso de insumos químicos, tecnologias que envolvam a utilização de microrganismos que conseguem disponibilizar esses nutrientes e atuarem como controle biológico de doenças, são de interesse ambiental e econômico, já que o custo dos insumos químicos, incluindo aqui fertilizantes e defensivos, é relativamente elevado, chegando a aproximadamente 5.000,00 R$/ha nas diferentes regiões produtoras do Brasil (Silva e Nonnenberg, 2023).

1.3 Bactérias promotoras de crescimento vegetal

1.3.1 Promoção de crescimento vegetal direto

No solo, associado às plantas, existem microrganismos que produzem diferentes tipos de substâncias que auxiliam no crescimento e no desenvolvimento vegetal. Entre esses microrganismos estão as rizobactérias, que podem habitar a rizosfera, bem como os espaços internos e externos das raízes, promovendo o crescimento de plantas. Estas bactérias que auxiliam no crescimento vegetal são comumente conhecidas como bactérias promotoras de crescimento vegetal (BPCV; Lopes et al., 2021).

Dentre as características promotoras das BPCV estão a fixação biológica de nitrogênio, a solubilização de fosfatos, a produção de fitohormônios e sideróforos

(Eldos et al., 2024; Cassán et al., 2020). Tais microrganismos têm se mostrado fundamentais para o desenvolvimento de bioinsumos visando a promoção de crescimento vegetal (Fiuza et al., 2023).

Diversas espécies bacterianas podem ser consideradas promotoras de crescimento vegetal. Como exemplo, bactérias diazotróficas, que incluem o gênero *Azospirillum*, contribuem para o desenvolvimento da planta através da fixação biológica de nitrogênio, na produção de fitormônios e solubilização de fosfatos (Cassán et al., 2020). Outras espécies de bactérias também têm se destacado capacidade em melhorar o desenvolvimento de raízes, levando a um aumento da biomassa são *Azotobacter, Pseudomonas* e *Bacillus* (Minut et al., 2022).

Bacillus tem se destacado na promoção de crescimento vegetal e entre as bactérias usadas na agricultura como bioinsumos (Bini et al., 2024). Estas bactérias são gram-positivas, possuem formato de bastonete e são capazes de formar endósporos, que possibilitam a sobrevivência em diferentes ambientes estressantes (Poveda e González-Andrés, 2023).

O gênero *Bacillus* tem se mostrado bastante eficiente na solubilização de fosfato de diferentes fontes inorgânicas, promovendo maior absorção pelas raízes das plantas, inclusive em algodão (Ahmad et al., 2021; Alaylar et al., 2020; Barrera-Ortiz, 2023). Uma das razões para isso é a capacidade de diversas espécies e cepas desse gênero em produzir diferentes ácidos orgânicos que atuam na solubilização de fosfato pela troca iônica ou como quelantes dos cátions ligados aos fósforos, como Al, Ca ou Fe (Alori et al., 2017; Ahmad et al., 2022). A liberação de prótons pelas células microbianas durante a assimilação de amônio ou a respiração microbiana também pode contribuir para a solubilização de fosfato inorgânico (Rawat et al., 2021).

O gênero *Bacillus* também pode realizar a mineralização de fosfato orgânico pela ação das enzimas fosfatases e fitases secretadas por este gênero (Zhao et al., 2022; Torres et al., 2024). Essas enzimas hidrolisam o fósforo orgânico total no solo, disponibilizando-o para as plantas e para a própria comunidade microbiana (Rawat et al., 2021).

O fósforo é uma parte do difosfato de adenosina (ADP) e do trifosfato de adenosina (ATP), que é essencial para a transformação e armazenamento de energia (Ahmad et al., 2022). Também está envolvido na formação de ácido nucleicos,

formação da membrana das células, fotossíntese das plantas, respiração, e na regulação de algumas enzimas, como por exemplo as quinases, que estão envolvidas na transferência dos grupos fosfato para onde for necessário (Ma et al, 2023). Neste sentido, ambientes agrícolas recebem cada vez mais fertilizantes ricos em fósforo, porém este nutriente apesar de estar abundante no solo e na água, não se encontra de forma assimilável no ambiente, pois a solubilidade do P é ditada por várias reações químicas e interações biológicas que ocorrem no solo (Mendoza-Arroyo et al., 2020).

Outra característica importante de *Bacillus* promotores de crescimento vegetal é a capacidade de produzir auxinas, como por exemplo, o ácido indol-3-acético (AIA), que é uma das principais moléculas que contribuem para o desenvolvimento das plantas (Mahdi et al., 2020; Galeano et al., 2021). Essas substâncias podem melhorar o desenvolvimento de raízes secundárias, aumentando a captação de nutrientes e água e, consequentemente, promovendo o crescimento vegetal (Zerrouk et al., 2020). As cepas de *Bacillus* também podem acelerar e aumentar as taxas de germinação de sementes (Sosa-pech et al., 2019), atuando também na regulação dos fitormônios em plantas (Wang et al., 2021). Por exemplo, em condições de estresse abiótico, como seca, altas temperaturas, salinidade e metais pesados, as plantas respondem aumentando a síntese de etileno que modula os seus transcritos para responder ao estresse (Glick, 2014; Kour et al., 2024). Entretanto, quando essas condições estressantes perduram, os altos níveis de etileno nos tecidos vegetais podem causar efeitos prejudiciais às plantas, como senescência e abscisão de órgãos e tecidos vegetais, inibindo o crescimento vegetal ou até mesmo à morte prematura (Glick, 2014; Gamalero et al., 2023). Algumas cepas de *Bacillus* são conhecidas por produzir a enzima 1-aminociclopropano-1-carboxilato (ACC) deaminase, que pode clivar o precursor do etileno, o ACC, nos tecidos vegetais (Etesami et al., 2023; Gamalero et al., 2023). Esse processo regula os níveis de etileno, mitigando seus efeitos adversos e melhorando a tolerância das plantas aos estresses ambientais (Kour et al., 2024).

1.3.2 Promoção de crescimento vegetal indireto

Além dos mecanismos diretos de promoção de crescimento vegetal, existem bactérias que podem promover o crescimento vegetal de forma indireta pelo controle do crescimento de fitopatógenos. Isolados do gênero *Bacillus*, por exemplo, vem se destacando como um Agente de Controle Biológico (ACB), pois são organismos considerados seguros e por serem distribuídos de maneira equilibrada no ambiente e

conseguem ter competitividade com microrganismos patogênicos presentes na rizosfera (Khan et al., 2022).

Espécies de *Bacillus*, por exemplo, têm sido estudadas como importantes produtoras de sideróforos em diferentes ensaios, com habilidade de sequestrar e solubilizar ferro, além da capacidade de inibir o crescimento de fitopatógenos (Sebastian et al., 2021; Ghazy e Elnahrawy, 2021), que ao sequestrar o ferro, este sideróforos bacterianos inibem o crescimento de patógenos ao privá-los desse elemento (Deb et al., 2024). Logo, essas moléculas de baixo peso molecular contribuem para a promoção indireta do crescimento vegetal, no controle de doenças (Ghosh et al., 2020) e tem recebido atenção por sua aplicação em diferentes ramos da agronomia, como ciência do solo, patologia de plantas e ciência ambiental (Ghazy e El-Nahrawy, 2021).

Além disso, muitas espécies de *Bacillus* possuem mecanismos de adaptação a ambientes desfavoráveis, como por exemplo a formação de endósporo, que pode proporcionar maior resistência a ambientes com escassez de água (Beskrovnaya et al., 2021). O controle biológico, realizado pelas bactérias, pode ocorrer tanto pela produção de compostos antimicrobianos quanto pela utilização de vias metabólicas, como a produção de enzimas que degradam a parede celular e que podem inibir o crescimento de patógenos (Tan et al., 2019; Les et al. (2020).

Bacillus podem produzir as enzimas N-acetil-β-D-glucosaminidases (NAGases), endoquitinases, β-1,3-glucanases e proteases, e inibir a germinação de esporos e alongamento do tubo germinativo de fungos patogênicos (Brzezinska et al., 2020). Recentemente, estudos demonstraram que a ação de quitinase, β-1,3-glucanase e protease de *B. velezensis* CE100 foi responsável pelo controle dos patógenos *Pestalotiopsis maculans* (agente causal da mancha foliar em carvalho japonês), *Macrophomina phaseolina* (agente causal da podridão do carvão) e *Fusarium oxysporum* f. sp. *fragariae* (agente causal da murcha de fusarium), aumentando a sobrevivência de mudas de morango e de carvalho japonês (Won et al., 2021; Hong et al., 2022). Na pesquisa de Liu et al. (2023) utilizando uma cepa de *B. amyloliquefaciens* YZU-SG146, eles demonstraram que a cepa produziu celulases, proteases e sideróforos e foi capaz de controlar a mancha de *Verticillium* e promover o crescimento do algodoeiro.

Além disso, existem outras doenças, como a mancha-alvo que é causada pelo

fungo *Corynespora cassiicola* e é caracterizada pela presença de lesões irregulares na folha com aneis concêntricos que se assemelha a um alvo e quando não são bem administradas podem gerar uma perda de até $15 milhões (Moore et al., 2021). Outra doença que também pode afetar outras culturas é *Fusarium oxysporum*, este fungo causa a murcha-de- fusarium, que é uma murcha vascular, especialmente o xilema, causando um apodrecimento da raiz, a sintomas de descoloração vascular, clorose, murcha e morte da planta (Diaz et al., 2021).

Tendo em vista as doenças causadas por fitopatógenos, há uma necessidade da busca de alternativas para o controle destes agentes patogênicos. Dentre as possibilidades, a produção de compostos voláteis pode ser considerada como um fator antimicrobiano ideal, visto que não há necessidade do contato físico entre o agente de biocontrole e o patógeno para a atuação de sua atividade (Contarino et al., 2019). Cepas do gênero *Bacillus* podem ser capazes de produzir substâncias como antifúngicos, que podem controlar o crescimento de fungos, sendo uma alternativa sustentável para evitar os impactos causados por agroquímicos, que tem altos custos, além disso impactam o meio ambiente (Ocegueda-Reyes et al., 2019). Além disso, a atuação no biocontrole de fitopatógenos também pode ser realizada pela competição por espaço e nutrientes na rizosfera, produção de ácido cianídrico (HCN), amônia e biossurfactantes e outros mecanismos que ainda estão sendo estudados (Eldos et al., 2024).

1.4 Inoculação com bactérias promotoras de crescimento vegetal

O processo de inoculação de plantas (sementes) com BPCV pode ser uma alternativa para um significativo desempenho da cultura, devido à capacidade dessas bactérias produzirem compostos que auxiliam no desenvolvimento vegetal (Escobar Diaz et al., 2019; Lopes et al., 2021). Cepas bacterianas promotoras de crescimento vegetal estão sendo sempre descobertas com o objetivo de produzir insumos biologicamente sustentáveis para serem comercializados, incluindo muitas cepas do gênero *Bacillus* (Backer et al., 2018). Um inoculante contendo cepas *Bacillus subtilis* (CNPMS B2084) e *Bacillus megaterium* (CNPMS B119) foi formulado e recomendado em culturas da soja, por promover a solubilização de fósforo e aumentar a produtividade em até 7,8%, ressaltando que este ganho provocado pela inoculação chega a ser dez vezes superior ao custo com a aplicação do produto (Oliveira-Paiva et al., 2021).

Apesar de atualmente serem encontrados inúmeros produtos que promovem o

crescimento vegetal, seja por esse microrganismo produzir reguladores de crescimento vegetal ou por fazer o biocontrole de fitopatógenos, há ainda muitos fatores que devem ser elucidados, como os mecanismos de interação entre eles, assim, todo o potencial das BPCV existente precisa ser mais bem estudado para que a escolha de estratégias adequadas resulte no sucesso da utilização desta nova alternativa em biotecnologia. Além disso, o uso de BPCV pode ser mais vantajoso economicamente e com baixo impacto ambiental, podendo reduzir custos com fertilizantes químicos (Gaspareto et al., 2023).

Objetivo Geral

Avaliar o potencial de isolados bacterianas do gênero *Bacillus* spp. em relação a promoção do crescimento e potencial de inibição *in vitro* dos fitopatógenos *Corynespora cassiicola* e *Fusarium oxysporum* em plantas de algodão.

