UNIVERSIDADE FEDERAL DO AMAZONAS - UFAM
PROGRAMA MULTI-INSTITUCIONAL DE PÓS-GRADUAÇÃO EM BIOTECNOLOGIA - PPGBIOTEC

MINERAÇÃO GENÔMICA E IDENTIFICAÇÃO DE MOLÉCULAS COM POTENCIAL BIOTECNOLÓGICO DA MICROBIOTA AMAZÔNICA

JENNIFER SALGADO DA FONSECA

MANAUS-AM
2024

JENNIFER SALGADO DA FONSECA

MINERAÇÃO GENÔMICA E IDENTIFICAÇÃO DE MOLÉCULAS COM POTENCIAL BIOTECNOLÓGICO DA MICROBIOTA AMAZÔNICA

Orientadora: Dr. Gilvan Ferreira da Silva (Embrapa)
Coorientador: Prof. Dr. Hector Henrique Ferreira Koolen (UEA)

Tese apresentada ao Programa de Pós-Graduação em Biotecnologia da Universidade Federal do Amazonas como requisito para obtenção de título de Doutor em Biotecnologia.

Manaus - AM
2024

Ficha Catalográfica

Ficha catalográfica elaborada automaticamente de acordo com os dados fornecidos pelo(a) autor(a).

F676m

Fonseca, Jennifer Salgado da
Mineração genômica e identificação de moléculas com potencial biotecnológico da microbiota amazônica / Jennifer Salgado da Fonseca . 2024
98 f.: il. color; 31 cm.

Orientador: Gilvan Ferreira da Silva
Coorientador: Hector Henrique Ferreira Koolen
Tese (Doutorado em Biotecnologia) - Universidade Federal do Amazonas.

1. Química de produtos naturais. 2. Bactérias aquáticas. 3. bgc. 4. Rio Negro. 5. Rio Solimões. I. Silva, Gilvan Ferreira da. II. Universidade Federal do Amazonas III. Título

JENNIFER SALGADO DA FONSECA

MINERAÇÃO GENÔMICA E IDENTIFICAÇÃO DE MOLÉCULAS COM POTENCIAL BIOTECNOLÓGICO DA MICROBIOTA AMAZÔNICA

Esta tese foi julgada para a obtenção do título de Doutora em Biotecnologia e aprovada em sua versão final pelo Programa de Pós-Graduação em Biotecnologia da Universidade Federal do Amazonas.

Prof. Dra. Rosany Piccolotto Carvalho
Coordenador PPGBIOTEC

Apresentada perante a Banca Examinadora composta pelos professores:

Dr. Gilvan Ferreira da Silva, Presidente
Empresa Brasileira de Pesquisa Agropecuária – Embrapa-AM

Dra. Marisa Vieira de Queiroz
Universidade Federal de Viçosa – UFV-MG

Dra. Ana Francisca Tibúrcia Amorim Ferreira e Ferreira
Universidade Federal do Amazonas – UFAM-AM

Dr. Rogério Eiji Hanada
Instituto Nacional de Pesquisa da Amazônia – INPA-AM

Dra. Claudia Afras de Queiroz
Instituto Nacional de Pesquisa da Amazônia – INPA-AM

Manaus, 30 de setembro de 2024

AGRADECIMENTOS

A Deus e Nossa Senhora por tudo.

Ao Dr. Gilvan Ferreira da Silva, meus mais sinceros agradecimentos. O senhor me apresentou inúmeros desafios ao longo do caminho, sempre acreditando que eu era capaz de superar cada um.

Ao Prof. Dr. Hector Koolen, pela oportunidade, apoio e ideias que contribuíram para o desenvolvimento deste trabalho. Mas obrigada, especialmente, por mostrar com muito bom humor que eu tinha motivos para me apaixonar também pela química.

Ao Prof. Dr. Rogério Hanada e ao técnico Luiz Alberto (Tirico), por abrirem as portas do Laboratório de Fitopatologia do INPA e dedicarem tempo para me ensinar sobre o manejo de plantas.

Aos meus pais, Walmiza Salgado e Waldemar Fonseca Filho (*in memorium*), muito obrigada pelo amor, carinho e apoio, sempre incondicionais, ao longo de toda minha vida. Vocês são os verdadeiros motivos pela minha paixão pela ciência e meus pilares em cada passo que dei. Quando o desespero ameaçava roubar meu ar, foram as palavras de vocês que me fizeram voltar a respirar.

Aos meus avós, Waldemar (*in memorium*), Rosalina, Walter (*in memorium*), Neomiza, Santina e Clóvis por acreditarem e apostarem sempre no meu potencial. Vocês sempre tiveram uma palavra de sabedoria e humor, que apenas os avós tem, capaz de me fazer erguer a cabeça e rir da situação.

Aos meus irmãos, Jéssica e Renato que demonstraram total apoio e comemoraram cada nova conquista na minha carreira como se fosse deles.

Aos meus sobrinhos, Sophia e Júlio que preencheram meu coração com muito amor e alegria.

Aos meus tios e padrinhos, Filomena e Walter Salgado (*in memorium*), por sempre vibrarem a cada nova conquista e dizer, constantemente, que esse é apenas o começo de um futuro que ainda está por vir.

Aos meus primos, Bárbara e Wlademir que compartilham da trajetória na ciência e compreendem os desafios de um doutorado, oferecendo aquele apoio silencioso, porém significativo, de quem não precisa de uma explicação e não vai te fazer perguntas.

Ao meu companheiro, Erick Vanucci por sempre acreditar no meu potencial e me oferecer um abraço quando meu dia foi difícil, além de oferecer suporte braçal

durante os experimentos com os tomateiros. Obrigada por compreender as longas horas de trabalho e tentar aprender sobre meu trabalho.

A todos os colegas do Laboratório de Genômica e Microbiologia Aplicada da Amazônia Legal da Embrapa que estiveram ao meu lado ao longo desta caminhada, sobretudo Claudia Afras, Jeferson Cruz e Thiago Fernandes que me ensinaram novas técnicas que foram importantes para o trabalho e me ajudaram a conduzir alguns dos experimentos.

A todos os colegas do Laboratório de Metabolômica e Espectrometria de Massas da UEA, em especial Eldrinei Gomes e Moysés Araújo por sempre estenderem a mão quando tive dificuldade com as análises químicas.

As amigas e parceiras Suene Almeida e Carina Nascimento que preencheram meus dias com risadas e companheirismo no Laboratório de Fitopalogia. Sem a colaboração de vocês, os tomateiros teriam sofrido muito nas minhas mãos.

Aos meus melhores amigos de uma vida inteira, Sayara Santos e Charles Marques, por terem ficado ao meu lado durante mais esta caminhada.

Aos amigos, Thiza Souza, Felipe Valente, Renan Azulay, Luiz Eduardo, Hallana Cristina, Waldomiro Magalhães, Matheus Lobo e Thiago Azevedo por tornarem minha vida mais leve e sempre se mostrarem dispostos a me ajudar a qualquer instante.

Aos membros do Programa de Pós-graduação em Biotecnologia da Universidade Federal do Amazonas, pelo apoio ao longo destes quatro anos, sobretudo aos professores que fazem parte do corpo docente e que de certo modo contribuíram para minha formação profissional.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) e Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM), pela concessão de Bolsa de Doutorado.

RESUMO

A Amazônia, com sua vasta biodiversidade, abriga uma rede complexa de ecossistemas interconectados por rios como o Negro e o Solimões. Esta dinâmica única favorece o surgimento e a diversificação de linhagens microbianas, tornando a região um hotspot para a descoberta de soluções biológicas inovadoras. Partindo deste contexto, esta tese investigou o potencial biotecnológico de 36 bactérias isoladas de sedimentos dos rios amazônicos, com foco em aplicações no biocontrole de fitopatógenos e na promoção do crescimento vegetal. Os isolados bacterianos, foram avaliados in vitro contra fitopatógenos de importancia agrícola, como *Corynespora cassiicola*, *Colletotrichum siamense*, *Rhizoctonia solani* e *Ralstonia solanacearum*. Esta seleção inicial deu origem a dois capítulos principais: o primeiro investiga o biocontrole de *R. solanacearum*, enquanto o segundo examina o potencial genômico e as aplicações agrícolas de *Alcaligenes nematophilus* SOL 109. No capítulo 1, três isolados - *Priestia aryabhattai* RN 11, *Streptomyces* sp. RN 24 e *Kitasatospora* sp. SOL 195 - demonstraram notável eficácia contra *R. solanacearum*, com inibição in vitro de 87-100%, redução da incidência da doença em 40-90% em mudas de tomateiro e promoção do crescimento das plantas. Análises filogenômicas baseadas em ANI e dDDH revelaram que RN 11 pertence à espécie *Priestia aryabhattai* (ANI: 98,61%, dDDH: 88,3%), enquanto RN 24 e SOL 195 apresentaram valores abaixo dos pontos de corte para novas espécies, sendo potencialmente novas espécies dos gêneros *Streptomyces* e *Kitasatospora*, respectivamente. O capítulo 2 revela o potencial multifacetado de *A. nematophilus* SOL 109, incluindo a inibição in vitro de 74 a 93% contra os fungos fitopatogênicos. A avaliação em casa de vegetação indica que a linhagem SOL 109 foi capaz de controlar o patógeno *R. solani* e promover o crescimento em tomateiros. Análises genômicas e químicas de SOL 109 identificaram clusters de genes biossintéticos (BGCs) únicos e compartilhados no gênero *Alcaligenes*, genes de resistência a antibióticos e metais pesados, e metabólitos com propriedades antimicrobianas. Os resultados desta tese destacam o potencial inexplorado dos microrganismos aquáticos amazônicos, revelando novas espécies de actinobactérias (RN 24 e SOL 195) e relatando pela primeira vez a ocorrência de *A. nematophilus* no Brasil. Este estudo contribui significativamente para o desenvolvimento de estratégias sustentáveis de manejo de doenças em plantas, oferece insights valiosos sobre o metabolismo secundário do gênero *Alcaligenes* e abre novas perspectivas para aplicações biotecnológicas na agricultura, reforçando a importância da conservação e estudo da biodiversidade microbiana amazônica.

Palavras-chaves: Química de produtos naturais; bactérias aquáticas; BGC.

ABSTRACT

The Amazon, with its vast biodiversity, harbors a complex network of ecosystems interconnected by rivers such as the Negro and Solimões. This unique dynamic favors the emergence and diversification of microbial lineages, rendering the region a hotspot for the discovery of innovative biological solutions. Within this context, this thesis investigated the biotechnological potential of 36 bacteria isolated from Amazonian river sediments, focusing on applications in phytopathogen biocontrol and plant growth promotion. The bacterial isolates were evaluated in vitro against agriculturally significant phytopathogens, including *Corynespora cassiicola*, *Colletotrichum siamense*, *Rhizoctonia solani*, and *Ralstonia solanacearum*. This initial screening gave rise to two main chapters: the first investigates the biocontrol of *R. solanacearum*, while the second examines the genomic potential and agricultural applications of *Alcaligenes nematophilus* SOL 109. In Chapter 1, three isolates - *Priestia aryabhattai* RN 11, *Streptomyces* sp. RN 24, and *Kitasatospora* sp. SOL 195 - demonstrated remarkable efficacy against *R. solanacearum*, with *in vitro* inhibition of 87-100%, reduction of disease incidence by 40-90% in tomato seedlings, and promotion of plant growth. Phylogenomic analyses based on ANI and dDDH revealed that RN 11 belongs to the species *Priestia aryabhattai* (ANI: 98.61%, dDDH: 88.3%), while RN 24 and SOL 195 presented values below the cut-off points for new species, potentially representing novel species within the genera *Streptomyces* and *Kitasatospora*, respectively. Chapter 2 elucidates the multifaceted potential of *A. nematophilus* SOL 109, demonstrating *in vitro* inhibition ranging from 74 to 93% against phytopathogenic fungi. Under greenhouse conditions, evaluations indicate that the SOL 109 effectively controlled the pathogen *R. solani* and promoted growth in tomato plants. Genomic and chemical analyses of SOL 109 identified unique and shared biosynthetic gene clusters (BGCs) within the genus *Alcaligenes*, genes conferring resistance to antibiotics and heavy metals, and metabolites with antimicrobial properties. The results of this thesis highlight the unexplored potential of Amazonian aquatic microorganisms, revealing new actinobacterial species (RN 24 and SOL 195) and reporting for the first time the occurrence of *A. nematophilus* in Brazil. This study significantly contributes to the development of sustainable plant disease management strategies, offers valuable insights into the secondary metabolism of the genus Alcaligenes, and opens new perspectives for biotechnological applications in agriculture, reinforcing the importance of conservation and study of Amazonian microbial biodiversity.