Objetivos específicos

I. Isolar bactérias do gênero *Bacillus* de raízes de algodão;
II. Realizar testes *in vitro* para detectar características relacionadas à promoção de crescimento vegetal dos isolados bacterianos;
III. Avaliar a capacidade dos isolados bacterianos selecionados em promover o crescimento *in vivo* em plantas de algodão;
IV. Estimar o potencial provocado pela inoculação através de análises físico-químicas e fitoquímicas;
V. Analisar *in vitro* o potencial de inibição de crescimento frente aos fitopatógenos *C. cassiicola* e *Fusarium oxysporum*;

Capítulo I

Potential of cotton (*Gossypium hirsutum* L.) *Bacillus* isolates to promote plant growth

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Potential of cotton (*Gossypium hirsutum* L.) *Bacillus* isolates to promote plant growth

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ABSTRACT: Cotton is socially and economically important worldwide. Notably, research on eco-friendly technologies that increase the sustainability of this production system is increasing. We explored the potential of bacterial strains isolated from cotton roots to promote plant growth. Bacteria were isolated from cotton roots by using a pasteurization method and cultivated on nutrient agar for strain purification and selection. The bacterial strains were subjected to *in vitro* tests to verify their ability to solubilize calcium phosphate, producing indole-3-acid acetic, siderophores, and cellulase. Potential strains were selected for the *in vivo* experiments in a greenhouse with cotton cultivars FM 985 and TMG 47. Treatments were conducted using 1 mL (10^8 CFU/mL) of bacterial strain culture and a commercial inoculant, with nine replicates observed for 110 d. The plants were evaluated for physical, phytochemical, and macronutrient parameters. The morphological parameters indicated a positive relationship between cultivars and microorganisms, increasing the height, fresh mass of the shoot, dry mass of fruits and roots, root volume and length, and number of leaves. Phytochemical parameters showed improvements in total sugars, ascorbate peroxidase, phenolic compounds, free amino acids, total sugar, chlorophyll A, reducing sugars, flavonoids, catalase, and chlorophyll B. In conclusion, our results showed that our bacterial isolates from cotton roots had many biochemical features related to plant growth promotion and were able to promote the growth of cotton cultivars in a greenhouse experiment. Future research could conduct field tests to evaluate their use for improving cotton productivity on a large scale.

Key words: microorganism, physical parameters, phytochemical parameters, root volume, solubilizing calcium phosphate.

Potencial dos isolados de *Bacillus* do algodão (Gossypium hirsutum L.) para promover o crescimento de plantas

RESUMO: O algodão é uma cultura social e economicamente importante em todo o mundo. Notavelmente, a investigação sobre tecnologias amigas do ambiente que aumentam a sustentabilidade deste sistema de produção está aumentando. Exploramos o potencial de cepas bacterianas isoladas de raízes de algodão para promover o crescimento das plantas. As bactérias foram isoladas de raízes de algodão usando um método de pasteurização e cultivadas em ágar nutriente para purificação e seleção de cepas. As cepas bacterianas foram submetidas a testes *in vitro* para verificar sua capacidade de solubilizar fosfato de cálcio, produzindo ácido indol-3-acético, sideróforos e celulase. Cepas potenciais foram selecionadas para experimentos *in vivo* em casa de vegetação com cultivares de algodão FM 985 e TMG 47. Os tratamentos foram conduzidos utilizando 1 mL (108 UFC/mL) de cultura de cepas bacterianas e um inoculante comercial, com nove repetições observadas por 110 dias. As plantas foram avaliadas quanto a parâmetros morfológicos, fitoquímicos e macronutrientes. Os parâmetros físicos indicaram relação positiva entre cultivares e microrganismos, aumentando a altura, massa fresca da parte aérea, massa seca de frutos e raízes, volume e comprimento de raízes e número de folhas. Os parâmetros fitoquímicos mostraram melhorias nos açúcares totais, ascorbato peroxidase, compostos fenólicos, aminoácidos livres, açúcar total, clorofila A, açúcares redutores, flavonoides, catalase e clorofila B. Em conclusão, nossos resultados mostraram que nossos isolados bacterianos de raízes de algodão tinham muitas características bioquímicas relacionadas à promoção do crescimento vegetal e foram capazes de promover o crescimento de cultivares de algodão em

experimento em casa de vegetação. Pesquisas futuras poderiam realizar testes de campo para avaliar seu uso para melhorar a produtividade do algodão em larga escala.

Palavras-chave: microrganismo, parâmetros físicos, parâmetros fitoquímicos, volume radicular, solubilização de fosfato de cálcio.

INTRODUCTION

Cotton (G*ossypium hirsutum* L.) is an agricultural commodity of substantial social and economic importance worldwide (XU & ZHANG, 2022). The main reason for its importance is that cotton cultivation is developed in locations where cotton is produced because of the complex, high-value production chain, such as cotton feathers in weaving fabrics and cotton seeds for animal feed (SOARES et al., 2022). Cotton cultivation is one of the most important agricultural activities for fiber production. Since 2016, Brazil has had the same amount of production as the largest producers, such as China, India, the United States, and Pakistan, and cotton cultivation has occupied approximately 35 million ha of planted area worldwide (ABRAPA, 2023). Mato Grosso comprises a large part of the cotton cultivation area, with 30% of companies in Brazil in this business (DA SILVA JÚNIOR et al., 2023).

Brazil's high productivity in cotton cultivation is facilitated by its high use of chemical products (MENG et al., 2019; NISAREN et al., 2022), soil plowing techniques, and the absence of crop rotation, which affect soil health, namely the degradation of the physical, chemical, and biological components of the soil (YANG et

al., 2019). To minimize the negative effects of agriculture, researchers are exploring methods based on the inoculation of beneficial microorganisms into plants (DIAZ-VALLE et al., 2019). Plant Growth-Promoting Rhizobacteria (PGPR; LOPES et al. 2021) can directly stimulate crop growth through indole compound production, phosphate solubilization, siderophore production, and enzyme production and can colonize the rhizosphere and stimulate plant growth and tolerance to abiotic stresses. Among PGPR, the genus *Bacillus* is one of the most studied, it is frequently isolated from the rhizosphere and within plant tissues and can promote plant growth even in environments subjected to environmental stress (HASHEM et al., 2019; MILJAKOVIĆ et al., 2020; NARAYANASAMY et al., 2020; MANASA et al., 2021; BARRERA-ORTIZ et al., 2023; ZHU et al., 2023).

Inoculation technology using bacteria that promote plant growth is a relevant strategy for achieving sustainability in agriculture because it does not cause environmental damage (CARVALHO et al., 2016). HUNGRIA et al. (2016) reported that approximately 16 companies produce commercial inoculants in Brazil. In *Bacillus* species, BETTIOL et al. (2022) showed that some strains of *Bacillus amyloliquefaciens, Bacillus licheniformis, Bacillus methylotrophicus, Bacillus pumilus, Bacillus subtilis*, and *Bacillus velezensis* represent bioproducts registered by the Brazilian Ministry of Agriculture. Some studies have shown that *Bacillus* efficiently solubilizes phosphate from different inorganic phosphate sources and promotes an increase in cotton roots (ALAYLAR et al., 2020; AHMAD et al., 2021). The *Bacillus* genus is one of the most abundant in root environments. Its strains are considered PGPR owing to their ability to increase nutrient solubility in agricultural soils (SAEID et al. 2018). Research on *Bacillus* and its benefits provides an opportunity to design strategies for developing PGPR for sustainable agriculture (FRANCO-SIERRA et al., 2020). This PGPR

efficiently produces siderophores and phytohormones; they are responsible for improving plant root development (WAGI & AHMED, 2019; SHAH et al., 2020) and regulating plant physiological processes (JIANG et al., 2019), helping establish plants in agricultural systems.

Understanding the mechanisms of interaction between microorganisms and plants and the full potential of PGPR might improve agriculture's use of this alternative biotechnological tool. Therefore, PGPR promotes plant growth and is a profitable and environmentally friendly tool. PGPR are environmentally friendly because they are natural inhabitants of these environments, the rhizosphere of cultivated and uncultivated plants. Therefore, our goal is to evaluate the potential of *Bacillus* spp. Strains isolated from cotton (*Gossypium hirsutum*) were used to promote plant growth by analyzing several biochemical features of the isolates and their ability to promote the plant growth of a cotton cultivar *in vivo*.

MATERIAL AND METHODS

Plant sampling

Twenty-four cotton root samples (*Gossypium hirsutum* L.) were obtained from three plots in an experimental area in Sapezal, Mato Grosso, Brazil (58° 68' 79" E and 13° 26' 34" N). Samples were identified, placed in sterile plastic bags in a thermal box, and sent to the Microbiology and Genetics Laboratory at the Federal University of Mato Grosso do Sul, Pantanal *Campus*, Corumbá, Mato Grosso do Sul, Brazil.

Bacteria isolation

Ten grams of roots from each sample were mixed and crushed with 90 mL of sterile saline solution (0.9% NaCl) and then agitated for 30 min at room temperature. The dilutions were from 10^{-1} to until 10^{-6}. The last two dilutions, 10^{-5} and 10^{-6}, were pasteurized at 80 °C for 12 min to isolate the endospore-forming bacteria (NIHORIMBERE & ONGENA, 2017), such as the *Bacillus* bacteria. Aliquots of 100 µL from each dilution were subsequently inoculated into Petri dishes with nutrient agar (Himedia, HiMedia Laboratories) containing 5 g peptic digest of animal tissue, 5 g NaCl, 1.5 g beef extract, 1.5 g yeast extract, and 15 g agar, with pH 7.4. The inoculated Petri dishes were incubated at 37 °C for 2 d. Single colonies representing different morphotypes, considering format, margin, color, brightness, and elevation, were selected to obtain pure cultures by successively transferring the cultures onto new agar plates. The isolated microorganisms were stored in glycerol (70%) at -20 °C.

Molecular identification of the isolates

To identify the isolated bacteria, we amplified and sequenced the 16S rRNA gene region by using the specific oligonucleotides: P0 5'- AAG AGT TTG ATC CTG GCT CAG - 3' (FURUSHITA et al., 2003) and P6 5'- CTA CGG CTA CCT TGT TAC GA - 3' (DI CELLO et al. 1997). Genomic DNA was extracted using the protocol of Versalovic et al. (1994). The 16S rRNA gene region was amplified for each sample by using polymerase chain reaction (PCR): 1X buffer, 1.5 mM MgCl2, 0.1 mM dNTP, 0.3 µM P0 and P6 primers, 1.5 U Taq polymerase (Invitrogen), and 10 ng DNA sample in a total volume of 50.0 µl. We also used the following cycle: 1x initial denaturation cycle

at 94 °C for 3 min, 30x cycles (i.e., denature at 94 °C for 45 s, annealing at 55 °C for 30 s, and extend at 72 °C for 1.5 min), and a 1x final extension cycle at 72 °C for 10 min. PCR products were designed to sequence the 16S rRNA gene by using the Sanger method at GoGenetic (Curitiba, Brazil). The sequences were analyzed using BLASTn software (NCBI database), and all sequences were deposited in GenBank (Supplementary Table 1). A phylogenetic tree was constructed using MEGA 6 software.

Bacterial biochemical qualitative and quantitative analysis

The bacterial isolates were subjected to biochemical assays. The bacteria isolates had been cultivated in DYGS medium (RODRIGUEZ NETO et al., 1986) at 120 rpm for 24 h at 30 °C so that the bacteria were in an approximate concentration of 10^6 at the time of the tests.

Calcium phosphate solubilization: The isolates were inoculated (100 µL) in 10 mL Pikovskaya's broth medium modified (PIKOVSKAYA, 1948) under agitation at 120 rpm for 7 d at 30 °C. The calcium phosphate precipitate was formed with dipotassium phosphate 0.57 M and calcium chloride 0.9 M. A quantitative assay was performed using the methodology of MURPHY & RILEY (1962) with modifications. The analyses were performed using polystyrene microplates with 96 wells, and the alteration of the volume and solutions was performed using a microplate reader (SpectraMax Plus 384®) at 880 nm. The standard curve used to determine soluble phosphorus released at the sample was (y= 0.0088x+0.2985), where "y" is the bacteria sample absorbance and "x" is the concentration in µg mL^{-1} of free phosphorus prepared

previously with known amounts of 10, 20, 40, 80, and 100 μg mL^{-1} of phosphorus.