Keywords: Natural products chemistry; aquatic bacteria; BGC

LISTA DE FIGURAS

LISTA DE TABELAS

Introdução

Capítulo 1

Capítulo 2

SUMÁRIO

Introdução

1. INTRODUÇÃO

O uso abusivo de compostos químicos nos setor agrícola representam um risco significativo ao meio ambiente e sistema de saúde devido as implicações na segurança alimentar e sustentabilidade agrícola (Shuqin; Fang, 2018).

Os químicos utilizados no setor agrícola estão relacionados tanto ao controle de pragas (insetos, roedores, ácaros e ervas-daninhas) quanto de microrganismos fitopatogênicos, assim como para a correção de nutrientes do solo, aceleramento do amadurecimento e estimulação de floração das plantas, contudo estes químicos representam um risco à saúde humana e animais, acarretam no desequilíbrio da comunidade rizosférica natural do solo, uma vez que a maioria possui na composição metais pesados como mercúrio (Hg), cádmio (Cd), chumbo (Pb), cobre (Cu) e níquel (Ni), e estudos indicarem o acúmulo de espécies de nitrato, como as nitrosaminas (cancerígenas), e fosfato que contaminam o solo e a água (Adedayo et al., 2022; Ajilogba; Babalola, 2013; Sehwarat et al., 2022; Savci, 2012ab; Schiesari, 2012).

Como alternativa a estes químicos, os microrganismos e os produtos naturais que produzem são visados tanto para promover o crescimento vegetal, através da produção de compostos como sideróforos, auxinas e solubilizadores de fosfato e zinco, quanto para fazer o biocontrole de fitopátogenos através de mecanimos de indução de resistência, antibiose (antimicrobianos e enzimas hidrolíticas) e competição por nutrientes e espaço (Abo-Zaid et al, 2023; Deveau et al., 2018; Legein et al., 2020; Zengler; Zaramela, 2018).

Segundo o Ministério da Agricultura e Pecuária do Brasil (2022ab), o Brasil é referência mundial na utilização de defensivos biológicos aplicados em campo e possui ao todo 552 defensivos registrados disponíveis para comercialização no país, sendo alguns a base microrganismos como *Bacillus amyloliquefaciens, Bacillus subtilis, Bacillus pumilus, Bacillus velezensis, Paecilomyces lilacinus, Pseudomonas flurorescens, Pseudomonas chlroraphis, Isaria fumosorosea, Beauveria bassiana, Metarhizium anisopliae, Trichodema asperellum* e *Trichoderma harzianum*

No Amazonas, além da presença de fitopatógenos amplamente pesquisados como *Rhizoctonia solani* e *Corynespora cassiicola*, há outros que não estão disseminados por todo o país como, *Ralstonia solanacerum*, bactéria responsável pela murcha-bacteriana em plantas como as solonáceas e banana (Coelho Netto et al., 2003, 2004; Gasparotto, 2022; Leroy; Lourd, 1989). Neste contexto, o trabalho se focou em aplicar bactérias isoladas dos sedimentos dos rios Negro e Solimões

com potencial de biocontrole de fitopatógenos e de produção de promotores de crescimento vegetal em cultivares de tomate, além de fazer a mineração genômica e anotação dos compostos químicos produzidos pelas bactérias.

2. REFERENCIAL TEÓRICO

2.1 BACTÉRIAS

As bactérias, mesmo sendo procariotos relativamente simples, possuem um potencial biotecnológico elevado para a produção de bioativos de alto valor. Estes microrganismos estão amplamente distribuídos nos ecossistemas e podem ser encontradas inclusive em ambientes considerados inóspitos devido ao elevado índice de elementos como ferro ou enxofre ou a baixa ou nula presença de oxigênio. Por terem essa versatilidade, a probabilidade de terem genes biossintetizadores de metabólitos secundários (smBGC) é considerada alta (Cordero; Datta, 2016; Jackson et al, 2018; Netzker et al, 2015)

Assim como outros microrganismos, contribuem para a manutenção do ciclo de carbono e nitrogênio e balanço de outro nutrientes presentes no ecossistema. Apesar de serem procariotos, possuem alta capacidade adaptativa, o que permite encontrar representantes deste grupo nos mais diversos ambientes, tais como rochas, geleiras, desertos, etc (Timmusk et al., 2011).

Apesar de amplamente distribuídos e com estudos em laboratório há mais de 150 anos, os especialistas apontam que ainda há muitos gêneros desconhecidos, uma vez que há cerca de 12 mil espécies já catalogadas e a estimativa global seja entre 10^7 e 10^9. Destes, estimasse que pelo menos 54 mil estejam amplamente distribuídos no solo e 37 mil nos oceanos (Overmann et al., 2017; Banerjee et al., 2018).

A genômica microbiana, correlacionada com estudos enzimáticos, indica um grande potencial de biossíntese de produtos naturais, especialmente terpenoides, pelas bactérias. Entre 2010-2013, ocorreu um pico de descoberta de novas moléculas desta classe (Figura 1A) e, aproximadamente, na mesma época, os estudos para alternativas de hospedeiros para a produção dos mesmos iniciou (Moser; Pichler, 2019; Rudolf et al., 2021).

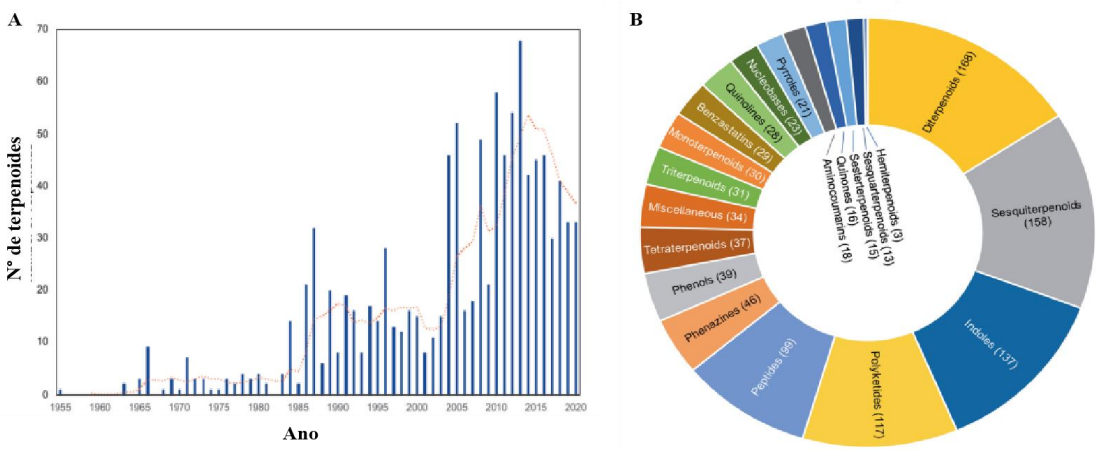

Figura 1. Resumo dos terpenóides bacterianos na literatura. (A) Número de terpenóides descobertos em bactérias por ano. A linha laranja é um movimento médio de cinco anos. A contagem para 2020 inclui apenas até meados de 2020. (B) Distribuição de terpenóides. Os números entre parênteses representam número de terpenóides bacterianos compilados na revisão de Rudolf et al. (2021).

Dentre as bactérias já descritas como hospedeiros para a produção de terpenoides, pode-se mencionar *Bacillus subtilis, Escherichia coli, Streptomyces sp, Pseudomonas putida, Rhodobacter sphaeroides, Corynebacterium glutamicum* e *Methylobacterium extorquens*, sendo os dois primeiros os principais utilizados (Moser; Pichler, 2019).

Apesar do ligeiro declínio na elucidação na novas moléculas, estima-se que é possível encontrar novas vias biossintéticas e características bioquímicas desconhecidas ao explorar linhagens bacterianas pouca exploradas, tendo portando o potencial de oferecer novas e inovadoras soluções para biotecnologia, agricultura e saúde pública (Pilla; Tang, 2018; Jackson et al., 2018).

2.2 FERTILIZANTES

Todas as plantas precisam receber os nutrientes necessários para o seu desenvolvimento em cada etapa do ciclo de vida. Elas absorvem o que precisam do solo, contudo, nem sempre existe a disponibilidade de todas as substâncias, sendo necessário a suplementação destes nutrientes através da fertilização do solo. Os fertilizantes químicos são os produtos mais utilizados, contudo representam um problema à saúde pública, políticas de sustentabilidade e poluição ambiental (Adedayo et al., 2022; Ajilogba; Babalola, 2013; Savci, 2012ab; Shuqin; Fang, 2018)

Existem três tipos de fertilizantes químicos: minerais (nitrogenados, potássicos, fosfatados, mistos ou corretivos), orgânicos (a partir de restos de animais

ou vegetais) e organominerais (mistura de material orgânico com minérios), sendo comercializados em formato sólido, líquido ou quelato. Muitos possuem na sua composição metais pesados como mercúrio (Hg), cádmio (Cd), chumbo (Pb), cobre (Cu) e níquel (Ni), e estudos indicam o acúmulo de espécies de nitrato, como as nitrosaminas (cancerígenas), e fosfato que contaminam o solo e a água (Fixen;West, 2002; Jiao et al, 2012; Savci, 2012ab; Sehwarat et al., 2022; Sharma; Chetani, 2017; Schiesari, 2012; Souri, 2016; Wang et al, 2018; Zörb et al., 2014).

A indústria de fertilizantes leva em conta alguns fatores para estabelecer o consumo global, tais como crescimento populacional (estimativas de chegar 9,7 bilhões até 2050), redução de áreas próprias para cultivo, crescimento do Produto Interno Bruto (PIB) mundial, política e uso alternativo das plantações como, por exemplo, a formulação de biocombustíveis, acarretando na alta volatilidade dos preços do mercado. Além destes fatores, entre 2020 e 2024, outros acontecimentos contribuíram para o desabastecimento e alto preço de fertilizantes: pandemia por COVID-19, o bloqueio na exportação de tecnologias pela China e o conflito entre Ucrânia e Rússia (Arndt et al., 2023; Azuizion, 2020; Hebebrand; Glauber, 2023; Höhler; Lansink, 2020; Poudel et al., 2020; Pujiharto; Wahyuni, 2023; Stamenković et al., 2018).

Devido a pandemia com o coronavírus (COVID-19), a economia mundial foi duramente afetada, principalmente nos setores de fornecimento de alimentos e redes de suprimentos. As companhias líderes de fornecimento de fertilizantes, principalmente as situadas na Rússia, declararam uma relativa estabilidade nesse período, porém o preço de mercado dos produtos aumentou devido as políticas de *lockdown* na tentativa de conter os efeitos do coronavírus até a formulação de vacinas e estabilização de práticas sanitárias eficazes (Höhler; Lansink, 2020; Ilinova et al., 2021; Paudel et al., 2023; Poudel et al., 2020; Sridhar et al., 2023).

Antes da estabilização econômica e sanitária, em 2022, a Rússia invadiu a Ucrânia, iniciando um conflito que dura até os dias atuais. Além de afetar os preços de fertilizantes, também afetou o setor de alimentos e combustíveis (Arndt et al., 2023; Hebebrand; Laborde, 2022; Shanini et al., 2022). Devido aos altos preços, recursos esgotáveis para a produção e a instabilidade do mercado de fertilizantes químicos, além das políticas de sustentabilidade, novas tecnologias estão emergindo para competir e, possivelmente, substituir os químicos, como o uso de microrganismos e seus compostos para atuarem como fertilizante (Bala, 2022;

Bargaz et al., 2018; Bamdad et al., 2022; Stamenković et al., 2018; Vurukonda et al, 2024).

Segundo Malusá et al. (2012), os três principais grupos de microrganismos considerados benéficos para a nutrição das plantas são os fungos micorrízicos arbusculares (FMA), as rizobactérias promotoras do crescimento das plantas (PGPR) e os rizóbios fixadores de nitrogênio. Pathak e Kumar (2016) dizem que, ao fazer uma bioformulação de base microbiana, pode-se classificar estas formulações em quatro tipos: (1) bactérias fixadoras de nitrogênio, (2) microrganismos solubilizadores/mobilizadores de P, (3) microrganismos de compostagem e (4) biopesticidas.

Segundo Bamdad et al. (2022), os microrganismos do solo podem ser divididos em dois grupos principais: indígenas ou estrangeiros. Os micróbios indígenas são espécies nativas de uma determinada região, enquanto aqueles considerados estrangeiros são espécies cultivadas em condições otimizadas de laboratório. Também afirma que a adição de culturas microbianas pré-cultivadas ao solo melhora as suas propriedades, aumenta o rendimento do crescimento e degrada compostos indesejados, como por exemplo fazer biorremediação de metais pesados. Além disso, a injeção de nutrientes/componentes suplementares pode induzir a propagação da população microbiana nativa a uma taxa elevada, aumentando os benefícios adquiridos pela planta.

Para desenvolver os biofertilizantes, além avaliar se possuem os mecanismos básicos de fixação de nitrogênio e a solubilização de fosfato, deve-se levar em consideração os benefícios da interação planta- micróbio e o microbioma natural do solo e se possuem a habilidade de produzir hormônios vegetais (AIA, giberilina, etc), sideróforos (pyoverdine, pyochelin, etc), cianeto (HCN) e enzimas líticas que têm efeito fitoestimulante na planta (Bambad et al., 2022; Stamenković et al., 2018; Timmusk et al., 2017). Alguns gêneros de bactérias, que demonstraram potencial para serem bactérias promotoras de crescimento vegetal (PGPB), são amplamente estudados tais como *Bacillus, Pseudomonas, Azospirillum, Bradyrhizobium, Rhizobium* (Araujo et al., 2020; Egamberdieva et al., 2017; Pastor-Bueis et al., 2019; Rékási et al., 2019).

Além de serem estudadas para a formulação de biofertilizantes, bactérias destes gêneros, em alguns casos, também atuam como biodefensivos contra microrganismos fitopatógenos, como no caso.

2.3 DEFENSIVOS AGRÍCOLAS

Defensivos agrícolas são produtos químicos, físicos ou biológicos usados no controle de seres vivos considerados nocivos ao homem, sua criação e suas plantações. São também conhecidos por agrotóxicos, pesticidas, praguicidas ou produtos fitossanitários.

Entre os defensivos agrícolas são encontrados produtos que controlam plantas invasoras (herbicidas), insetos (inseticidas), fungos (fungicidas), bactérias (bactericidas), ácaros (acaricidas) e ratos (rodenticidas). Também são considerados defensivos agrícolas os reguladores de crescimento, que aceleram o amadurecimento e floração de plantas, por exemplo. Os mesmos são categorizados em quatro classes quanto ao risco de toxicidade ao meio ambiente e ao ser humano, sendo I o mais perigoso e o IV o mais seguro para uso (Schiesari, 2012).

Dito isto, busca-se alternativas aos produtos químicos comercialmente utilizados, sendo estes os chamados agentes microbiológicos de controle (AMCs), também conhecidos como biodefensivos. Estes agentes são microrganismos capazes de promover uma barreira de proteção natural contra pragas e outras doenças que atingem plantações, não agride o homem e nem ataca de forma maléfica as estruturas das plantas. A capacidade de colonizar a rizosfera das plantas é um ponto chave neste processo, uma vez que um agente de controle biológico que não apresente capacidade de crescer na rizosfera não poderá competir por espaço e nutrientes (Almeida, 2020; Halfeld-Vieira et al., 2016)

No mercado se encontram produtos a base de espécies de *Trichoderma, Bacillus, Pseudomonas* e algumas *Streptomyces,* que são aplicados não apenas para o controle biológico, mas também para a promoção de crescimento das plantas, uma vez que estes microrganismos produzem compostos promotores de crescimento vegetal. Além disso, há produtos há base produtos naturais, como no caso de Baryon, Bioativus, Bionautus, Simetria e Stimutrop (Biotrop, 2024; Desangoosse, 2024; Sadeghi et al, 2009; Karima; Nadia, 2012).

2.4 PRODUTOS NATURAIS

O uso de produtos naturais é retratado ao longo da história da humanidade, principalmente, para uso medicinal na forma de chás, óleos, emplastros e unguentos preparados com insumos terrestres e marinhos usando conhecimento tradicional (Dias et al., 2012).

Por definição, são considerados produtos naturais os metabólitos que são extraídos de organismos vivos (plantas, animais e microrganismos), assim como os produtos derivados destes que possuem atividade biológica. Alguns grupos se destacam, tais como NRPS, PKS e terpenos(Abdel-Razeck et al., 2020; Bernadini et al., 2018; Dias et al., 2012).

Estes bioativos possuem uma variedade química diversa que permitem inúmeras aplicações biológicas (antimicrobiano, imunosupressivo, anticâncer, anti-inflamatório, etc), sendo possível alterar completamente propriedade e/ou inviabilizar ao mudar a posição, remover e/ou acrescentar elementos químicos em uma molécula (Abdel-Razeck et al., 2020; Pham et al., 2019).

No levantamento com dados entre 1981 e 2014, foi observado que entre as 1211 moléculas aprovadas como fármacos pelo FDA (*Food and Drug Administration*) dos Estados Unidos, cerca de 65% possuem uma profunda correlação com as estruturas encontradas nos produtos naturais ou são derivados diretos de alguma fonte biológica (Paulo et al., 2019). É importante destacar que nem todo produto natural, independente da sua fonte, é benéfico para uso humano, uma vez que muitos compostos podem ser tóxicos. Por este motivo, todos os estudos que envolvem o desenvolvimento de produtos com bioativos passa por uma série de ensaios de toxicidade e caracterização química (Shen, 2015).

O campo de estudo de produtos naturais nos últimos dez anos vem passando por uma rápida inovação e avanço tecnológico ao combinar abordagens químicas, biológicas e de bioinformática para elucidar a diversidade, distribuição e função dos produtos naturais (Santen et al., 2019; Pham et al., 2019).

2.4.1 Policetídeo sintase (PKS)

Os policetídeos são uma classe de produtos naturais com uma gama de atividades biológicas biossintetizados por grandes blocos enzimáticos multimodulares, denominados policetídeos sintases (PKS). Apesar de se tratar de um grupo heterogêneo de estruturas, que compreende compostos como os poliéteres, polienos, polifénois, macrolídeos e enediinos, todos os policetídeos são derivados de um dos blocos construtores mais simples, o acetato o (CH_3COO^-), qual é usado para formar os precursores empregados na construção das cadeias: metilmalonil-CoA, propanoil-CoA e acetil-CoA (Kohlhaas et al., 2013; Miyanaga, 2017; Paulo et al., 2019).

Como mencionado anteriormente, as PKS são formadas por diversos blocos enzimáticos, conhecidos como domínios (Tabela 1). Alguns são peças essenciais para obter um policetídeo funcional, enquanto outros são facultativos, acrescentando maior complexidade à molécula (Herbst et al., 2018; Paulo et al., 2019).

Tabela 1. Domínios essenciais e facultativos mais comuns em policetídeos sintases. Fonte: Adaptado de Herbst et al., 2018, Kohlhaas et al., 2013 e Paulo et al., 2019.

Domínio (sigla)	Nome	Função	Essencial
AT	Acetiltransferase	Recrutar uma unidade extensora de acetil-CoA	Sim
ACP	Proteína carreadora de acila	Carregador da cadeia policetídica em formação	Sim
KS	Cetoacila sintase	Catalisar a formação de ligação C-C através da condensação descarboxilativa	Sim
KR	Cetorredutase	Redução da carbonila ao álcool	Não
ER	Enoila redutase	Converte olefina na cadeia saturada	Não
DH	Desidratase	Elimina água, levando a formação de olefina	Não
TE	Tioesterase	Tranesterificação do policetídio, isto é, removendo a molécula do complexo enzimático.	Não
MET	Metiltransferase	Recrutar CH_3 para "adornar" o policetídeo	Não
MAT	Malonil-acetil transferase	Recrutar uma unidade extensora de malonil-CoA	Não

As PKS são classificadas em três tipos: PKS I, PKS II e PKS III (Figura 2). As do tipo I são encontradas em fungos e bactérias e são caracterizadas por ter múltiplos módulos subdivididos em vários domínios. As do tipo II são encontradas em bactérias e são caracterizadas por terem domínios catalíticos discretos. As do tipo III são, comumente, encontradas em plantas e bactérias e são enzimas do tipo chalcona sintase com um único sítio ativo (Nivina et al., 2019; Paulo et al., 2019).

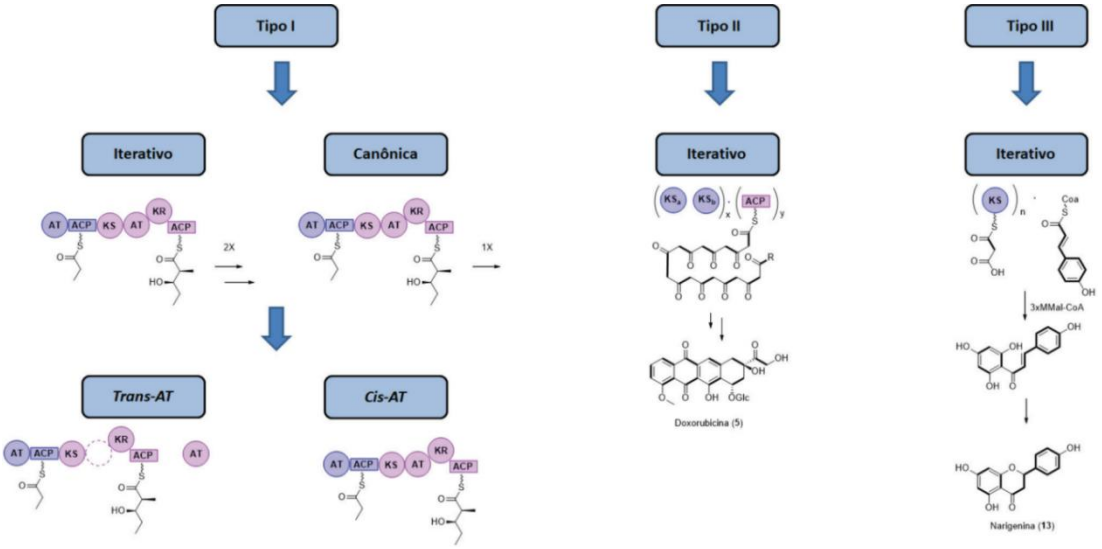

Figura 2. Classificação das sintases de policetídeos com relação a sua estrutura (tipo I, tipo II e tipo III), do modo de condensação dos substratos (iterativo ou canônico) e com relação a colinearidade dos domínios AT (trans-AT ou cis-AT). Fonte: Paulo et al., 2019.