Indole-3-Acetic Acid (IAA) production: Aliquots of the bacteria culture (25 μL) were transferred to an essay tube containing DYGS medium (5 mL) with and without the addition of *L*-tryptophan (100 mg L^{-1}), using the protocol of Sarwar and Kremer (1995), and were stored for 72 h at 30 °C in the dark. The samples were analyzed using the reader microplates (SpectraMax Plus 384®) at 540 nm. The IAA concentration was estimated using a standard curve (y= 0.0159x+0.064) of IAA commercial (Sigma-Aldrich®) where "y" is the bacteria sample absorbance and "x" is the concentration in μg mL^{-1} IAA produced by the sample with concentrations of 1, 5, 10, 20, 40, 80, and 100 μg mL^{-1}.

Carboxymethyl cellulase production: The cellulose degradation assay in solid medium was performed as KASANA et al. (2008) recommended. Specifically, 10 μL of the bacteria culture was inoculated in the Petri dishes with CMC medium and incubated at 30 °C for 96 h. The presence of a halo around the colonies showed the production of carboxymethyl cellulase. The cellulolytic activity (ICA) of HANKIN & ANAGNOSTAKIS (1975) was calculated as follows:

$$ICA = (TDC + HZ) / TDC$$

where:

ICA = Index Cellulolytic Activity

TDC = Total Diameter of the Colony

HZ = Hydrolysis Zone

The diameters of the colonies and hydrolysis zones were measured using a standard pachymeter.

Siderophore production: The siderophore synthesis was evaluated using the method of SCHWYN & NEILANDS (1987) with some adaptation concerning the use of the polystyrene microplate and some adjustments to the reagent volumes, in that an aliquot of 100 µL from supernatant culture was used and 100 µL of Chrome Azurol S solution was added. The samples were quiescent for 1 h at room temperature. The positive samples were orange or yellow and were analyzed using the reader microplates (SpectraMax Plus 384®) at 630 nm. The percentage of siderophore unit (SU) was calculated using the following formula of MACHUCA & MILAGRES (2003):

$$\%SU = ((RSA - SA)/(RSA)) \times 100$$

where:

%SU = percentage of Siderophore Unit

RSA = Reference sample absorbance

SA = Sample absorbance

All the flasks used in the assay were submerged in chloric acid (10%) for 48 hours and washed with distilled water to remove any metal remnants.

Statistical analysis: All characterization experiments regarding plant growth promotion were performed in triplicate. Data are expressed as means and standard

deviations. The mean of each analysis was submitted to variance analysis (ANOVA) and compared with the Scott-Knott Test (SCOTT & KNOTT, 1974) with 5% probability. SISVAR software (FERREIRA, 2019) was used to analyze the data.

In vivo experiment

For the *in vivo* experiment, four strains were selected after the statistical analysis of the biochemical, qualitative, and quantitative characteristics of plant growth promotion, four isolates were selected. These were then used to verify their capacity to promote plant growth in pots in a greenhouse. The cotton cultivars used in the greenhouse experiment were FM 985 (late cycle) and TMG 47 (mid-late cycle). The treatments used with both varieties as follows: 1) non-inoculated control, 2); commercial inoculant (containing *Bacillus pumilus* strain CCTB05 = CNPSo3203, *Bacillus subtilis* strain CCTB04 = CNPSo2720, and *Bacillus amyloliquefaciens* strain CCTB09 = CNPSo3602); 3) Isolate 1; 4) Isolate 2; 5) Isolate 3; and 6) Isolate 1. The experiment was done in randomized blocks into nine replicates for each treatment. Treatments were performed for both cotton varieties, for a total of 12 treatments in the greenhouse experiment.

The bacterial strains were cultivated in nutrient broth containing 5 g peptone, 5 g sodium chloride, 1.5 g meat extract, and 1.5 g yeast extract. Bacterial colonies were counted by employing the drop plate technique (OLSEN & BAKKEN, 1987), using 0.01 mL bacterial culture, incubated at 30 °C for 12, 24, and 36 h. The bacteria were cultivated for 3 d until they reached 10^8 CFU/mL. The commercial inoculant presented

an assurance of $1.0x10^8$ CFU/mL. An inoculation of 1 mL was performed in the groove on the first day of the experiment. After 14 d, 1 mL of the bacterial culture (10^8 CFU/mL) was reapplied to the plant collar.

The soil used to perform the *in vivo* experiment was collected at the rural area settlement 72 (19° 4' 7.5" E and 57° 34' 45.9" N), near Corumbá, MS. This area is usually used for planting agroecological vegetables, free from chemical fertilizers and pesticides. The physicochemical characteristics of the soil were evaluated at the Sial Solo Laboratory (Laboratory Analysis LTDA; Table 1). The soil was composed of NPK: 7.2 g nitrogen (urea), 38.4 g P_2O_5 (Simples super), and 16.8 g K_2O (potassium chloride) per planting bag (8 L) containing approximately 8 kg soil. After 40 and 99 d, 44.4 mg of urea was reapplied.

One day after fertilization, five seeds were placed in each planting bag. After 14 d, the plants were thinned, leaving only one plant per planting bag. The experiment lasted for 110 d. Irrigation was performed twice or thrice daily to maintain soil moisture.

Effects of PGPRs on morphological and phytochemical parameters

Physical parameters: The physical parameters used to evaluate the efficiency of the strains in promoting plant growth *in vivo* were plant height, number of fruits and leaves, fresh weight of shoots, dry weight of fruits and roots, and length and volume of roots. The plant and root lengths were measured using a measuring tape. The weight of the plant and root was determined using an electronic digital scale (Filizola model BPS-15). The plant material was dried using a conventional drying oven at 60 °C for 72 h. Root volume was measured using the method of PANG et al. (2011), using water

displacement after root submersion. Fruits and leaves were counted manually.

Phytochemical parameters: The phytochemical parameters evaluated comprised total and reducing sugars, amino acids, phenolic compounds, flavonoids, chlorophyll, carotenoids, ascorbate peroxidase, and catalase. For the flavonoid analysis, phenolic compounds, sugars, amino acids, 100 mg fresh leaves macerated with liquid nitrogen, and 5 mL methanol (80% v/v) were added to it. The methanol extracts were centrifuged at 3,000 rpm for 20 min at 25 °C and stored at -20 °C for subsequent analysis.

Total sugar concentration was determined using the sulfuric acid-phenol method (DUBOIS et al., 1951). The methanol extracts were diluted (1:10), and 0.5 mL aliquots were added to the test tubes. Phenol (0.5 mL) and concentrated sulfuric acid were added to the tubes, and the tubes were placed in an ice bath for 20 min. The absorbance of the samples was read at 490 nm by using a spectrophotometer (GENESYS 10S), and the values were calculated using a standard curve with different glucose concentrations. The results were expressed in milligrams of total sugars per g^{-1} of fresh plant weight. The total sugar content of the leaf extracts was evaluated using 3,5-dinitrosalicylic acid) as MILLER (1959) described. Sample absorbances were read at 540 nm in a microplate reader (SpectraMax Plus 384®), and calculations were performed based on a standard curve using glucose: 0, 5, 10, 20, 40, 50, 60, 70, and 80 µg μL^{-1}. Values are expressed as milligrams of reducing sugars per g^{-1} of fresh weight.

The concentration of free amino acids (AA) in the extracts was measured using the protocol of SANDHYA et al. (2010) with modifications. In test tubes, 500 µL extract, diluted previously with methanol (1:9), was mixed with 500 µL 0.2 M citrate buffer (pH 5.2) and 1.0 mL ninhydrin solution (1% w/v). The samples were incubated at 100 °C for 15 min and cooled; next, their absorbances were read at 570 nm (SpectraMax Plus 384®). Known concentrations 0, 5, 10, 20, 30, and 40 µg μL^{-1} of the

amino acid *L*-leucine were used as a standard. The results are presented as milligrams of total free amino acids per g^{-1} fresh weight.

The assay of phenolic compounds was performed using the protocol of LÓPEZ ARNALDOS et al. (2001). In brief, 200 µL of the supernatant extracts were collected from each sample and placed in an essay tube; next, 2 mL of solutions Na_2CO_3 (2% w/v) was added. Moreover, 150 µL of the Folin-Ciocalteau reagent and the samples were incubated for 45 min in the dark and evaluated using a spectrophotometer (SpectraMax Plus 384®) at 750 nm. A standard curve was prepared using different concentrations of gallic acid, starting from the mother solution at a concentration of 1 mg/mL^{-1}. The results were expressed by calculating the concentration of phenolic compounds and expressed as milligrams of gallic acid equivalents.

$$GAE = (\alpha \times 25)/0.1$$

Where:

GAE: gallic acid equivalent,

α: the value obtained from the equation of the line (extract diluted 10x),

25: the numerical value for the total methanol extraction volume, and

0.1: the fresh weight of the leaves in grams used for the extraction.

The method used for the flavonoids analysis was described in DE ASSIS et al. (2020) with modifications. First, 500 µL solution $AlCl_3$ (2% w/v) was added to 500 µL extract and mixed in a vortex. Subsequently, 2 mL ethanol was added to each sample. The samples were incubated in the dark for 1 h and evaluated using a spectrophotometer (SpectraMax Plus 384®) at 420 nm. Different concentrations of rutin were used for the calibration curve. Flavonoid values are expressed in milligrams of routine equivalents.

For the chlorophyll and carotenoids analysis, fresh leaves (100 mg) were macerated in liquid nitrogen, extracted with 1.5 mL acetone (80%) for pigment extraction, and centrifuged at 12,000 rpm for 3 min. After centrifugation, the extracts were transferred to clear centrifuge tubes. The extraction process was repeated until the pellets became colorless. The volume extract was adjusted to 10 mL with acetone and analyzed in three wavelengths by using a spectrophotometer (SpectraMax Plus 384®) at 663 nm for chlorophyll *a*, 647 nm for chlorophyll *b*, and 470 nm for carotenoids. The Lichtenthaler and Buschmann (2001) formula was used to determine pigment concentrations as follows: Chlorophyll *a*: CA = 12.25 A663-2.79 A647 (ug per mL solution); Chlorophyll *b*: CB = 21.50 A663-5.10 A647 (ug per mL solution); Total chlorophyll: Chlorophyll *a*+Chlorophyll *b* (ug per mL solution); Total carotenoids: [(1000 A470)–(1.82 CA)–(85.02 CB)]/198 (ug per mL solution).

The ascorbate peroxidase assay was performed using the method of Nakano and Asada (1981). To analyze catalase activity, we used the method of HAVIR & MCHALE (1987) with some modifications to the volume. Previously, a solution containing 700 µL buffer KH_2PO_4 (100 mM; pH 7) and 250 µL H_2O_2 (50 mM) was prepared for each sample to analyze the decomposition of H_2O_2. In this solution, 50 µL extracted enzymes were added after it was analyzed in a spectrophotometer (SpectraMax Plus 384®) at 240 nm for 90 s to verify the decrease in the absorbance. The solution used the molar extinction coefficient of 39 mM^{-1} cm^{-1}, and the enzyme activity was expressed in enzyme units per milligram of protein.

For the evaluation of macronutrients, leaves were dried at 60 °C sent in paper bags for analysis to the Paulista State University, Faculty of Agricultural Sciences (UNESP, Botucatu-São Paulo), where N, P, K, Ca, Mg, and S were evaluated.

Statistical analysis: The data of each analysis was submitted to variance analysis (ANOVA). The means were compared using the Scott-Knott Test (SCOTT & KNOTT, 1974) with 1 and 5% of significance and the SISVAR software (FERREIRA, 2019) was used for statistical analysis of the data.

The mean number of each morphological parameter was analyzed using a 5% probability for the number of fruits and dry mass of fruits, and a 1% probability for plant height, fresh mass of shoots, dry mass of roots, volume and length of roots, and number of leaves. Each treatment was repeated thrice for all phytochemical parameters. The mean of each analysis was subjected to variance analysis (ANOVA) and compared using the Scott-Knott Test (SCOTT & KNOTT, 1974) with a 1% probability.