As PKS tipo I podem atuar de modo iterativo, isto é, os domínios se repetem durante a biossíntese, ou não-iterativo (canônico), onde cada ciclo de extensão da cadeia policetídica é realizado por um módulo específico e os domínios não se repetem. Outra particularidade está relacionada ao domínio AT que pode ser *trans*-AT (não está alinhado aos demais domínios) ou *cis*-AT (alinhado com os domínios).

Os trans-AT PKSs são notórias por sua alta diversidade de módulos não-canônicos que, muitas vezes, contêm novos domínios enzimáticos, produzindo bioativos de relevância terapêutica e responsáveis por fatores patogenicidade, simbiose e regulação de bactérias. Muitos sistemas trans-AT incluem módulos de peptídeo não-ribossomal sintase (NRPS), resultando em híbridos NRPS – PKS. Em casos como esse, os domínios KS *downstream* aceitam intermediários derivados de NRPS atípicos (Kohlhaas et al., 2013; Helfrich et al., 2019).

Em sistemas cis-AT, existem poucas informações a respeito dos fatores que promovem a associação física entre PKS e NRPS, acrescentando um tópico de estudo no campo de engenharia genética (Kohlhaas et al., 2013).

Dentre os policetídeos já presentes no mercado, alguns se destacam pela sua alta taxa de eficiência com atividades biológicas diversas: lovastatina (redutor do nível de colesterol), eritromicina (antibiótico), anfotericina (antifúngico), avermectina (anti-tuberculose), doxorubicina e salinomicina (antineoplásicos) (Paulo et al., 2019).

2.4.2 Peptídeo não-ribossomal sintase (NRPS)

O ribossomo é uma importante organela para a produção de peptídeos e proteínas, sendo descoberto o funcionamento do mecanismo de síntese de proteínas por volta de 1960. Em 1963, ao realizar experimentos com espécies de *Bacillus*, descobriram que a produção do peptídeo tirocidina não era afetada mesmo com o uso de inibidores ribossomais, o que levou a hipótese de que haveria uma outra rota alternativa para a produção de peptídeos fora do ribossomo (Mach; Reich; Tatum, 1963; Sussmuth; Mainz, 2017)

Peptídeos não-ribossomais sintases são complexos multienzimáticos, isto é, com múltiplos domínios com funções específicas, com diversas aplicações (antibactericida, antitumor, imunosupressor, etc), similar ao que observamos nas policietídeos sintases, usando aminoácidos como bloco construtores, assim como os peptídeos produzidos em ribossomos. São comuns em bactérias e fungos (Izoré; Cryle, 2018; Sussmuth; Mainz, 2017).

O sistema de produção de peptídeos não-ribossomais (NRP), além de fazer uso de uma ampla variedade de monômeros (L-α-aminoácidos e não-proteinogênicos α e β aminoácidos), permite que sejam feitas modificações através de domínios catáliticos específicos encontrados em algumas NRPS. Dentre os monômeros não-proteinogenicos α e β aminoácidos, pode-se citar fenil-glicina, guanidinas cíclicas e aminoácidos contendo grupos alceno, alcino, halo, hidroxil ou ciclopropil (Izoré; Cryle, 2018).

Como mencionado anteriormente, as NRPS são formadas por diversos blocos enzimáticos, conhecidos como domínios (Tabela 2), que se organizam em módulos. Em suma, cada módulo corresponde a um ciclo completo para a extensão da cadeia peptídica. Alguns domínios são peças essenciais para obter uma NRPS funcional, enquanto outros são facultativos, acrescentando maior complexidade à molécula (Pichi et al., 2009; Sussmuth; Mainz, 2017).

Tabela 2. Domínios essenciais e facultativos mais comuns em peptídeos não-ribobossomais sintases (NRPS). Fonte: Pichi et al., 2009; Sussmuth; Mainz, 2017.

Domínio (sigla)	Nome	Função	Essencial
A	Adenilação	Reconhecimento e ativação de aminoácidos específicos através de reações de adenilações com Acyl-CoA, acarretando na formação de um aminoacil adenilato.	Sim
PCP	Proteína carreadora de peptídeo	Carregador da cadeia peptídica em formação através de reações de tiolação contendo um grupo prostético 4'-fosfopanteína.	Sim
C	Condensação	Catalisar a formação de ligação peptídica dos entre os mônomeros de aminoácidos e o peptídeo formado nos módulos iniciais da NRPS através do ataque nucleofílico do grupo α-amino livre de um substrato no sítio aceptor à ligação tioéster de um substrato vizinho (aminoácido ou peptídeo) no sítio doador, promovendo o elongamento da cadeia peptídica.	Sim
TE	Terminação	Está presente na porção C-terminal da NRPS (último módulo) e é responsável pela tioesterificação do peptídeo formado e consequente liberação da molécula.	Sim
E	Epimerização	Catalisa a racemização do L-aminoácidos, permitindo a presença de D-aminoácidos nos peptídeos formados.	Não
Mt	Metilação	Acrescentar grupos metila no peptídeo em formação tanto em grupo amino como carboxi.	Não
R	Redução	Substitui o domínio TE e reduz a porção aldeído ou alcóolica do peptídeo com uma redutase dependente de NADPH.	Não
F	Formilação	Acrescenta grupos formila	Não
Cy	Ciclização	Homólogo do domínio C com função adicional de heterociclização, porém atacando o resíduo de asparagina (Asp) ao invés da serina (Ser)	Não

Para ilustrar o que foi descrito acima, usaremos como exemplo a formação de virginiafactina (Figura 3) produzida por *Pseudomonas* sp. QS1027.

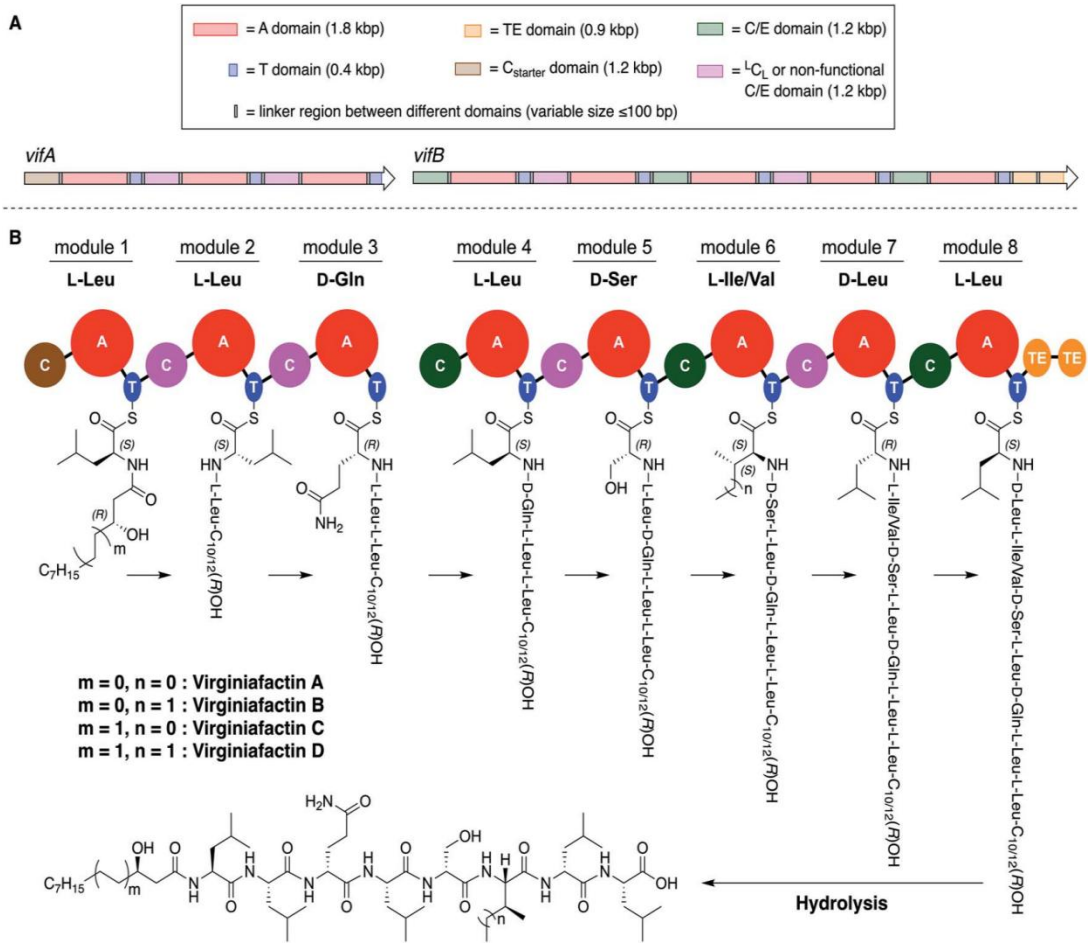

Figura 3. (A) Organização dos múltiplos domínios que compõem o gene para biosíntese de virginiafactinas. (B) Proposta da biossíntese de virginiafactinas. As diferentes cores do dominío C indicam a família a qual pertencem (Marrom - Cstarter; Rosa - LCL; Verde - epimerase). Fonte: Gotze et al., 2019.

Como visto, cada domínio A recebe um aminoácido em específico para incorporar na cadeia peptídica, selecionando inclusive se é L ou D. Em alguns casos, é possível ocorrer o que é observado no módulo 6 onde mais de um aminoácido é reconhecido (Gotze et al., 2019).

Através dos estudos do genoma usando ferramentas de bioinformática, algumas rotas de biossintese de NRPS já foram elucidadas tanto em bactérias quanto em fungos (Tabela 3), tendo alguns grupos com estudos mais completos devido sua relevância médica, agrícola e/ou industrial (Sussmuth; Mainz, 2017).

Tabela 3. Importantes filos e gêneros bacterianos e fúngicos que contém genes de NRPS. Fonte: Adaptado de Sussmuth; Mainz, 2017.

Microrganismo	Filo/classe	Gênero	Compostos relevantes
Bactéria Gram-positiva	Actinobacteria	*Streptomyces*	Glicopeptídeos
		Mycobacterium	Micobactina (sideróforo)
	Cianobacteria	*Microcystis, Planktothrix, Anabaena, Oscillatoria* e *Nostoc*	Microcistina, Cianopeptolinase e Criptoficina
	Firmicutes	*Bacillus*	Lipociclopeptídeos
		Staphylococcus	Aureusimina
		Streptococcus	Mutanobactina
Bactéria Gram-negativa	β-Proteobacteria	*Burkholderia*	Malleobactina (sideróforo)
		Pseudomonas	Siringomicina e Pioverdina
		Escherrichia	Enterobactina
		Salmonella	Samochelina
		Yersinia	Yersibiabactina
		Vibrio	Vibriobactina
		Serratia	Surfactina
	γ-Proteobacteria	*Photorhabdus*	Peptídeos lineares e cíclicos
	δ-Proteobacteria	Myxobacterium (ordem)	Argirina e híbridos de PK-NRP
Fungo	Ascomycota	*Talypocladium*	Ciclosporina A
		Fusarium	Eniatina
		Penicillium	Penicilina
		Acremonium	Cefalosporina C
		Claviceps	Ergopeptinas
		Trichoderma	Peptaiboles
	Basidiomycota	*Ustilago*	Ferrichrome

2.4.3 Terpeno

Historicamente, terpenos foram primeiro identificados em óleos essenciais de plantas, porém já foi comprovado a presença em fungos, algas, plantas, alguns animais (insetos e organismos marinhos, principalmente) e bactérias (actinomicetos, cianobactérias e mixobactéria, principalmente). São conhecidos mais de 55 mil diferentes metabólitos secundários constituídos de terpenos atuando, por exemplo, como: aromatizante, composto de defesa contra vírus, bactérias e parasitas em plantas, antioxidante, anti-inflamatório e anti-cancerígeno (Gonzales-Burgos; Gómez-Serranillos, 2012; Rabe et al., 2013; Salha et al., 2019).