RESULTS

Bacteria isolation and molecular identification

We isolated 54 bacteria from cotton and of these 46 were sequenced. The sequencing showed 43 of which belonged to *Bacillus*, and three isolates were identified as *Pseudoroseomonas ludipueritiae* (Figure 1). We confirmed that this methodology was efficient for isolating *Bacillu*s spp. All 16s RNA sequences were deposited in GenBank (accession numbers: OR461758–OR461802) (Supplementary Table 1).

Bacterial biochemical characteristics in vitro analysis

Considering the ability of bacterial isolates to produce different compounds *in vitro*, our results show the potential of several isolates to promote plant growth. From the 45 isolates tested, 17 showed activity for calcium phosphate solubilization, and the P6T01B7 isolate (*Pseudoroseomonas ludipueritiae*) presented the significant result: 64.77 μg mL^{-1} free phosphorus (Table 2). In the IAA assay, 30 isolates were positive producing this hormone, mostly the P6T32B7 isolate (*Bacillus* sp.) that produced 11.72 μg mL^{-1} of IAA (Table 2).

The carboxymethyl cellulase production assay showed a major number of positive isolates (30). The P6T32B7 isolate (*Bacillus* sp.) also showed the best results, with an Index Cellulolytic Activity (ICA) of 54.25. For siderophore analysis, five isolates produced this compound, and the P3T01B4 isolate (*Bacillus* sp.) presented the best result: 72.31% SU (Table 2). The isolates that tested positive for all tests were P1T01B1 (*Bacillus* sp.), P3T01B4 (*Bacillus* sp.), P6T32B2 (*Bacillus* sp.), and P6T32B4 (*Bacillus* sp.; Table 2).

The statistical analysis showed that the isolates P6T01B7 (*Pseudoroseomonas ludipueritiae*), P6T32B7 (*Bacillus* sp. 2), and P3T01B4 (*Bacillus* sp. 1) presented significant results in the tests conducted (Table 2) and were the first three selected for the greenhouse experiment. The fourth bacteria selected was the P6T32B4 (*Bacillus megaterium*) because it showed positive results for all tests (Table 2) and was the best for calcium phosphate solubilization (47.00 μg mL^{-1}) and siderophores (68.58% SU). The sequences of the strains selected for the greenhouse experiment were used to construct a phylogenetic tree, as were the sequences that showed genetic similarity according to the database.

Greenhouse experiment

The four isolates selected were: P3T01B4 (*Bacillus* sp. 1 - Isolate 1), P6T01B7 (*Pseudoroseomonas ludipueritiae* - Isolate 2), P6T32B4 (*Bacillus megaterium* - Isolate 3), and P6T32B7 (*Bacillus* sp. 2 - Isolate 4).

Morphological parameters: The results of the greenhouse experiment showed that the bacterial isolated promoted plant growth, establishing an important relationship between the cultivars and microorganisms. The analysis of variance (ANOVA) indicated that the use of cultivars, bacteria, and their interaction (cultivars x bacteria) influence the variables of height, fresh mass of the shoot, dry mass of fruits and roots, root volume and length, and number of root leaves (Table 3). The number of fruits was influenced only by the cultivar factor and the cultivar x bacteria interaction (Table 3). The use of the cultivar or bacteria in isolation increased the fresh mass of fruits, and the fresh mass of roots was influenced only using the bacteria. The dry mass of the shoots was not influenced by the treatments (Table 3).

The differences between P3T01B4 isolate and commercial inoculant treatment were nonsignificant. P3T01B4 isolate showed better results for plant height, with values of 104.6 and 101.6, respectively, than FM 985 cultivar, and the height of the control was 88.6 cm (Table 3, Figure 2a). For TMG 47 cultivar, after the P6T32B7 isolate had a value of 105.2 cm (an increase of 26.44%) like the commercial inoculant, was observed, and a value of 102.2 cm (an increase was 22.8%) when compared with the control. The commercial inoculant showed significant results for both cotton cultivars, considering plant height (Table 3, Figure 2b).

The P6T01B7 and P6T32B7 isolates showed positive results for FM 985 cultivar for the number of fruits, with increments of 27.27% and 13.63%, respectively. For TMG 47 cultivar, the significant results were for the commercial inoculants, P6T32B4 and

P3T01B4 isolates, with an increase in the number of fruits of 60.00, 20.00, and 13.33%, respectively, compared with the control (Table 3). For the fresh mass of shoots, there were no significant differences between the treatments of FM 985 cultivar (Table 3). However, for TMG 47 cultivar, the treatment with the commercial inoculant showed significant result for fresh mass of the shoot, an 81.66% increase, followed by the P6T32B7 isolate, with a 66.66% increase (Table 3).

Regarding the dry mass of the fruits, the P3T01B4, P6T32B4 and P6T32B7 isolates, and commercial inoculant showed similar results, with an increase of 47.3, 52.63, 44.73, and 44.73%, respectively, for FM 985 cultivar (Table 3). For TMG 47 cultivar, the P6T01B7 and P6T32B4 isolates presented significant results, increasing the fruit dry mass by 80 and 60%, respectively. For the other treatments, no significant differences were observed (Table 3).

The analysis of root dry mass did not show significant differences for FM 985 cultivar. For TMG 47 cultivar, the P6T01B7 isolate and commercial inoculant presented similar results, with 120 and 100% increases in root dry mass, respectively (Table 3).

The P6T32B4 isolate showed significant result, an increase of 74.07% (Figure 3a) in the root volume, followed by the P6T01B7 and P6T32B7 isolates, showing increases of 51.85 and 48.14%, respectively, for FM 985 cultivar, when compared with the control (Table 3). The P6T01B7 and P6T32B7 isolates presented significant results for the root volume of TMG 47 cultivar, showing increases of 77.41 and 61.29%, respectively, compared with the control (Table 3, Figures 3b and 3c).

The strains P6T32B4 and P6T32B7 isolates and the commercial inoculant presented significant differences for cultivar FM 985 cultivar, considering the root length, which increased 11.76, 12.29 and 8.5%, respectively (Table 3, Figures 3a and 3c). For TMG 47 cultivar, the P6T32B7 isolate showed the significant result for this

parameter (Figure 3c), with an increase of 37.57%, followed by the P6T01B7 and P6T32B4 isolates, which also presented significant differences, with increases of 19.47 and 15.26%, respectively, when compared with the control (Table 3). The number of leaves was bigger after the P6T32B7 isolate in FM 985 cultivar, 20.98% greater than that of the control. The P6T32B4 isolate had the greater result in TMG 47 cultivar considering the number of leaves, 69.42% higher than that of the control (Table 3).

Phytochemical parameters: Considering the phytochemical parameters, our results indicated that the use of cultivar, bacteria, and their interaction (cultivars × bacteria) influenced the variables of total sugars, ascorbate peroxidase, phenolic compounds, free amino acids, total, and chlorophyll A (Table 4). Reducing sugars, flavonoids, catalase, and chlorophyll B were influenced only by the bacteria and the cultivar *versus* bacteria interactions (Table 4).

The treatments showed significant differences in reducing sugar content. The P6T32B7 isolate had a significant result for the FM 985 cultivar, 68.20% higher than that of the control, followed by P6T01B7 isolate, which also showed a significant improvement, 45.12% higher than that of the control (Table 4). For TMG 47 cultivar, P6T32B7 isolate also showed significant results, with an 86.88% increase. The P3T01B4 and P6T01B7 isolates also produced significant results, with increases of 51.91 and 55.73%, respectively, when compared with the control (Table 4).

For FM 985 cultivar, the P6T32B7 isolate had the best results for total sugars compared with the control, increasing from 2.29 to 16.83 mg. g^{-1} fresh weight (634.93%). The commercial inoculant and P6T32B4 isolate also showed significant results, with improvements ranging from 2.29 to 15.51 (577.29%) and 15.87 mg. g^{-1} (593.01%) fresh weight, respectively (Table 4). In TMG 47 cultivar, the P3T01B4

isolate presented the significant result, increasing 27.81% compared with the control, followed by P6T01B7 and P6T32B4 isolates, increasing 13.94 and 14.83%, respectively, compared with the control.

The P6T01B7 isolate showed the best result for FM 985 cultivar for ascorbate peroxidase, increasing from 1.97 to 7.47 U. mg^{-1} (279,18%), followed by P6T32B4 and P6T32B7 isolates, increasing from 1.97 to 5.66 (187.30%) and 5.69 U. mg^{-1} (188.83%). For TMG 47 cultivar, a significant result was observed after the P6T32B7 isolate, with an improvement ranging from 1.68 to 10.57 U. mg^{-1} (529.16%). The P3T01B4 isolate also showed satisfactory results, increasing from 1.68 to 5.21 U. mg^{-1} (210.11%) (Table 4).

For the phenolic compounds, significant result for FM 985 cultivar was after the P6T32B7 isolate: a 75.35% increase. The commercial inoculant and P6T01B7 also presented significant results, increasing 28.71 and 27.60%, respectively. For TMG 47 cultivar, the commercial inoculant and P6T32B7 isolate showed significant results, improving 67.31 and 66.06%, respectively. Moreover, the P3T01B4 isolate showed a good result, increasing 46.29% compared with the control (Table 4).

In the evaluation of flavonoids, the P6T32B7 isolate showed significant results: a 64.41% increase for FM 985 cultivar. The commercial inoculant, P3T01B4, and P6T01B7 isolates showed increases of 17.75, 16.79, and 21.02%, respectively, and the only treatment that showed similar results to the control was the P6T32B4 isolate (Table 4). For TMG 47 cultivar, the P3T01B4 isolate presented the best result, increasing 34.19%, followed by the P6T32B4 and P6T32B7 isolates, with increases of 27.20 and 20.20%, respectively (Table 4).

Regarding free amino acids, P6T32B4 isolate presented significant results for FM 985 cultivar, increasing from 0.43 to 1.27 mg. g^{-1} (195.34%). The P6T01B7 and

P6T32B7 isolates also presented good results, increasing from 0.43 to 1.07 (148.83%) and 1.06 mg. g^{-1} (146.51%). The P3T01B4 isolate had a significant result for TMG 47 cultivar, 52.68% higher than that of the control. The P6T32B4 and P6T32B7 isolates also showed significant results, increasing 30.10 and 25.80%, respectively (Table 4).

The catalase activity had a significant result after the P6T01B7 isolate of FM 985 cultivar, increasing from 1.94 to 9.03 U.mg^{-1} (365.46%). The P6T32B4 and P6T32B7 isolates also presented significant results, increasing from 1.94 to 5.66 (191.75%) and 5.69 U. mg^{-1} (193.23%), respectively. For TMG 47 cultivar, a significant result was in response to the P6T32B7 isolate increasing from 1.66 to 10.84 U. mg^{-1} (553.01%), followed by P3T01B4 isolate, increasing from 1.66 to 5.21 U. mg^{-1} (213.85%) (Table 4).

The P6T32B4 isolate presented significant results for chlorophyll A, with an increase of 16.6%. The other treatments did not present significant differences in FM 985 cultivar. For TMG 47 cultivar, the P6T01B7 isolates presented a significant result, increasing 34.4% compared with the control. Regarding chlorophyll B, the P6T32B4 isolate showed a significant result for FM 985 cultivar, increasing 51.42% compared with the control, followed by P3T01B4 isolate with an increase of 17.14%. For TMG 47 cultivar, after the P6T01B7 isolate, an increase of 28.57% was observed, and for the P6T32B4 isolate and commercial inoculant, a 20% increase was observed; these three treatments showed a significant results when compared with the results of the control (Table 4). The P3T01B4 isolate showed the best results for FM 985 cultivar for total chlorophyll, with a 16.77% increase. The control group showed results similar to those of the other three treatment groups. TMG 47 cultivar showed a significant result for the P6T01B7 isolate, increasing 33.12% compared with the control. The control showed similar results to those of the other two treatments.

Chlorophyll A and total chlorophyll were the only assays for which the control showed results similar to those of the commercial inoculant and P6T32B4 isolate (Table 4). The macronutrient analysis using ANOVA showed significant differences only for nitrogen and calcium concerning the cultivar *versus* bacteria. The analysis also showed that cultivar variables had significant effects on nitrogen, Mg, and K. For the nitrogen parameter and the FM 985 cultivar, no significant differences were observed between treatments. For TMG 47 cultivar, the P6T01B7, P6T32B4, P6T32B7 isolates and commercial inoculant treatments showed significant results, increasing 6.9, 13.11, 11.06 and 8.08%, respectively, for the nitrogen parameter compared with the control (Table 5). The P3T01B4, P6T01B7, and P6T32B4 isolates showed significant results for calcium: for FM 985 cultivar, increasing 15.18, 7.62, and 19.66%, respectively. For TMG 47 cultivar, the treatments did not show significant differences (Table 5).