São compostos por unidades de funcionais de isoprenos (pirofosfato de isopentenilo), sendo divididos (Figura 4) em hemiterpenos (1 unid. de isopreno - C_5), monoterpernos (2 unid. de isopreno - C_{10}), sesquiterpenos (3 unid. de isoprenos - C_{15}), diterpenos (4 unid. de isopreno - C_{20}), triterpenos (6 unid. de isopreno - C_{30}), tetraterpenos (8 unid. de isopreno - C_{40}) e politerpenos (8 unid. de isopreno <n>30.000 - C_{5n}) (Salha et al., 2019).

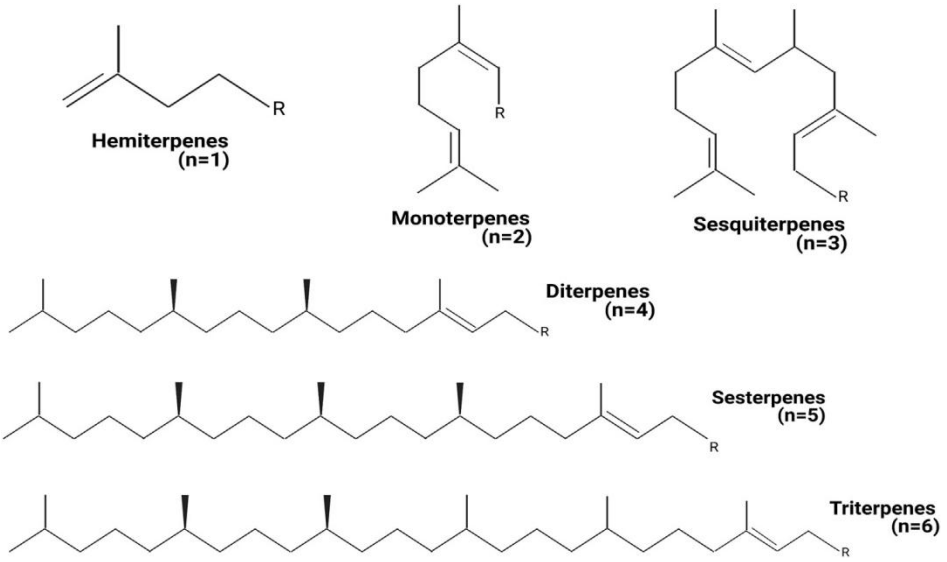

Figura 4. Classificação de terpenos. Fonte: Prado-Audelo et al., 2021.

No sistema biológico, terpenos são gerados usando a enzima terpeno ciclase a partir de precursores lineares: geranil difosfato (GPP, monoterpenos), farnesil difosfato (FPP, sequiterpenos) e geranil-geranil disfosfato (GGPP, diterpenos) (Rabe et al., 2013).

Pode-ser citar alguns terpenos com propriedades conhecidas e amplamente comercializados, tais como: borneol (cardioproteção), eugenol (gastroproteção), guaiazulene (hepatoproteção), eremanthin (diabetes), ent-3ß-butanoyloxykaur-15-en-17-ol (neuroproteção), carnosol (hepato e gastroproteção), ácido 23-hydroxytormentic (nefroproteção), platycodin A (neuroproteção), ß-caroteno (neuro e cardioproteção e diabetes), licopeno (proteção ocular, hepato, nefro e cardiológica e controle de diabetes) e crocin (proteção neuro, nefro e cardiológica e controle de diabetes) (Gonzales-Burgos; Gómez-Serranillos, 2012).

Dentre os terpenos mais conhecidos que são sintetizados por bactérias (Figura 5), destacam-se alguns como o pentalene (antiaromático), albaflavenone (atividade antibiótica e aromatizante), geosmin (aromatizante e alerta bioquímico

quanto a interação de predação microbiana) (Yamada et al., 2015; Zaroubi et al., 2022; Zhao et al., 2008).

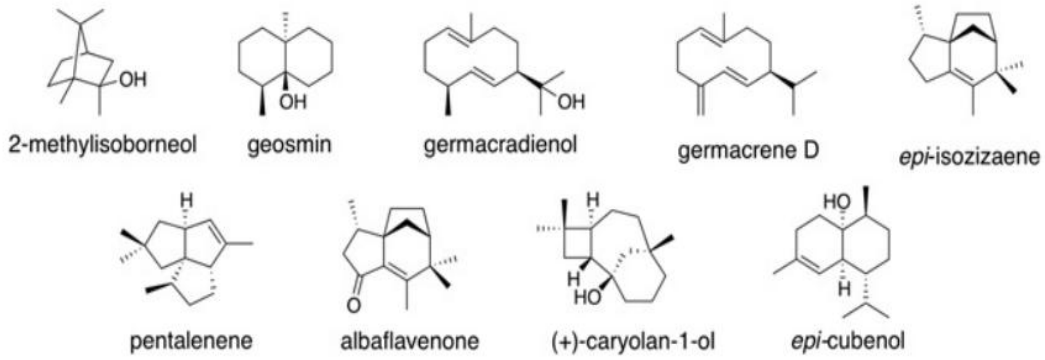

Figura 5. Estruturas dos terpenos produzidos por bactérias mais conhecidos. Fonte: Yamada et al., 2015.

2.5 TOMATEIRO

A cultura do tomateiro (*Solanum lycopersicum L.*) é originária da América do Sul, e está amplamente distribuída pelo mundo inteiro para consumo tanto *in natura* quanto industrializado, sendo fonte de antioxidantes como o licopeno (Dahlke et al., 2019; Duma et al., 2015).

O tomateiro é uma planta herbácea de porte arbustivo e possui caule flexível, piloso e com abundante ramificação lateral. Suas flores são hermafroditas, pequenas e reunidas em cachos. Os frutos são bagas carnosas de tamanhos e formatos variáveis conforme a cultivar e, quando maduros, apresentam coloração vermelha. Possuem sementes pequenas, pilosas e imersas na mucilagem placentária (Aguiar, 2015; Clemente; Boiteux, 2012).

A planta possui dois hábitos de crescimento que condicionam o tipo de cultivo: o hábito indeterminado, onde ocorre predominância da gema apical sobre as gemas laterais, comum na maioria das cultivares para a produção de frutos para mesa; e o hábito determinado, onde há crescimento vegetativo menos vigoroso e a planta assume a forma de uma moita, predominante na produção de frutos para a agroindústria (Aguiar, 2015; Filgueira, 2013).

Em 2022, a produção brasileira de tomate chegou a cerca de 3,8 milhões toneladas, sendo os maiores estados produtores Goiás (27,5%), São Paulo (24,3%), Minas Gerais (14,2%) e Paraná (7,1%). O Amazonas ocupa o 3º lugar relativo ao consumo de tomate no Brasil, porém mais de 90% do tomate comercializado em

Manaus vem de outros estados (Almeida et al., 2023; Gama et al., 2008; IBGE, 2022ab).

A produção agrícola no Amazonas é prejudicada por fatores climáticos (altas temperaturas e precipitações), incidência de doenças causadas por microrganismos (*damping-off,* macha-alvo e murcha-bacteriana), solo pobre em nutrientes e área limitada para plantio, uma vez uma porção do território é afetada com inundações periodicamente. A população local, os chamados ribeirinhos, desenvolveram o sistema de cultivo, não apenas do tomate, mas também de outras hortaliças, baseado de acordo com o regime fluvial de cheia (dezembro-maio) e vazante (junho-novembro), o que impulsiona a migração de uma área para outra, assim como a adaptação das técnicas de manejo e culturas cultivadas a cada período (Albuquerque et al, 2023; Cardoso et al, 2001; Demosthenes; Bentes, 2011; Noda et al., 2012; Sales, 2011; Santos et al, 2022, Silva, 2011; Vasconcelos et al, 2022).

2.5.1 Doenças causadas por microorganismos

2.5.1.1 Podridão-radicular

Rhizoctonia solani é um dos fungos causadores de podridão-radicular mais estudados, uma vez que causa doença em mais de 2643 espécies de plantas e acarreta na perda de 20-50% da produção. É usualmente encontrado nas camadas superficiais do solo, principalmente nos primeiros 10 cm devido à dependência de oxigênio, onde pode sobreviver saprofiticamente ou através de estruturas de repouso (escleródios) por longos períodos. A doença é influenciada, principalmente, por umidade, temperatura, concentração do inóculo, supressividade do solo e vigor da plântula (Ajayi-Oyetunde; Bradley, 2018; Akber et al., 2023; Farr e Rossman, 2021; Oliveira et al., 2008; Senapati et al., 2022; Spurlok e Rothrock, 2015).

Os sintomas podem se manifestar em pré ou pós-emergência. No primeiro caso, as sementes morrem no solo antes de germinarem, por conta de necroses nos tegumentos ou pela perda de rigidez e apodrecimento, ou germinam com falhas. No caso da pós-emergência, as plântulas apresentam folhagem seca ou murcha, lesões marrons nas hastes e prostamento das hastes (tombamento) devido à constrição do tecido vegetal. A penetração do fungo nas plântulas se dá diretamente pela parede celular da epiderme da raiz ou hipocótilo, ao produzir enzimas extracelulares (celulase, lignase e pectinase), com a subsequente invasão do micélio nos tecidos da planta (Ajayi-Oyetunde; Bradley, 2018; Aklber et al, 2023; Lopes; Ávila, 2005).

O controle químico deste fitopatógeno é realizado, por exemplo, por triadimenol, ácido salicílico, pencycuron, ácido sorbitol, tolylfluanid, carboxin, thiram, carbendazim e vitavax, porém não é possível sem estar associado a um programa integrado de controle, uma vez que o uso contínuo de um mesmo químico pode induzir o desenvolvimento de resistência por parte do fitopatógeno (El-Mohamedy et al., 2014; Goulart, 2002; 2021; Sadeghi et al., 2009).

Como alternativa ao uso de químicos, biocontroladores a base de espécies de *Trichoderma* (Bio-cure-F®, Ecotrich ES® e SoilGard 12 G®), *Bacillus* (FZB24® e Yield Shield®) e *Streptomyces* (Actinovate® SP e Mycostop®) já se encontram no mercado para o controle da doença (Bettiol et al., 2012; Karima; Nadia, 2012; Sadeghi et al., 2009).

2.5.1.2 Murcha-bacteriana

A murcha-bacteriana é causada pelo complexo de espécies de *Ralstonia solanacearum* (RSSC), anteriormente classificado em filotipos (I, II, III, IV) que estão distribuídos em diferentes regiões pelo mundo. Possuem mais de 450 hospedeiros conhecidos sem qualquer relação com o filotipo, uma vez que todos são capazes de causar a doença. Além da murcha-bacteriana observada em solonáceas, a bactéria também é responsável pela doença de Moko da banana. Estudos apontam o Brasil como um possível centro do filotipo II, apesar de linhagens do filotipo I serem encontradas (Bergsma-Vlami et al, 2018; Cellier e Prior, 2010; Coelho et al, 2004; Lebeau et al., 2011; Lopes; Rossato, 2018; Romo et al., 2012; Wicker et al., 2012; Santiago et al., 2017; Rodrigues et al., 2018).

Atualmente, o RSSC foi reclassificado por meio de análise genômica baseada na identidade média dos nucleotídeos (ANI) em três espécies: *R. solanacearum*, correspondente ao filotipo II; *R. pseudosolanacearum*, correspondente aos filotipos I e III e *R. syzygii*, correspondente ao filotipo IV. Posteriormente, *R. syzygii* foi dividido em três subespécies: subsp. *syzygii*, subsp. *indonesiensis* e subsp. *celebesensis*, cada um correspondendo a grupos ainda restritos de linhagens com características ecológicas particulares (Vailleau; Genin, 2023).

A bactéria *R. solanacearum* é comumente encontrada em regiões tropicais e sub-tropicais, cujo mecanismo de infecção se dá pela penetração nas raízes das plantas, invadindo o xilema e se espalhando rapidamente para as partes aéreas da planta através do sistema vascular onde seu alto nível de multiplicação e de produção

de exopolisacarídeos (EPS) levam a sintomas de murcha e, em última instância, a morte da planta (Genin, 2010; (Genin; Denny, 2012; Huet, 2014; Kaari et al, 2022a; Saputra et al, 2020). O estilo de vida da bactéria é uma dos principais problemas para o controle da doença, pois permite que se adapte a diferentes nichos ecológicos, como solo, água e planta (rizosfera da planta não-hospedeira e xilema do hospedeiro) e se mantenha na forma saprófita no solo por longos períodos (Huet, 2014; Kaari et al., 2022ab; Lebeau et al., 2011).

No Brasil, as perdas devido a murcha-bacteriana não ocorrem em todo o território, estando concentradas na região norte, a exemplo do estado do Amazonas onde são relatadas as perdas entre 40-80% de produção de hortaliças como tomate, pimentão, jiló, pimenta, berinjela e em outras culturas como a banana, *Melanthera discoidea* e *Moringa oleifera.* No caso do tomate, essa bactéria é um dos principais fatores por inviabilizar o cultivo a céu aberto na Amazônia, sendo muito empregado o cultivo protegido (Assefa et al., 2015; Coelho Netto et al., 2003, 2004; Elnaggar et al., 2018; Gasparotto, 2022; Katafiire et al., 2005; Kurabachew; Ayana, 2016; Lopes; Rossato, 2018).

A maioria dos trabalhos voltados para o combate da murcha-bacteriana em tomateiro estão concentrados no melhoramento genético de cultivares, como no caso da cultivar Yoshimatsu desenvolvida no INPA, que tem boa adaptação ao estado do Amazonas com resistência poligênica ao patógeno causador da doença (Costa et al, 2023; Noda et al., 2013). Além disso, há trabalhos que avaliam como alternativa o uso de enxertia de cultivares de solonáceas suscetíveis a doença em solonáceas silvestres (cubiu e jurubeba) que são resistentes a doença (Fernandes; Bentes, 2018).

Não há disponível no mercado até o momento nenhum defensivo químico para o controle de *R. solanacearum*, porém há alguns estudos com tomateiros que apontam a possibilidade do uso de validamycin A, validoxylamine e uma mistura de lipopeptídeos (iturina, surfactina e fengycin) para o controle do fitopatógeno (Chen et al, 2019; Yuliar et al., 2015)

Para a formulação de biodefensivos voltados para o biocontrole de *R. solanacearum,* há diversos trabalhos com espécies de *Bacillus*, *Pseudomonas* e *Streptomyces* que, possivelmente, suprimem o fitopatógeno por mecanismos de competição, indução de resistência, antibiose e produção de sideróforos e de enzimas que degradam a parede celular da bactéria (Boukaew et al, 2011; El-sayed et al.,

2020; Ling et al., 2020; Raza et al., 2016; Tan et al, 2006, 2011; Zhao et al, 2019; Zhuang et al, 2020).

3. OBJETIVOS

3.1 GERAL

Realizar mineração genômica e identificação química de moléculas produzidas pela microbiota amazônica com potencial antimicrobiano visando o desenvolvimento de produtos e processos biotecnológicos.

3.2 ESPECÍFICOS

- Selecionar de bactérias produtoras de antimicrobianos e indutores de crescimento visando o desenvolvimento de bioinoculantes para controle biológico e promoção de crescimento vegetal;
- Realizar mineração com foco na identificação de vias para a produção de metabólitos secundários;
- Analisar os produtos do metabolismo secundário por meio de espectrometria de massas visando a identificação dos compostos produzidos;
- Realizar testes *in planta* com as bactérias com potencial antimicrobiano e produtoras de indutores de crescimento.

4. REFERÊNCIAS

Abdel-Razek, A. S., El-Naggar, M. E., Allam, A., Morsy, O. M., Othman, S. I. Microbial Natural Products in Drug Discovery. **Processes,** vol. 8, n. 4, 470, 2020. https://doi.org/10.3390/pr8040470;

Abo-Zaid, G. A., Abdullah, A. S., Soliman, N. A-M., El-Sharouny, E. E., Al-Askar, A. A, Su, Y., Abdelkhalek, A., Sabry, S. A-F. Evaluation of Bio-Friendly Formulations from Siderophore-Producing Fluorescent *Pseudomonas* as Biocontrol Agents for the Management of Soil-Borne Fungi, *Fusarium oxysporum* and *Rhizoctonia solani*. **Agriculture**, vol. 13, n. 7, 1418, 2023. https://doi.org/10.3390/agriculture13071418;

Adedayo, A. A., Babalola, O. O., Prigent-Combaret, C., Cruz C., Stefan M., Kutu F., Glick B. R. The application of plant growth-promoting rhizobacteria in *Solanum lycopersicum* production in the agricultural system: a review. **PeerJ**, 2022. https://doi.org/ 10.7717/peerj.13405;

Aguiar, F.M. **Caracterização de isolados de *Corynespora cassiicola* e avaliação da sensibilidade *in vitro* a fungicidas**. Brasília, Universidade de Brasília, Tese de Doutorado, 109p, 2015;

Ajayi-Oyetunde, O. O., Bradley, C. A. *Rhizoctonia solani*: taxonomy, population biology and management of rhizoctonia seedling disease of soybean. **Plant Pathology**, vol. 67, p. 3–17, 2018. https://doi.org/10.1111/ppa.12733;

Ajilogba, C. F., Babalola, O O. Integrated Management Strategies for Tomato *Fusarium* Wilt. **Biocontrol Science**, vol. 18, n. 3, p. 117−127, 2013;

Akber, M. A., Mubeen, M., Sohail, M. A., Khan, S. W., Solanki, M. K., Khalid, R., Divvela, P. K., Zhou, L. Global distribution, traditional and modern detection, diagnostic, and management approaches of *Rhizoctonia solani* associated with legume crops. **Frontiers in Microbiology**, vol. 13, 1091288, 2023. https://doi.org/10.3389/fmicb.2022.1091288;

Albuquerque, K.C, Côrrea, G. R., Campos, P. V. Ethnoknowledge in the amazon: Traditional agriculture in the middle amazon. **Verde Grande**, vol. 5, n. 2, 2023. https://doi.org/10.46551/rvg2675239520232487508;

Almeida, S. F. **Avaliação de linhagens de *Trichoderma* na promoção de crescimento de raízes de tomateiro e no controle de *Meloidogyne enterolobii***. Brasília, Universidade de Brasília, Tese de Doutorado, 103p, 2020;

Almeida, G. V. B., Pedroso, M. T. M., Ferreira, Z. R. Tomate no Brasil: dados recentes de produção, comercialização e consumo. **Revista Campos & Negócios**, 2023.

Araujo, J., Díaz-Alcántara, C. A., Urbano, B., González-Andrés, F. Inoculation with native *Bradyrhizobium* strains formulated with biochar as carrier improves the performance of pigeonpea (Cajanus cajan L.). **European Journal of Agronomy**, vol. 113, 125985, 2020. https://doi.org/10.1016/j.eja.2019.125985

Assefa, W., Tesfaye, B., Dessalegn, L. Influence of inter-intra row spacing on yield losses of tomato cultivars. **Ethiopian Journal of Agricultural Sciences**, v. 25, n. 2, p. 15-28, 2015.

Azuizion, 2020. **World's Population to Reach 9.7 Bln in 2050: UN Report**. https://en.azvision.az/news/107258/-worlds-population-to-reach-9.7-bln-in-2050-un-report-.html

Bala, K. Microbial Fertilizer as an Alternative to Chemical Fertilizer in Modern Agriculture. In: Prasad, R., Zhang, S. H. (eds) **Beneficial Microorganisms in Agriculture. Environmental and Microbial Biotechnology**. Springer, Singapore, 2022. https://doi.org/10.1007/978-981-19-0733-3_4

Bamdad, H., Papari, S., Lazarovits, G., Berruti, F. Soil amendments for sustainable agriculture: Microbial organic fertilizers. **Soil Use and Management**, vol. 38, p. 94–120, 2022. https://doi.org/10.1111/sum.12762

Banerjee, S, Schaeppi, K., Heijden, M. Keystone taxa as drivers of microbiome structure and functioning. **Nature Reviews Microbiology**, 2018;

Bargaz, A., Lyamlouli, K., Chtouki, M., Zeroual, Y., Dhiba, D. Soil Microbial Resources for Improving Fertilizers Efficiency in an Integrated Plant Nutrient Management System. **Frontiers in Microbiology**, vol. 9, 1606, 2018. doi: 10.3389/fmicb.2018.01606

Bergsma-Vlami, M., Van de Bilt, J. L. J., Tjou-Tam-Sin, N. N. A. Phylogenetic Assignment of *Ralstonia pseudosolanacearum* (*Ralstonia solanacearum* Phylotype I) Isolated from Rosa spp. **Plant disease**, vol. 102, n.11, p. 2258-2267, 2018. https://doi.org/10.1094/PDIS-09-17-1345-RE;

Bernardini, S., Tiezzi, A., Masci, V. L., Ovidi, E. Natural products for human health: an historical overview of the drug discovery approaches. **Natural Product Research**, vol. 32, n.16, p. 1926-1950, 2018. https://doi.org/10.1080/14786419.2017.1356838;

Bettiol. W, Morandi, M. A., Pinto, Z. V., Paula Júnior, T. J., Corrêa, E. B. Moura, A. B., Lucon, C. M. M., Costa, J. C. B., Bezerra, J. L. **Documentos 88: Produtos comerciais à base de agentes de biocontrole de doenças de plantas**. Embrapa Meio Ambiente, 155 p., 2012. ISSN 1516-4691;

Biotrop. 2024. Acessado em 24 de abril de 2024. Disponível em https://biotrop.com.br/produtos;

Boukaew, S., Chuenchit, S., Petcharat, V. Evaluation of *Streptomyces* spp. for biological control of Sclerotium root and stem rot and *Ralstonia* wilt of chili pepper. **BioControl**, 56, 365–374, 2011. https://doi.org/10.1007/s10526-010-9336-4;

Cardoso, M. C., Boher, B., Ávila, A. C., Assis, L. A. G. **Circular técnica 9: Doenças das Cucurbitáceas no Estado do Amazonas**. Embrapa, Manaus-AM, 2001;

Cellier, G., Prior, P. Deciphering phenotypic diversity of *Ralstonia solanacearum* strains pathogenic to potato. **Phytopathology**, vol. 100, p.1250-1261, 2010.