DISCUSSION

Most of the bacterial isolates obtained in this study were identified as *Bacillus* by using 16S rRNA gene region sequencing. The method of pasteurization used during the isolation selected only bacterial endospore formation; consequently, the genus *Bacillus* was the most frequently reported bacterium, indicating the efficiency of the method used in the isolation of bacterial strains. We observed that the bacterial isolates from cotton in this study were able to produce indole acetic acid, solubilized calcium phosphate, cellulase, and siderophores. These characteristics were important to select the microorganisms for *in vivo* testing.

The tested microorganisms were capable of solubilizing calcium phosphate ,

primarily P6T01B7 isolate (64.77 µg. mL^{-1}). Assays conducted with strains of *Bacillus cereus* and *B. megaterium*, subjected to growth in culture medium with different concentrations of NaCl, showed similar concentrations (68.3 ± 4l µg. mL^{-1}) to result in the literature (ABDELMOTELEB & GONZALEZ-MENDOZA, 2020). Lower values than ours were obtained in SHAHID et al. (2021), in which a strain of *B. amyloliquefaciens* presented a maximum free P value of 23.20 µg. mL^{-1}.

Phosphorus is a nutrient with low solubility in soil and is usually complexed with Zn, Al, Ca, and Mg (TORRES-CUESTA et al., 2023), making it unavailable to plants. TIAN et al. (2021) reported that the solubilization of calcium phosphate is an important function of microorganisms in soil systems. Solubilization can be achieved by microorganisms through the production of organic acids or phosphatase enzymes (TOUHAMI et al., 2020; WEI et al., 2021). These characteristics indicate the potential of *Bacillus* strains to promote plant growth (MAHDI et al., 2020; AHMAD et al., 2021). Therefore, the ability of bacterial isolates to solubilize insoluble phosphorus is considered an important characteristic, along with other attributes that can directly and indirectly promote plant growth (GALEANO et al., 2021).

Another important characteristic is the production of indole acetic acid (IAA) because this phytohormone can stimulate plant growth with improved development of the roots and adventitious roots due to the stimulation of cellular division (MIKE-ANOSIKE et al., 2018; YAMAUCHI et al., 2019; LIBAO et al., 2020). An increase in the root area allows for an increase in the absorption of water and nutrients from the soil (JING & STRADER, 2019). Studies have demonstrated the efficiency of rhizobacteria in the production of IAA (JIANG et al., 2020; LAIRD et al., 2020; KOUAM et al., 2023). Our isolates showed significant IAA production among 30 positive isolates. According to SOSA-PECH et al. (2019), *Bacillus* spp. has different

periods to synthesis of IAA and can produce IAA after 24 h of incubation, which remains constant during other incubation periods. In addition, these microorganisms can produce different amounts of IAA, depending on the concentration of *L*-Tryptophan in the medium (DO PRADO et al., 2019; WIDAWATI et al., 2020).

The P6T32B7 strain produced the highest amount of IAA (11.72 µg. mL^{-1}), with 100.0 mg. L^{-1} of *L*- Tryptophan. Similar results were found in *Bacillus* species, in which IAA production varied with changes in *L*-Tryptophan concentrations (DO PRADO et al., 2019). WAGI & AHMED (2019) observed that the concentration of IAA varied for each species, depending on the amount of the precursor, with *B. subtili*s and *B. cereus* producing 37.0 µg. mL^{-1} and 54.0 µg. mL^{-1}, in a concentration of 200.0 mg L^{-1} *L*-Tryptophan, twice the concentration used in this study. *Bacillus* isolates efficiently promote plant growth through the production of IAA, which can be affected by different concentrations of the precursor L-tryptophan (WAGI & AHMED, 2019).

Approximately 60% of all bacterial isolates in this work were able to produce cellulase. The strain with the highest ICA was P6T32B7 isolate 54.25, with significant differences from the other strains. The production of this enzyme means that the isolates can establish an efficient interaction between the bacteria and the host through the superficial penetration of the microorganism into plant tissues (HASSAN et al., 2017). In addition, cellulases can inhibit the growth of certain pathogens (TAPIA-VÁZQUEZ et al., 2020). Cellulase is an important hydrolytic enzyme involved in nutrient cycling, which provides carbon for glucose molecules (GUPTA et al., 2020).

The isolates capable of producing siderophores can collaborate with important processes in plants owing to their capacity to produce iron-chelating compounds, making it possible for them to play an important role in various physiological mechanisms of plants, such as respiration and photosynthesis (SINGH et al., 2022). In

addition, the production of siderophores by microorganisms reduces plant stress in iron-stressed soil and can function as biofertilizers and control phytopathogens (SINGH et al., 2022). Five bacterial isolates produced siderophores, and the isolate P3T01B4 produced the highest value (72.31% SU). YU et al. (2017) found similar amounts of siderophores in tests of *Bacillus* strains using different carbon sources and observed the presence of 80.68% SU in medium using glucose. Despite the decrease in the number of isolates positive for siderophore production, also observed in KUMAR et al. (2021), in which among the five isolates tested, only one was positive for siderophores, the importance of this compound can directly assist in the plant's cellular metabolism and improve plant growth.

The greatest increase in height in the cotton cultivars was 18.05 and 14.67%, supporting the results of ROMERO-PERDOMO et al. (2021), who found an increase of approximately 15% in the height of the aerial part for cotton after inoculation with PGPR. MAJID et al. (2020), using plant growth-promoting *Bacillus* and *Rhizobium* strains in cotton, found increases of approximately 30.87%, 19.59%, and 9.20% in plant height compared with the control. HAMED et al. (2019) inoculated cotton with *Bacillus* strains and reported a significant increase in plant height. These studies show that the use of inoculants containing plant growth bacteria can increase the height of cotton cultivars. The number of fruits was not a significant variable; however, the dry weight of the fruits had a relative improvement compared with the control, which may indicate an improvement in the quality and volume of the fruit. An improvement in the fruit variable also was found by HAMED et al. (2019) after inoculation with *Bacillus* strains in cotton.

One important result of this study regards root volume and length, which can influence plant development, such as biomass and nutrients. An increase of 74.07% in the root volume and 37.57% in the root length was observed after the P6T32B7

isolate. Notably, IAA production (11.72 μg. mL^{-1}) was observed for the P6T32B7 isolate and can

This study also showed an increase in chlorophyll, an important pigment responsible for plant photosynthetic metabolism, which responds to a wide range of environmental conditions by adjusting its chlorophyll production mechanism according to this condition (PÉREZ-BUENO et al., 2019), including its association with plant growth-promoting organisms. The best results for total chlorophyll in this study were for the P3T01B4 treatment, with a 16.77% increase, and for the P6T01B7 treatment, with an increase of 33.12%, indicating that the *Bacillus* can increase chlorophyll and, consequently, increase the photosynthetic rate, corroborating other studies that reported an increase in the chlorophyll content by using *Bacillus* and *Rhizobium* strains in cotton (HAMED et al., 2019; MAJID et al., 2020; ROMERO-PERDOMO et al., 2021).

This improvement in chlorophyll can influence photosynthesis rates and, consequently, the quantities of products resulting from photosynthesis, such as sugars (ELKELISH et al., 2020). P6T32B7 isolate presented a breakthrough (better) result considering total sugar compared with the control, with an increase of 2.29 (control) to 16.83 mg. g^{-1} fresh weight, indicating the potential use of these bacterial isolates for crops. The free amino acid content was significant in the three treatments in this study, indicating the capacity of *Bacillus* isolate to increase plant metabolites and corroborating previous studies that reported an increase in amino acids using *Bacillus* strains in other crops (KHAN et al., 2020; KAZEROONI et al., 2021). An increase in amino acids can influence the physiology of plants because amino acids are precursors of organic molecules, such as nucleic acids, which affect plants in stressful environments (KHAN et al., 2020). The morphological and phytochemical parameters showed

that PGPR inoculation had a positive effect on cotton. Thus, the use of PGPR as an inoculant is crucial for improving agriculture as an environment-friendly technology.

The use of plant growth-promoting bacteria has been elucidated, with new bacterial species being used for various crops worldwide. Because of the importance of improving crop development, this study showed that bacterial isolates can affect plant development and improve agribusiness input production. The P6T32B4(*Bacillus megaterium*) and P6T32B7 (*Bacillus* sp. 2) isolates showed a significant results for both cultivars, mainly related to root parameters, with 74.07% of increase to P6T32B4 and 48.14% to P6T32B7. The P6T32B7 isolate showed after a significant result about the IAA production in vitro, with 11.72 $\mu g\ mL^{-1}$. Thus, strain P6T32B4 and P6T32B7 could be tested in the field, because of the characteristics presented about increasing the root surface, providing greater nutrient uptake and consequently an increase in productivity.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS CONTRIBUTION

All authors contributed equally to the conception and writing of the manuscript. All authors critically revised and approved the final version of the manuscript.

DATA AVAILABILITY

DNA sequences can be found under the access number on NCBI according to Supplementary Table 1. Data will be made available on request.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi_____.

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FIGURE HEADINGS

Figure 1 - Phylogenetic tree of identified bacteria from roots of cotton by sequencing of 16S. The three was constructed with the Maximum Likelihood and 100 bootstrap in MEGA X (version 11).

Figure 2 - (a) FM 985 cultivar, from left to right: control, commercial inoculant, and P3T01B4 treatments; (b) TMG 47 cultivar, from left to right: control, commercial inoculant, and P6T32B7 treatments.

Figure 3 - (a) FM 985 cultivar, from left to right: control, commercial inoculant, and P6T32B4 treatments; (b) TMG 47 cultivar, from left to right: control, commercial inoculant, and P6T01B7 treatments; (c) TMG 47 cultivar, from left to right: control, commercial inoculant, P6T32B7 treatments.

Table 1 - Physicochemical characteristics of soils sampled from an agroecological farm, Corumbá, Mato Grosso do Sul state, Brazil.

	Soil Sample	
Variables	1	2
Clay (g/kg)	460.00	463.00
Silt (g/kg)	183.00	172.00
Sand (g/kg)	357.00	365.00
OM (g/dm^3)	31.50	33.30
Iron (mg/dm^3)	28.01	27.37
Av P Mehlich (mg/dm^3)	15.00	13.00
K (mg/dm^3)	265.90	275.40
Mn (mg/dm^3)	27.33	28.59
Ca ($mmolc/dm^3$)	265.00	278.00
Mg ($mmolc/dm^3$)	102.00	109.00
CEC ($mmolc/dm^3$)	393.00	411.00
pH $CaCl_2$	6.10	6.30

CEC – cation exchange capacity; OM – organic matter.

Table 2 - Biochemical characteristics and molecular identification based on the sequencing of the 16S rRNA gene region of the selected bacterial isolates.

	Isolates				
	P3T01B4	P6T01B7	P6T32B4	P6T32B7	Coefficient of variation (%)
Species	*Bacillus* sp. 1	*Pseudoroseomonas ludipueritiae*	*Bacillus megaterium*	*Bacillus* sp. 2	-
Access number (GenBank)	OR461774	OR461782	OR461795	OR461798	-
Amount of phosphate (µg mL^{-1})	46.09b	64.77a	47.00b	38.33c	13.13
Amount of IAA (µg mL^{-1})	4.56c	9.45b	4.29c	11.72a	26.61
Carboxymethyl Cellulase (ICA)	35.15c	41.38b	36.60c	54.25a	10.50
Siderophore production (% SU)	72.31a	Nd	68.58a	Nd	4.46

Different letters indicate significant differences between means ($P < 0.05$); nd = not detected; ICA = Index Cellulolytic Activity; SU = Siderophore Unit.

Table 3 - Morphological parameters obtained from the *in vivo* experiments considering different cotton cultivars and bacteria isolates.

Cultivars	**Treatments**					
	Control	**P3T01B4**	**P6T01B7**	**P6T32B4**	**P6T32B7**	***CI**
	Plant height (cm)					
CV (%)	4.49					
FM 985	88.6 aC	104.6 aA	96.6 aB	96.4 aB	97.8 bB	101.6 aA
TMG 47	83.2 aC	92.0 bB	89.2 bB	92.4 aB	105.2 aA	102.2 aA
	Number of fruits (unit)					
CV (%)	22.29					
FM 985	4.4 aB	4.4 aB	5.6 aA	4.0 aB	5.0 aA	3.8 aB
TMG 47	3.0 bA	3.4 aA	4.0 bA	3.6 aA	3.4 bA	4.8 aA
	Fresh mass of the shoot (g)					
CV (%)	6.28					
FM 985	0.080 aA	0.086 bA	0.089 aA	0.081 bA	0.087 bA	0.084 bA
TMG 47	0.060 bE	0.097 aC	0.087 aD	0.092 aC	0.100 aB	0.109 aA
	Dry mass of the fruits (g)					
CV (%)	25.90					
FM 985	0.0076 aB	0.0112 aA	0.0096 aB	0.0116 aA	0.0110 aA	0.0110 aA

TMG 47	0.0050 aB	0.0056 bB	0.0090 aA	0.0080 bA	0.0044 bB	0.0062 bB
	Dry mass of the root (g)					
CV (%)	30.48					
FM 985	0.0042 aA	0.0040 aA	0.0050 bA	0.0050 aA	0.0060 aA	0.0046 bA
TMG 47	0.0050 aB	0.0050 aB	0.0110 aA	0.0070 aB	0.0060 aB	0.0100 aA
	Root volume (mL)					
CV (%)	10.65					
FM 985	27.0 aC	27.0 bC	41.0 bB	47.0 aA	40.0 bB	29.0 bC
TMG 47	31.0 aC	42.0 aB	55.0 aA	38.0 bB	50.0 aA	43.0 aB
	Root length (cm)					
CV (%)	7.71					
FM 985	37.4 aB	37.8 aB	37.2 bB	41.8 aA	42.0 bA	40.6 aA
TMG 47	38.0 aC	36.4 aC	45.4 aB	43.8 aB	50.0 aA	41.2 aC
	Number of leaves (unit)					
CV (%)	7.26					
FM 985	32.4 aB	30.0 aB	31.4 aB	28.8 bC	39.2 aA	27.0 bC
TMG 47	24.2 bD	27.80 aC	27.2 bC	41.0 aA	30.4 bB	31.0 aB

*CI= Commercial Inoculant; Considering the ANOVA results, the same lowercase letters in the column and the same capital letters in the row do not differ statistically from each other at 5% level using the Scott Knott test. CV = Coefficient of variation.

Table 4 - Phytochemical parameters obtained from the *in vivo* experiments considering different cotton cultivars and the bacteria isolates.

Cultivars	**Treatments**					
	Control	**P3T01B4**	**P6T01B7**	**P6T32B4**	**P6T32B7**	**CI***
	Reducing sugars					
CV (%)	4.49					
FM 985	1.95 aE	2.32 bD	2.83 aB	2.66 aC	3.28 aA	2.41 aD
TMG 47	1.83 aE	2.78 aB	2.85 aB	2.64 aC	3.42 aA	2.25 bD
	Total sugars					
CV (%)	5.49					
FM 985	2.29 bE	7.87 bD	10.19 bC	15.87 aB	16.83 aA	15.51 aB
TMG 47	14.49 aC	18.52 aA	16.51 aB	16.64 aB	15.25 bC	15.03 aC
	Ascorbate peroxidase					
CV (%)	5.69					
FM 985	1.97 aD	4.14 bC	7.47 aA	5.66 aB	5.69 bB	4.01 bC
TMG 47	1.68 aD	5.21 aB	4.72 bC	4.70 bC	10.57 aA	4.50 aC
	Phenolic compounds					
CV (%)	3.43					
FM 985	16.23 bC	17.58 bC	20.71 bB	17.20 bC	28.46 bA	20.89 bB

TMG 47	24.84 aE	36.34 aB	28.05 aD	30.21 aC	41.25 aA	41.56 aA
			Flavonoids			
CV (%)			5.45			
FM 985	13.46 aC	15.72 bB	16.29 aB	13.79 bC	22.13 aA	15.85 aB
TMG 47	13.86 aD	18.60 aA	15.42 aC	17.63 aB	16.66 bB	15.71 aC
			Free amino acids			
CV (%)			7.62			
FM 985	0.43 bE	0.59 bD	1.07 aB	1.27 aA	1.06 bB	0.96 aC
TMG 47	0.93 aD	1.42 aA	1.06 aC	1.21 aB	1.17 aB	0.58 bE
			Catalase			
CV (%)			6.67			
FM 985	1.94 aD	4.14 bC	9.03 aA	5.66 aB	5.69 bB	4.21 aC
TMG 47	1.66 aD	5.21 aB	4.66 bC	4.56 bC	10.84 aA	4.50 aC
			Chlorophyll A			
CV (%)			5.40			
FM 985	1.26 aB	1.47 aA	1.23 bB	0.86 bC	1.22 aB	1.19 aB
TMG 47	1.25 aB	1.00 bC	1.68 aA	1.28 aB	1.07 bC	1.23 aB
			Chlorophyll B			
CV (%)			8.02			

FM 985	0.35 aC	0.41 aB	0.30 bD	0.53 aA	0.30 bD	0.34 bC
TMG 47	0.35 aC	0.27 bC	0.45 aA	0.42 bA	0.36 aB	0.42 aA
			Total chlorophyll			
CV (%)			5.12			
FM 985	1.61 aB	1.88 aA	1.53 bB	1.39 bC	1.52 aB	1.53 bB
TMG 47	1.60 aB	1.28 bC	2.13 aA	1.70 aB	1.44 aC	1.64 aB

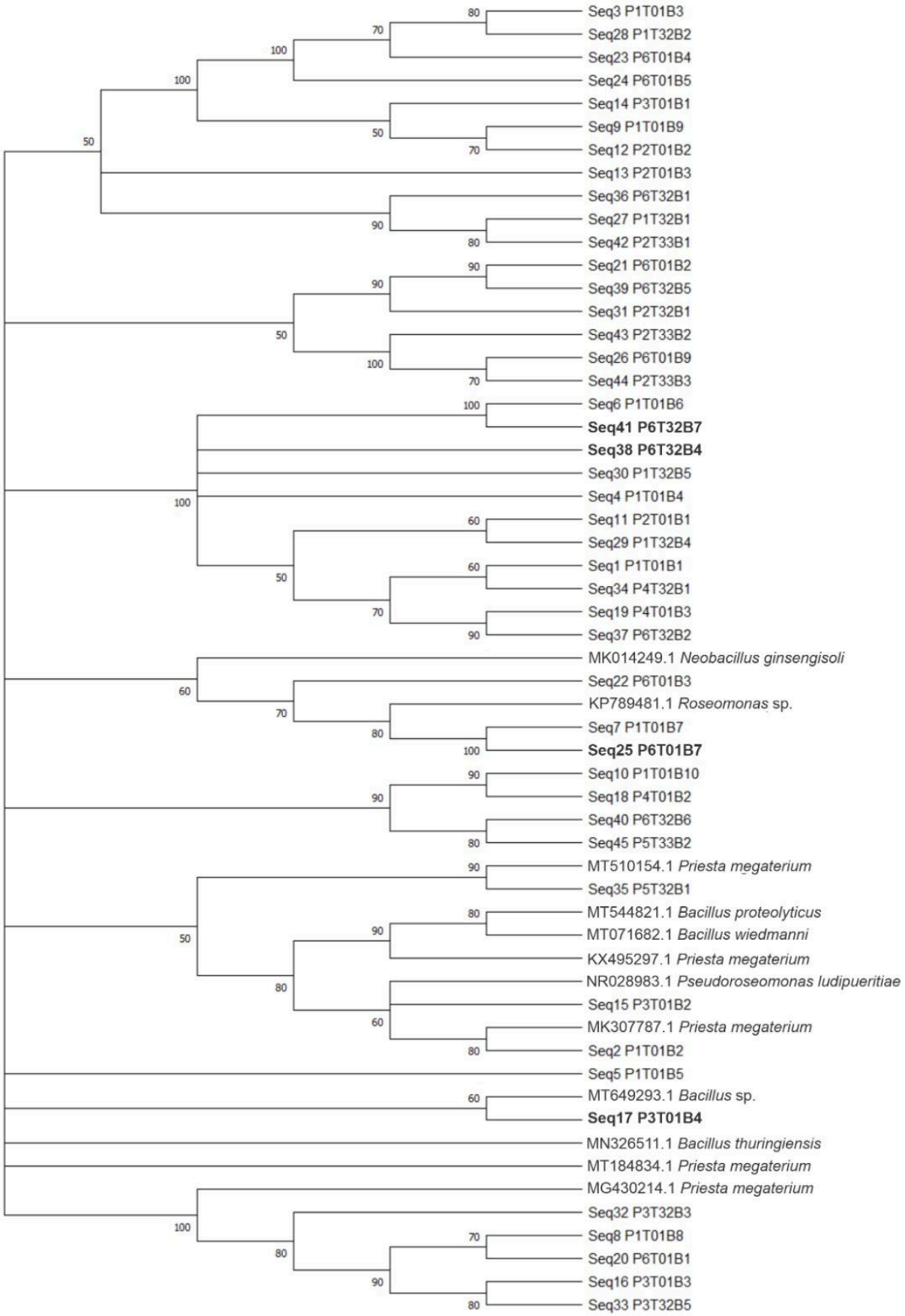
*CI= Commercial Inoculant; Considering the ANOVA results, the same lowercase letters in the column and the same capital letters in the row do not differ statistically from each other at 5% level using the Scott Knott test. CV = Coefficient of variation.

Table 5 - Macronutrients shoot content different cotton cultivars and the bacteria isolates.

Cultivars	Treatments					
	Nitrogen					
	Control	**P3T01B4**	**P6T01B7**	**P6T32B4**	**P6T32B7**	**CI***
FM 985	23.40 aA	23.01 aA	21.58 bA	23.21 bA	21.40 bA	21.07 bA
TMG 47	23.49 aB	22.66 aB	25.13 aA	26.57 aA	26.09 aA	25.39 aA
	Calcium					
FM 985	29.50 aB	33.98 aA	31.75 aA	35.30 aA	29.25 aB	27.80 bB
TMG 47	31.33 aA	30.83 aA	33.65 aA	30.60 aA	33.20 aA	33.60 aA

*CI= Commercial Inoculant; Considering the ANOVA results, the same lowercase letters in the column and the same capital letters in the row do not differ statistically from each other at 5% l

Fig. 1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Seq28 P1T32B2
Seq23 P6T01B4
Seq24 P6T01B5
Seq14 P3T01B1
Seq9 P1T01B9
Seq12 P2T01B2
Seq13 P2T01B3
Seq36 P6T32B1
Seq27 P1T32B1
Seq42 P2T33B1
Seq21 P6T01B2
Seq39 P6T32B5
Seq31 P2T32B1
Seq43 P2T33B2
Seq26 P6T01B9
Seq44 P2T33B3
Seq6 P1T01B6
Seq41 P6T32B7
Seq38 P6T32B4
Seq30 P1T32B5
Seq4 P1T01B4
Seq11 P2T01B1
Seq29 P1T32B4
Seq1 P1T01B1
Seq34 P4T32B1
Seq19 P4T01B3
Seq37 P6T32B2
MK014249.1 Neobacillus ginsengisoli
Seq22 P6T01B3
KP789481.1 Roseomonas sp.
Seq7 P1T01B7
Seq25 P6T01B7
Seq10 P1T01B10
Seq18 P4T01B2
Seq40 P6T32B6
Seq45 P5T33B2
MT510154.1 Priesta megaterium
Seq35 P5T32B1
MT544821.1 Bacillus proteolyticus
MT071682.1 Bacillus wiedmanni
KX495297.1 Priesta megaterium
NR028983.1 Pseudoroseomonas ludipueritiae
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MK307787.1 Priesta megaterium
Seq2 P1T01B2
Seq5 P1T01B5
MT649293.1 Bacillus sp.
Seq17 P3T01B4
MN326511.1 Bacillus thuringiensis
MT184834.1 Priesta megaterium
MG430214.1 Priesta megaterium
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Seq8 P1T01B8
Seq20 P6T01B1
Seq16 P3T01B3
Seq33 P3T32B5