Chen, M. C., Wang, J. P., Zhu, Y. J., Liu, B., Yang, W. J., Ruan, C. Q. Antibacterial activity against *Ralstonia solanacearum* of the lipopeptides secreted from the *Bacillus amyloliquefaciens* strain FJAT-2349. **Journal of Applied Microbiology**, vol. 126, n. 5, p. 1519-1529, 2019. https://doi.org/10.1111/jam.14213;

Clemente, F., Boiteux, L. S. **Produção de tomate para processamento industrial**. Embrapa, Brasília, 344p, 2012;

Coelho Netto, R. A., Pereira, B. G., Noda, H., Boher, B. Caracterização de isolados de *Ralstonia solanacearum* obtidos de tomateiros em várzea e em terra firme, no Estado do Amazonas. **Fitopatologia Brasileira**, vol. 28, p. 362-366, 2003;

Coelho Netto, R. A., Pereira, B. G., Noda, H., Boher, B. Murcha bacteriana no estado do Amazonas, Brasil. **Fitopatologia Brasileira**, vol. 29, p. 21-27, 2004;

Cordero, O. X., Datta, M. S. Microbial interactions and community assembly at microscales. **Current opinion in microbiology**, vol. 31, p.227-234, 2016;

Costa, K. D. S., Lima, E. S., Santos, P. R., Santos, A. M. M., Filho, J. L. S. C., Silva, M. O., Souza, A. R. E., Santana, S. R. A., Santana, J. T. S. Selection of tomato progenies F2:3 for resistance to bacterial wilt via mixed modeling. **Brazilian Journal of Development**, vol. 9, n. 1, p. 2488–2503, 2023. https://doi.org/10.34117/bjdv9n1-170;

Dahlke, I., Guerra, D., Souza, E. L., Lanzanova, M. E., Bohrer, R. E. G., Ramires, M. F. Desempenho produtivo do tomateiro sob cultivo protegido utilizando caldas agroecológicas. **Cultura Agronômica**, vol. 28, n. 2, p. 204-214, 2019. http://dx.doi.org/10.32929/2446-8355.2019v28n2p204-214;

Demosthenes, L. C. R., Bentes, J. L. S. Fontes de resistência à murcha bacteriana em germoplasma de *Capsicum* spp. do estado do Amazonas. **Acta Amazonica**, vol. 41, n. 3, p. 435–438, 2011. https://doi.org/10.1590/S0044-59672011000300016;

Desangoosse. 2024. **Baryon**. Acessado em 24 de abril de 2024. Disponível em https://www.desangosse.com.br/bio-solutions/baryon;

Deveau, A; Bonito, G.; Uehing, J.; Paoletti, M.; Becker, M.; Bindschedler, S.; Hacquard, S.; Hervé, V.; Labbé, J.; Lastovetsky, O. A.; Mieszkin, S.; Millet, L. J.; Vajna, B.; Junier, P.; Bonfante, P.; Krom, B. P.; Olsson, S.; Elsas, J.; Wick, L. Bacterial–fungal interactions: ecology, mechanisms and challenges. **FEMS Microbiology Reviews**, vol. 42, p. 335-352, 2018;

Dias, D. A.; Urban, S.; Roessner, U. A Historical Overview of Natural Products in Drug Discovery. **Metabolites**, vol. 2, p. 303-336, 2012;

Duma, M., Alsina, I., Dubova, L., Erdberga, I. Chemical composition of tomatoes depending on the stage of ripening. **Cheminė technologija**, vol. 66, n. 1, 2015. http://dx.doi.org/10.5755/j01.ct.66.1.12053;

Egamberdieva, D., Reckling, M., Wirth, S. Biochar-based *Bradyrhizobium* inoculum improves growth of lupin (*Lupinus angustifolius L.*) under drought stress. **European Journal of Soil Biology**, vol. 78, p. 38–42, 2017. https://doi.org/10.1016/j.ejsobi.2016.11.007;

El-Mohamedy, R. S., Jabnoun-Khiareddine, H., Daami-Remadi, M. Control of root rot diseases of tomato plants caused by *Fusarium solani*, *Rhizoctonia solani* and *Sclerotium rolfsii* using different chemical plant resistance inducers. **Tunisian Journal of Plant Protection**, vol. 9, n. 1, 56, 2014;

Elnaggar, S., Mohamed, A. M., Bakeer, A., Osman, T. A. Current status of bacterial wilt (*Ralstonia solanacearum*) disease in major tomato *(Solanum lycopersicum* L.) growing areas in Egypt. **Archives of Agriculture and Environmental Science**, vol. 3, n. 4, p. 399-406, 2018. https://doi.org/10.26832/24566632.2018.0304012;

Elsayed, T. R., Jacquiod, S., Nour, E. H., Sorensen, S. J., Smalla, K. Biocontrol of Bacterial Wilt Disease Through Complex Interaction Between Tomato Plant,

Antagonists, the Indigenous Rhizosphere Microbiota, and *Ralstonia solanacearum*. **Frontiers in Microbiology**, vol. 10, 2020. https://doi.org/10.3389/fmicb.2019.02835;

Farr, D. F.; Rossman, A. Y. **Fungal databases: U.S. National Fungus Collections, ARS, USDA**. 2021. Acessado em 10 de fevereiro de 2024. Disponível em https://nt.ars-grin.gov/fungaldatabases;

Fernandes, B. S., Bentes, J. L. S. Tomato grafting in wild solanaceae to control bacterial wilt. **Revista Agrária Acadêmica**, vol. 1, n. 3, 2018. https://doi.org/10.32406/v1n32018/26-38/agrariacad;
Filgueira, F. A. R. **Novo Manual de Olericultura – agrotecnologia moderna na produção e comercialização de hortaliças**, vol. 3, Editora UFV, Viçosa-MG, 2013.

Gama, A. D. S., Lima, H., Gonçalves, J., Teixeira, W., Bentes, J. D. S. Sistema de produção de tomate em substrato para agricultura familiar na região metropolitana de Manaus. In: Dezordi, C., Teixeira, W. G. (eds). **Seminário de Pós-Graduação na Embrapa Amazônia Ocidental: Integrando Esforços para o Desenvolvimento da Amazônia**, Embrapa Amazônia Ocidental, Manaus-AM, 1ª ed., 124 p., 2008, ISBN 978-85-89111-05-8;

Gasparotto, L. Plant pathogens that inhabit or pass through the soil in Amazonia. **Studies in Environmental and Animal Sciences**, v.3, n.4, p. 953-968, 2022;

Genin, S. Molecular traits controlling host range and adaptation to plants in *Ralstonia solanacearum*. **New Phytologist**, vol. 187, p. 920-928, 2010. https://doi.org/10.1111/j.1469-8137.2010.03397.x;

Genin, S., Denny, T. P. Pathogenomics of the *Ralstonia solanacearum* Species Complex. **Annual Review of Phytopathology**, vol. 50, n. 1, p. 67–89, 2012. https://doi.org/10.1146/annurev-phyto-081211-173000;

González-Burgos, E.; Gómez-Serranillos, M. P. Terpene compounds in nature: A review of their potential antioxidant activity. **Current Medicinal Chemistry**, vol. 19, p. 5319-5341, 2012;

Götze, S., Arp, J., Lackner, G., Zhang, S., Kries, H., Klapper, M., García-Altares, M., Willing, K., Günther, M., Stallforth, P. Structure elucidation of the syringafactin lipopeptides provides insight in the evolution of nonribosomal peptide synthetases. **Chemical Science**, vol. 10, n. 48, p. 10979-10990, 2019. https://doi.org/10.1039/c9sc03633d;

Goulart, A. C. P. Efeito do tratamento de sementes de algodão com fungicidas no controle do tombamento de plântulas causado por *Rhizoctonia solani*. **Fitopatologia Brasileira**, vol. 27, n.4, p. 399-402, 2002;

Goulart, A. C. P. Influence of previous crops and fungicide seed treatment in the incidence and control of damping-off caused by *Rhizoctonia solani* in cotton seedlings under greenhouse conditions. **Summa Phytopathologica**, v. 47, n. 3, p. 157-161, 2021;

Halfeld-Vieira, B. A., Marinho-Prado, J. S., Nechet, K. L., Morandi, M. A. B., Bettiol, W. **Defensivos Agrícolas Naturais - Uso e perspectivas**. Embrapa, Brasília- DF, 854p, 2016;

Hebebrand, C., Laborde, D., 2022. **High Fertilizer Prices Contribute to Rising Global Food Security Concerns**. International Food Policy Research Institute, Washington D.C. H. Disponível em: https://www.ifpri.org/blog/high-fertilizer-prices-contribute-rising-global-food-security-concerns;

Hebebrand, Charlotte; and Glauber, Joseph W. 2023. The Russia-Ukraine war after a year: Impacts on fertilizer production, prices, and trade flows. In: Glauber, J., David Laborde Debucquet, D. L. (eds.). **The Russia-Ukraine Conflict and Global Food Security,** Section One: A Conflict with Global Consequences, Chapter 8, Pp. 43-47. https://doi.org/10.2499/9780896294394_08;

Helfrich, E.J.N.; Ueoka, R.; Dolev, A.; Rust, M.; Meoded, R.A.; Bhushan, A.; Califano, G.; Costa, R.; Gugger, M.; Steinbeck, C.; Moreno, P.; Piel, J. Automated structure prediction of trans-acyltransferase polyketide synthase products. **Nature Chemistry and Biology,** vol. 15, n. 8, p. 813-821, 2019;

Herbst, D.A.; Townsend, C.A.; Maier, T. The architectures of iterativa type I PKS and FAS. **Natural Product Reports**, vol. 35, 2018;

Höhler J, Oude Lansink A. Measuring the impact of COVID-19 on stock prices and profits in the food supply chain. **Agribusiness**, vol. 37, p. 171–186, 2021. https://doi.org/10.1002/agr.21678;

Huet, G. Breeding for resistances to *Ralstonia solanacearum*. **Frontiers in Plant Science**, vol. 5, 2014. https://doi.org/10.3389/fpls.2014.00715;

Ilinova, A., Dmitrieva, D., Kraslawski, A. Influence of COVID-19 pandemic on fertilizer companies: The role of competitive advantages. Resources Policy, v. 71, p. 102019, 2021;

Instituto Brasileiro de Geografia e Estatística (IBGE), **Produção agropecuária**, 2022 https://www.ibge.gov.br/explica/producao-agropecuaria/tomate/br;

Instituto Brasileiro de Geografia e Estatística (IBGE). **Indicadores IBGE - Levantamento Sistemático da Produção Agrícola Estatística da Produção Agrícola**, 2022;

Izoré, T., Cryle, M. J. The many faces and important roles of protein–protein interactions during non-ribosomal peptide synthesis. **Natural product reports**, vol. 35, n. 11, p. 1120-1139, 2018. https://doi.org/10.1039/c8np00038g;

Jackson, S.; Crossman, L.; Almeida, E.; Margassery, L.; Kennedy, J.; Dobson, A. Diverse and abundant secondary metabolismo biosynthetic gene clusters in the genome of marine sponge derived Streptomyces spp. Isolates. **Marine drugs**, vol. 16, n 67, 2018;

Jiao, W., Chen, W., Chang, A. C., Page, A. L. Environmental risks of trace elements associated with long-term phosphate fertilizers applications: A review. **Environmental Pollution**, vol. 168, p. 44-53, 2012. https://doi.org/10.1016/j.envpol.2012.03.052;

Kaari, M., Joseph, J., Manikkam, R., Sreenivasan, A., Venugopal, G., Alexander, B., Krishnan, S. Anti-Biofilm Activity and Biocontrol Potential of *Streptomyces* Cultures Against *Ralstonia solanacearum* on Tomato Plants. **Indian Journal of Microbiology**, vol. 62, n. 1, p. 32-39, 2022. https://doi.org/10.1007/s12088-021-00963-1;