Fig. 2

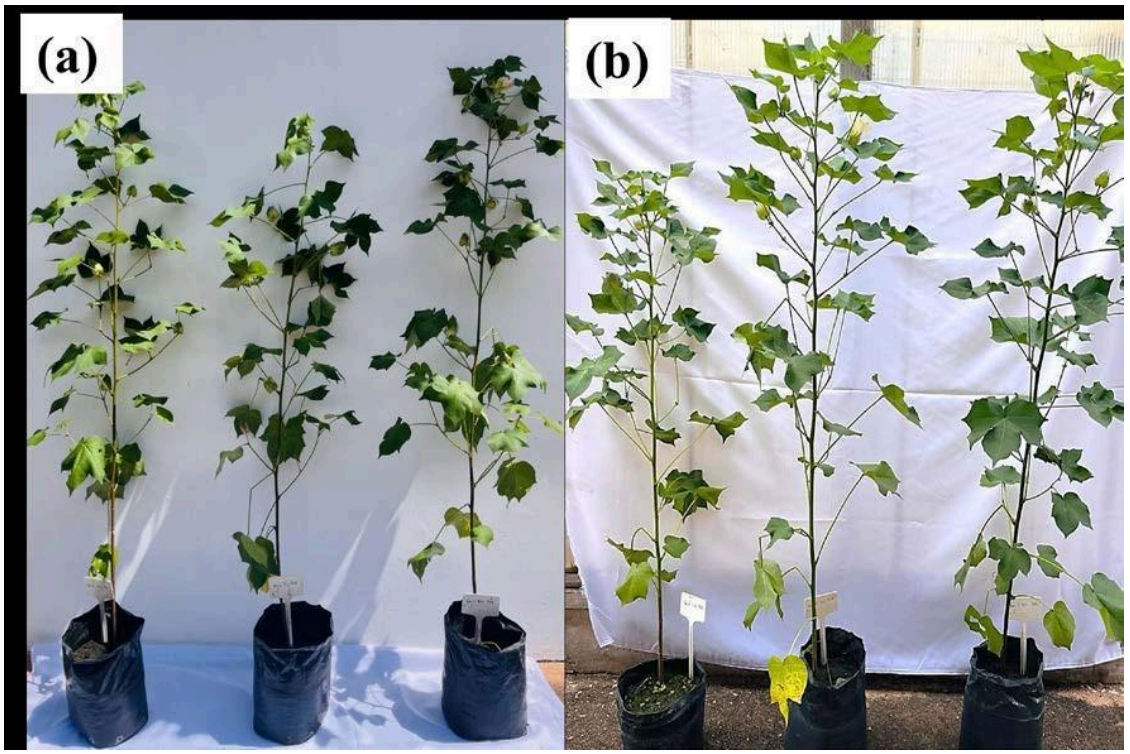
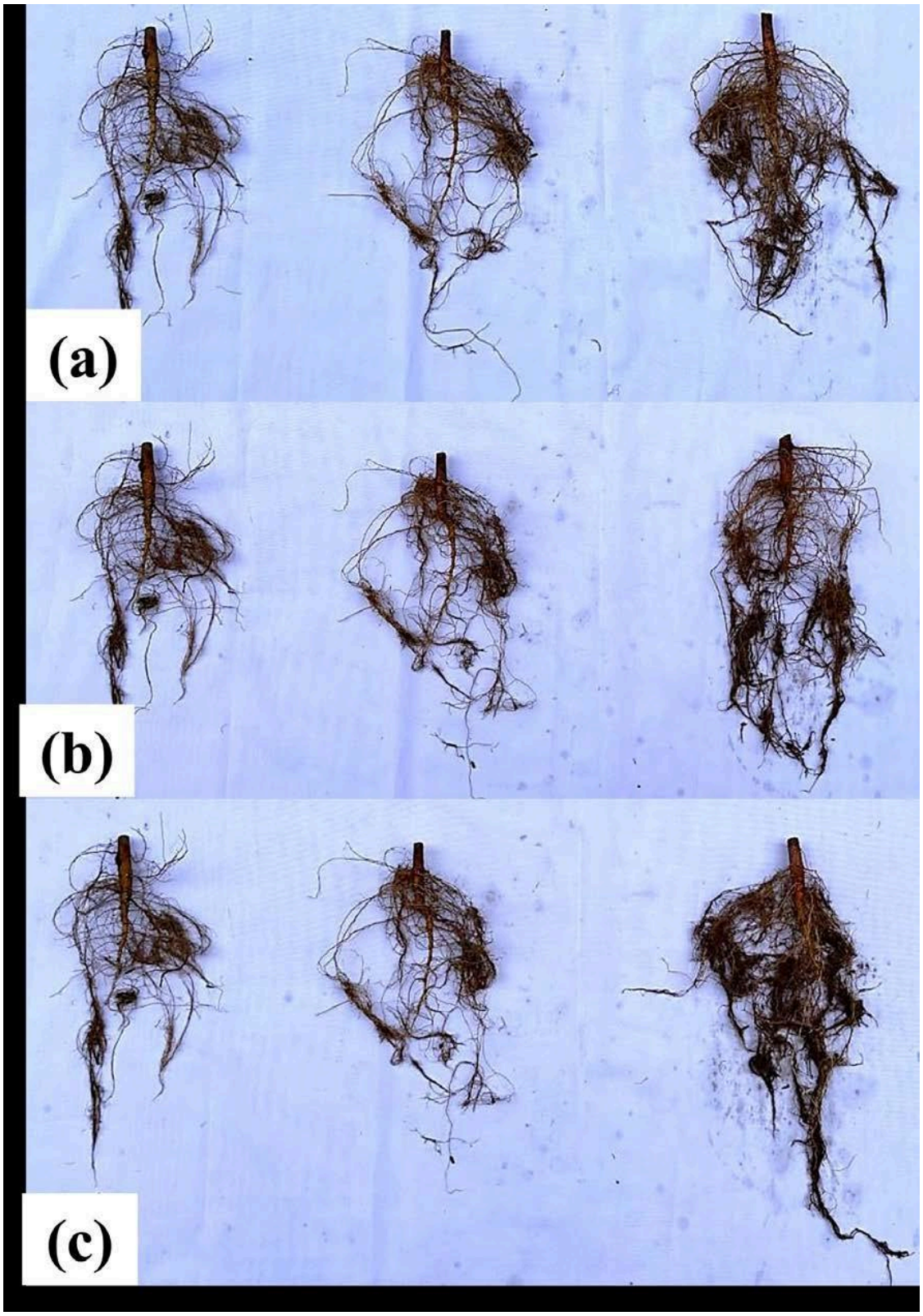


Fig. 3



Capítulo II

Inibição *in vitro* do crescimento de dois fitopatógenos do algodão por bactérias isoladas do algodão

Artigo a ser submetido para a revista "Biocontrol Science and Technology".

Inibição *in vitro* do crescimento de dois fitopatógenos de algodoeiro por bactérias isoladas do algodão

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Resumo

No Brasil, o clima tropical favorece o crescimento de microrganismos fitopatogênicos que causam doenças e limitam a produtividade do algodoeiro. Nesse contexto, o uso de rizobactérias promotoras de crescimento vegetal (RPCV) como agentes de controle biológico pode ser uma alternativa mais segura e sustentável aos agrotóxicos para controlar doenças. Portanto, o objetivo deste estudo foi verificar o potencial de isolados bacterianos no controle de fitopatógenos do algodoeiro *in vitro*. Para isso, 46 isolados obtidos de algodoeiro foram testados quanto ao potencial de inibição do crescimento micelial dos fitopatógenos *Corynespora cassiicola* e *Fusarium oxysporum* f. sp. *vasinfectum* em meio de cultura batata-dextrose-ágar (BDA). Ao final de cada ensaio, a

porcentagem de inibição foi calculada usando placas controle sem a presença dos antagonistas. Os patógenos também foram inoculados em plantas de algodoeiro por aspersão de suspensões de micélios triturados nas folhas e colmo e pelo uso de discos (1 mm) de culturas crescidas em meio BDA, que foram fixados em fissuras nos tecidos vegetais. Os resultados mostraram que 19 cepas bacterianas apresentaram taxas de inibição variando entre 39,43% e 71,89%. Destas, 18 mostraram antagonismo contra o fitopatógeno *C. cassiicola*, com destaque para o isolado P1T01B3 (71,89%). Enquanto dez cepas também demonstraram potencial antagonista contra *F. oxysporum*, sendo que cinco delas apresentaram inibição superior a 60%, com destaque para o isolado P1T01B5, com 69,5%. Os resultados obtidos *in vitro* indicam o potencial dessas cepas para o controle biológico desses fitopatógenos na cultura do algodoeiro. No entanto, é necessário realizar ensaios para investigar esses efeitos nas plantas, utilizando cepas de fitopatógenos que estejam com sua patogenicidade ativa.

Palavras-chave: microrganismos, controle biológico, algodoeiro, *Corynespora cassiicola* e *Fusarium oxysporum* f. sp. *vasinfectum*

1. Introdução

A cotonicultura, que é a produção da fibra de algodão, é uma prática comum em mais de cem países. A concentração de 78% da produção mundial e 90% da área colhida é proveniente de cinco países principalmente: Estados Unidos, China, Índia, Paquistão e Brasil (ABRAPA, 2023). A cultura do algodoeiro no Brasil enfrenta grandes problemas devido ao clima tropical, que favorece o crescimento de muitos microrganismos fitopatogênicos (Chohan et al., 2020). Os fungos *Ramularia griseola* (Mancha-de-ramularia), *Myrothecium roridum* (Mancha-de-mirotécio), *Corynespora cassiicola* (Mancha Alvo) e *Fusarium oxysporum* (Murcha de fusarium), que causam doenças relacionadas a outros fatores críticos que limitam a alta produtividade dessa cultura (Chohan et al., 2020).

Na agricultura brasileira e mundial, há ainda o uso intensivo e sistêmico de agrotóxicos para o controle de fitopatógenos, principalmente por ser uma estratégia atraente devido sua simplicidade na aplicação e pelo conhecimento prévio sobre o amplo espectro de patógenos afetados por esta técnica (Tudi et al., 2021). No entanto, o uso desse produto pode acarretar desde a contaminação de alimentos, rios e lençois freáticos, solos e animais que vivem ao redor, como também pode ocasionar o surgimento de doenças a humanos associadas ao uso de agrotóxicos (Sarker et al., 2021).

A demanda da sociedade por alimentos saudáveis e livres de defensivos artificiais tem aumentado a cada dia. O controle biológico de pragas e doenças tem se tornado uma alternativa possível parceira nessa busca pela agricultura mais sustentável (Ramakrishnan et al., 2021; Wang et al., 2023). O controle biológico de patógenos vegetais atraiu atenção significativa recentemente, por ser uma estratégia

ambientalmente correta para o controle de doenças (Raymaekers et al., 2020; Sood et al., 2020). Dentro dessa perspectiva, atualmente, há o uso de microrganismos que oferecem o controle biológico de diversas doenças. As Rizobactérias Promotoras de Crescimento Vegetal (RPCV), são conhecidas por colaborar direta ou indiretamente com o desenvolvimento da planta, como por exemplo, através da competição com organismos patogênicos na rizosfera (Saeed et al., 2021).

Dentre os Agentes de Controle Biológico (ACB), destacam-se bactérias do gênero *Bacillus*, pois são organismos seguros e que estão bem distribuídos no ambiente, bem como sobrevivem em ambientes com condições desfavoráveis, devido a produção de endósporos (Beskrovnaya et al., 2021). Estes bacilos são capazes de produzir uma variedade de antimicrobianos que é a primeira linha de defesa contra fitopatógenos e, além desse mecanismo, o controle biológico pode ser feito pelas bactérias por meio produção de compostos capazes de inibir o crescimento de diferentes patógenos (Liu et al., 2020; Alharbi et al., 2024). Tan et al. (2020) relataram a produção de enzimas que degradam estruturas vitais de determinados patógenos, como as paredes das células de fungos fitopatogênicos. Tendo em vista os problemas com fungos patogênicos enfrentados pela cultura do algodão, o objetivo foi verificar o potencial de isolados bacterianos de algodoeiro de inibir o crescimento *in vitro* de fitopatógenos comuns na cultura do algodão.

2. Material e métodos

2.1. Teste de antagonismo aos fitopatógenos *in vitro*

Foram utilizados para a triagem de antagonismo 56 isolados bacterianos provenientes do algodão; o isolamento ocorreu conforme descrito no Capítulo I desta tese. Os fitopatógenos *Corynespora cassiicola* e *Fusarium oxysporum* f. sp. *vasinfectum* foram isolados de plantas doentes e fornecidos pela Empresa Scheffer e Cia. Ltda.

Discos de 5 mm contendo micélio do fitopatógeno foram inoculados no centro de placas de Petri (90 mm de diâmetro), contendo meio BDA (Batata-Dextrose-Agar; Himedia), 24h antes da inoculação das bactérias ao experimento, mantidos a 28 °C. Após 24h, alíquotas de cultura de isolados bacterianos crescidos anteriormente em meio líquido DYGS (Rodriguez Neto et al., 1986) foram inoculados em quatro pontos simétricos a 2,5 cm do centro da placa de BDA (Figura 1A e 1C). Os discos inoculados apenas com o patógeno foram usados como controle nas mesmas condições de crescimento. As placas foram incubadas a 28 °C no escuro até que as placas do controle fossem completamente cobertas pelo patógeno (Figura 1A e 1C) (Zhang et al., 2017). Cada ensaio foi feito em triplicatas e o teste de inibição foi repetido duas vezes.

2.2. Cálculo de Taxa de Inibição (TI)

A Taxa de Inibição (TI) do crescimento do fitopatógeno foi calculada seguindo recomendação de Khedher et al. (2015), da seguinte forma:

$$TI\ (\%) = [(DC - DFC)/DC] \times 100$$

Sendo:

DC: Diâmetro do Controle

DFC: Diâmetro do Fitopatógeno Confrontado

2.3. Análise estatística

Os dados foram analisados utilizando o software SISVAR versão 5.8 (Build 92) (Ferreira, 2011), em que foram realizadas três repetições para cada ensaio, três repetições para cada estirpe bacteriana, sendo calculadas as médias de cada resultado utilizando a análise de variância (ANOVA) e comparadas por Teste de Tukey (1974) a 5% de probabilidade.

3. Resultados

Dos 56 isolados bacterianos utilizados para o ensaio de antagonismo aos fitopatógenos *Corynespora cassiicola* e *Fusarium oxysporum* f. sp. *vasinfectum,* 19 cepas bacterianas tiveram potencial antagonista com Taxa de Inibição (TI) que variaram de 39.43% a 71.89% (Tabela 1). Destes 19 isolados, 18 apresentaram antagonismo ao fitopatógeno *C. cassiicola*, que causa a doença mancha-alvo, e 10 tiveram potencial antagonista também para *F. oxysporum*, responsável pela murcha da parte aérea do algodão (Tabela 1).

Se tratando de antagonismo a *C. cassiicola*, das 18 bactérias positivas para inibição ao fungo, 10 isolados bacterianos apresentaram TI ≥ 60%, com destaque para o isolado PT01B3 que apresentou melhor resultado comparado às outras cepas (Tabela 1). Já para o antagonismo a *F. oxysporum*, das 10 positivas ao antagonismo, cinco apresentaram TI ≥ 60%, ressaltando o isolado P1T01B5 que apresentou melhor TI

comparando-se aos outros isolados bacterianos.

É possível verificar que foram obtidos resultados promissores tanto para o fitopatógeno *Corynespora cassiicola,* quanto para o *Fusarium oxysporum* f. sp. *vasinfectum*. O isolado P1T01B3 foi a melhor antagonista para *C. cassicola* e a segunda

melhor ao *F. oxyporum*, e o isolado P1T01B5 foi o que teve a maior TI para *F. oxyporum* e a segunda melhor para *C. cassicola* (Figura 1 B e D).

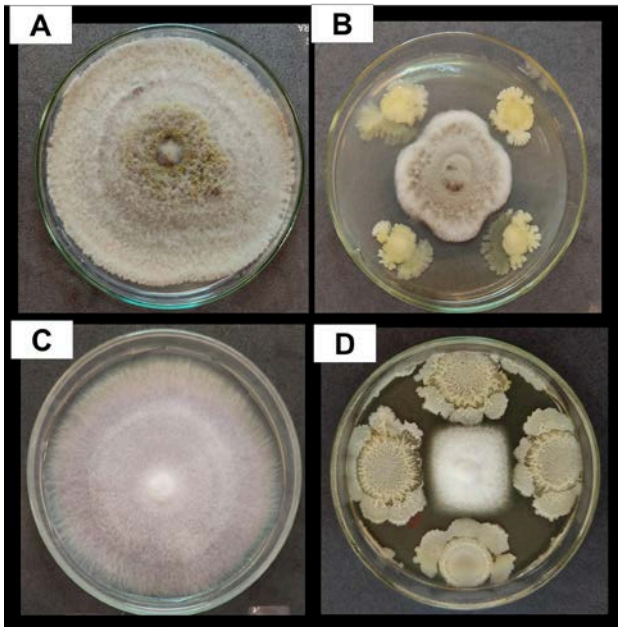

Figura 1 A: Fungo *Corynespora cassicola*, controle*;* C: Fungo F*usarium oxysporum*, controle; B: Antagonismos doisolado bacteriano P1T01B3 a *C. cassicola*; D: Antagonismos do isolado P1T01B5 a *F. oxysporum*.

Pode-se verificar que os isolados que não obtiveram resultados observados para algum dos fitopatógenos, como P5T01B1, P1T01B4, P1T32B6, P3T32B1, P1T01B8, P3T01B4, P1T01B10, P6T01B2, P6T32B1 e P1T01B6, apresentaram taxa de inibição ≥ 50%, o que mostrou que mesmo estas cepas não sendo positivas para os dois fungos, ainda assim ela tem um impacto no crescimento do fitopatógeno para o qual foi positiva (Tabela 1).

Com relação ao teste de agressividade do fungo, os observou-se que este não se mostrou capaz de causar sintomas da doença na planta. Dessa maneira, concluímos que há a necessidade de realizar outros métodos de inoculação além do uso concomitante de outras cepas de fungos, capazes de causar sintomas de doença, além do uso de cepas isoladas patogênicas isoladas de plantas adoecidas. Desta forma, poderão ser feitos testes de biocontrole *in vivo* em casa de vegetação para verificar a ação das bactérias

selecionadas nos testes *in vitro* e verificar seu potencial no controle do crescimento do fitopatógeno no tecido vegetal afetado.

Tabela 1 Taxas de Inibição dos isolados bacterianos aos fitopatógenos *Corynespora cassiicola* e *Fusarium oxysporum* f. sp. *vasinfectum*.

Taxa de Inibição (%)		
Isolado bacteriano	***C. cassiicola***	***F. oxysporum***
P2T01B3	53,04b	62,14ab
P5T01B1	53,95b	NO
P6T01B10	57,03ab	61,34ab
P1T01B4	57,43ab	NO
P6T32B7	58,25ab	39,43cd
P1T32B6	58,48ab	NO
P1T32B7	59,26ab	58,51ab
P3T32B4	59,81ab	64,58ab
P3T32B1	NO*	49,99bc
P1T01B8	60,58ab	NO
P5T32B1	60,91ab	56,66abc
P3T01B4	61,88ab	NO
P2T32B1	62,50ab	59,57ab
P1T01B10	63,03ab	NO
P6T01B2	64,36ab	NO
P6T32B1	65,39ab	NO
P1T01B6	66,69ab	NO
P1T01B5	70,05ab	69,50a
P1T01B3	71,89a	67,82ab

*NO: Não Observado. Letras minúsculas que acompanham os valores das Taxas de Inibição indicam diferenças estatísticas pelo teste de Tukey a 5% de probabilidade.

4. Discussão

Fitopatógenos são um dos principais causadores de danos à produção agrícola, afetando a qualidade e a quantidade gerada da produção. Assim, para evitar que os danos sejam ainda maiores, os produtores agrícolas utilizam agroquímicos que visam o controle de fitopatógenos de origem fúngica (Les et al., 2020). No entanto, a demanda de produtos que tenham uma produção mais sustentável está cada vez mais sendo validada pelos consumidores destes produtos e o uso de microrganismos na agricultura tem cada vez mais se tornado uma alternativa mais sustentável na melhora da produção (Djebaili et al., 2020).

Os testes *in vitro* representam uma ferramenta que possibilita selecionar microrganismos que podem desempenhar o papel de biocontrole contra microrganismos patogênicos (Hiremani et al., 2020; Clough et al., 2022). Pode-se verificar que no presente estudo há um grupo de isolados bacterianos que tem o potencial de serem bons biocontroladores, sendo que as cepas PT01B3 e P1T01B5 mostraram os melhores resultados *in vitro* no controle dos fitopatógenos, com taxas de inibição de crescimento que ficaram entre 67,82%, para *Fusarium oxysporum* e 71,89% para *Corynespora cassiicola* (Tabela 1).

Dos 56 isolados testados, 19 apresentaram alguma taxa de inibição com valores significativos, variando de 39,43% a 71,89%. No trabalho de Ocegueda-Reyes et al. (2019) foram verificados que de 656 cepas isoladas, apenas 23 apresentaram taxa de inibição contra o fitopatógeno *Sclerotium cepivorum*, e estes isolados apresentaram taxas que variaram de 21 a 24%. Esta observação mostrou que nem sempre os isolados irão apresentar taxas maiores de inibição na observação *in vitro*, porém aqueles que tiverem valores mais altos podem apresentar um potencial de controle que pode ou não

refletir nos testes *in vivo*.

Os resultados do presente trabalho mostraram que se pode obter isolados que desempenharam taxa de inibição de 71,89% para o fungo *Corynespora cassicola*, um bom resultado se comparado a outros trabalhos que tiveram taxas menores para o mesmo fungo. Os estudos de Zhang et al. (2023) observaram cepas que chegaram a inibir o crescimento de *C. cassicola* em até 67%.

É importante ressaltar que neste estudo pode-se observar que os isolados com resultados significantes tiveram potencial de inibição para ambos fitopatógenos, o que é de grande importância na agricultura, já que os isolados podem desempenhar controle de duas doenças na planta. Porém, nem sempre este resultado é constatado, como observado por Khalil et al. (2021), onde nenhum isolado teve o potencial de inibir duas cepas do fitopatógeno do gênero *Fusarium* testadas.

Não foi possível verificar a patogenicidade dos fungos estudados, pois os sintomas da doença não se apresentaram no tecido vegetal, mostrando que neste caso os métodos utilizados não foram suficientes para que houvesse sucesso no teste. Resultados positivos foram encontrados no trabalho de Nascimento et al. (2019) que obtiveram plantas com sintomas, utilizando métodos semelhantes aos usados no presente estudo. Portanto, é necessário realizar diferentes metodologias para alcançar resultados positivos, porém muitas das vezes fatores abióticos poderão interferir nesse resultado (Li et al., 2023).

De acordo com os resultados obtidos pode-se verificar que a obtenção de bactérias com potencial para bio controlar fitopatógenos pode ser um importante início para realizar estudos *in vivo*. Obteve-se mais de 30% de isolados bacterianos que tiveram potencial para inibição de crescimento dos fitopatógenos, alguns isolados com

mais de 60% de taxa de inibição. Porém, há a necessidade de realizar novamente testes relacionados à patogenicidade das cepas de fungos, verificando se este se encontra agressivo ou não com relação à doença.

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Considerações Finais

O presente trabalho mostrou o potencial de bactérias promotoras de crescimento vegetal que apresentaram a capacidade de promover o crescimento vegetal *in vitro* e *in vivo*. Esta característica pode indicar o uso destas cepas bacterianas em testes em campo, que pode trazer benefícios tanto para o meio ambiente, devido a diminuição do uso de insumos químicos, quanto para o agricultor, que poderá ter maior produtividade com menor custo com insumos. Além disso, foi observado o potencial destas cepas em controlar o crescimento de dois fitopatógenos de algodão *in vitro*, que também pode indicar a possibilidade do uso destes isolados como produto biológico no controle de doenças de origem fúngica. No entanto, é necessário ainda que se faça testes primeiramente *in vivo* em casa de vegetação para certificação deste potencial observado em laboratório.

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