Kaari, M., Joseph, J., Manikkam, R., Sreenivasan, A., Venugopal, G. Biological control of *Streptomyces* sp. UT4A49 to suppress tomato bacterial wilt disease and its metabolite profiling. **Journal of King Saud University- Science**, vol. 38, 101688, 2022. https://doi.org/10.1016/j.jksus.2021.101688;

Karima. H.; Nadia, G. *In vitro* study on *Fusarium solani* and *Rhizoctonia solani* isolates causing the damping-off and root rot diseases in tomatoes. **Nature and Science**, vol. 10, 2012;

Katafiire, M., Adipala, E., Lemaga, B., Olanya, M., El-Bedewyand, R., Ewell, P.. Management of bacterial wilt of potato using one-season rotation crops in southwestern Uganda. In: Allen, C., Prior, P., Hayward, A.C. (Eds.). **Bacterial Wilt Disease and the *Ralstonia solanacearum* Species Complex**. American Phytopathological Society, St. Paul, Minnesota USA. pp. 197-203, 2005;

Kohlhaas, C.; Jenner, M.; Kampa, A. Briggs, G. S.; Afonso, J.P.; Piel, J.; Oldham, N.J. Amino acid-accepting ketosynthase domain from a trans-AT polyketide synthase exhibits high selectivity for predicted intermediate. **Chemistry Science**, vol. 4, p. 3212–3217, 2013;

Kurabachew, H., Ayana, G. Bacterial wilt caused by *Ralstonia solanacearum* in Ethiopia: Status and management approaches: A review. **International Journal of Phytopathology**, vol 5, n. 3, p. 107-119, 2016;

Lebeau, A., Daunay, M.-C., Frary, A., Palloix, A., Wang, J.-F., Dintinger, J., Chiroleu, F., Wicker, E., Prior, P. Bacterial wilt resistance in tomato, pepper, and eggplant: Genetic resources respond to diverse strains in the *Ralstonia solanacearum* species complex. **Phytopathology**, vol. 101, p.154-165, 2011.

Legein, M., Smetsm W,, Vandenheuvel, D., Eilers, T., Muyshondt, B., Prinsen, E., Samson, R., Lebeer, S. Modes of Action of Microbial Biocontrol in the Phyllosphere. **Frontiers in Microbiology**, vol. 11, n. 1619, 2020. https://doi.org/10.3389/fmicb.2020.01619;

Leroy, M.; Lourd, M. Doença foliar do tomateiro causada por *Corynespora cassiicola* em Manaus. **Fitopatologia Brasileira**, Brasília, v.14, n.1, p.32-36, 1989;

Ling, L., Han, X., Li, X., Zhang, X., Wang, H., Zhang, L., Cao, P., Wu, Y., Wang, X., Zhao, J., Xiang, W. A *Streptomyces* sp. NEAU-HV9: Isolation, Identification,

and Potential as a Biocontrol Agent against *Ralstonia solanacearum* of Tomato Plants. **Microorganisms**, vol. 8, 2020. https://doi.org/ 10.3390/microorganisms8030351

Lopes, C. A., Ávila, A.C. **Doenças do tomateiro**. Embrapa Hortaliças, Brasília-DF, p.11-15, 2005;

Lopes, C. A., Rossato, M. History and Status of Selected Hosts of the *Ralstonia solanacearum* Species Complex Causing Bacterial Wilt in Brazil. **Frontiers in Microbiology**, vol. 9, 2018. https://doi.org/ 10.3389/fmicb.2018.01228;

Mach, B.; Reich, E.; Tatum, E. L. Separation of the biosynthesis of the antibiotic polypeptide tyrocidine from protein biosynthesis. **Proceedings of the National Academy of Sciences**, vol. 50, n. 1, p. 175–181, 1963. https://doi.org/10.1073/pnas.50.1.175;

Ministério da Agricultura e Pecuária do Brasil. 2022a. **MAPA registra 46 defensivos agrícolas**. Acessado em 24 de abril de 2024. Disponível em https://www.gov.br/agricultura/pt-br/assuntos/noticias-2022/mapa-registra-46-defensivos-agricolas-incluindo-sete-produtos-biologicos

Ministério da Agricultura e Pecuária do Brasil. 2022b. **Diário oficial da união, ato nº 38, de 12 de agosto de 2022**. Acessado em 24 de abril de 2024. Disponível em https://www.in.gov.br/en/web/dou/-/ato-n-38-de-12-de-agosto-de-2022-425197453

Miyanaga, A. Structure and function of polyketide biosynthetic enzymes: various strategies for production of structurally diverse polyketides, **Bioscience, Biotechnology, and Biochemistry**, vol. 81, n. 12, p. 2227-2236, 2017. https://doi.org/10.1080/09168451.2017.1391687;

Moser, S., Pichler, H. Identifying and engineering the ideal microbial terpenoid production host. **Applied Microbiology and Biotechnology**, vol. 103, p. 5501-5516, 2019. https://doi.org/10.1007/s00253-019-09892-y;

Netzker, T., Fischer, J., Weber, J., Mattern, D. J., König, C. C., Valiante, V., Schroeckh, V., Brakhage, A. A. Microbial communication leading to the activation of silent fungal secondary metabolite gene clusters. **Frontiers in Microbiology**, vol. 6, n. 299, 2015. https://doi.org/10.3389/fmicb.2015.00299;

Nivina, A.; Yuet, K. P.; Hsu, J.; Khosla, C. Evolution and diversity of assembly-line polyketide synthases. **Chemical reviews**, vol. 119, p. 12524-12547, 2019;

Noda, H., Souza, L. A. G. D., Silva Filho, D. F. D. **Pesquisas Agronômicas para a Agricultura Sustentável na Amazônia Central**, Manaus-AM, Nerua-INPA, 2013. ISBN 978-85-66808-03-2;

Noda, S. N., Martins, A. L. U., Noda, H., Silva, A. I. C., Braga, M. D. S. Paisagens e etnoconhecimentos na agricultura Ticuna e Cocama no alto rio Solimões, Amazonas. **Boletim do Museu Paraense Emílio Goeldi. Ciências Humanas**, v. 7, n. 2, p. 397-416, 2012;

Oliveira, A. C. C., Souza, P. E. E., Pozza, A., Manerba, F. C., Lopes, M. F. Metodologias de inoculação de *Rhizoctonia solani* na cultura da cenoura. **Ciências e Agrotecnologia**, vol. 32, p. 992-995, 2008;

Overmann, J., Abt, B., Sikorski, J. Present and future of culturing bacteria. **Annual review of microbiology**, vol. 71, p. 711-730, 2017. https://doi.org/10.1146/annurev-micro-090816-093449;

Pastor-Bueis, R., Sánchez-Cañizares, C., James, E. K.,González, A. F. Formulation of a highly effective inoculant for common bean based on an autochthonous elite strain of *Rhizobium leguminosarum* bv phaseoli, and genomicbased insights into its agronomic performance. **Frontiers in Microbiology**, vol. 10, 2019. https://doi.org/10.3389/fmicb.2019.02724;

Pathak, D. V., Kumar, M. Microbial inoculants as biofertilizers and biopesticides. In: Singh, D. P., Prabha, R., Singh, H. B. (eds). **Microbial Inoculants in Sustainable Agricultural Productivity: Research Perspectives**, vol. 1, Delhi: Springer, 197–209, 2016. doi: 10.1007/978-81-322-2647-5;

Paudel, D., Neupane, R.C., Sigdel, S., Poudel, P., Khanal, A. R. COVID-19 Pandemic, Climate Change, and Conflicts on Agriculture: A Trio of Challenges to Global Food Security. **Sustainability**, vol. 15, 8280, 2023. https://doi.org/10.3390/su15108280;

Fixen, P. E., West, F. B. Nitrogen Fertilizers: Meeting Contemporary Challenges. **AMBIO: A Journal of the Human Environment**, vol. 31, n. 2, p. 169-176, 2002. https://doi.org/10.1579/0044-7447-31.2.169;

Paulo, B. S., Sigrist, R., Oliveira, L. G. Avanços recentes em biossíntese combinatória de policetídeos: Perspectivas e desafios, **Química Nova**, vol. 42, n. 1, p. 71-83, 2019;

Pham, J. V., Yilma, M. A., Feliz, A., Majid, M.T., Maffetone, N., Walker, J. R., Kim, E., Cho, H. J., Reynolds, J. M., Song, M. C., Park, S. R., Yoon, Y. J. A Review of the Microbial Production of Bioactive Natural Products and Biologics. **Frontiers in Microbiology**, vol. 10, n. 1404, 2019. https://doi.org/10.3389/fmicb.2019.01404;

Pichi, D. G., Altei, W. F., Saito, M. S., Bolzani, V.S., Cilli, E. M. Peptídeos cíclicos de biomassa vegetal: Características, diversidade, biossíntese e atividades biológicas. **Química Nova**, vol. 32, n. 5, p. 1262-1277, 2009;

Pilla, G., Tang, M. Going around in circles: virulence plasmids in enteric pathogens. **Nature Reviews Microbiology**, vol. 16, n. 8, p. 484-495, 2018. https://doi.org/10.1038/s41579-018-0031-2;

Poudel, P. B., Poudel, M. R., Gautam, A., Phuyal, S., Tiwari, C. K., Bashyal, N., Bashyal, S. COVID-19 and its Global Impact on Food and Agriculture. **Journal of Biology and Today's World**, vol. 9, n. 5, 221, 2020;

Prado-Audelo, M. L. D., Cortés H., Caballero-Florán, I. H., González-Torres, M., Escutia-Guadarrama, L., Bernal-Chávez, S. A., Giraldo-Gomez, D. M., Magaña, J. J., Leyva-Gómez, G. Therapeutic applications of terpenes on inflammatory diseases. **Frontiers in Pharmacology**, vol. 2, 2021;

Pujiharto P, Wahyuni S. The impact of COVID-19 pandemic on business risks and potato commercial model. **Open Agriculture**, vol. 8, n. 1, 20220158, 2023. https://doi.org/10.1515/opag-2022-0158;

Rabe, P., Citron, C. A., Dickschat, J. S. Volatile terpenes from actinomycetes: A biosynthetic study correlating chemical analyses to genome data. **ChemBioChem**, vol. 14, p. 2345-2354, 2013;

Raza, W., Ling, N., Yang, L., Huang, Q., Shen, Q. Response of tomato wilt pathogen *Ralstonia solanacearum* to the volatile organic compounds produced by a biocontrol strain *Bacillus amyloliquefaciens* SQR-9. **Scientific Reports**, vol. 6, 2016. https://doi.org/10.1038/srep24856;

Rékási, M., Szili-Kovács, T., Takács, T., Bernhardt, B., Puspán, I., Kovács, R., Kutasi, J., Draskovits, E., Molnár, S., Molnár, M., Uzinger, N. Improving the fertility of sandy soils in the temperate region by combined biochar and microbial inoculant treatments. **Archives of Agronomy and Soil Science**, vol. 65, p. 44–57, 2019. https://doi.org/10.1080/03650340.2018.1482536;

Rodrigues, F., Nunes, A. C. P., Carvalho, D. D. C., Ribeiro, M. C. Indução de tolerância à murcha bacteriana em híbridos de tomate por aplicação de giberelina. **Revista de Ciências Agroveterinárias**, vol. 17, n.1, 2018. https://doi.org/10.5965/223811712018054;

Romo, J. P., Osorio, J. G., Yepes, M. S., Identification of new hosts for *Ralstonia solanacearum* (Smith) race 2 from Colombia. **Revista de Protección Vegetal**, vol. 27, n. 3, 2012;

Rudolf, J. D., Alsup, T. A., Xu, B., Li, Z. Bacterial terpenome. **Natural product reports**, vol. 38, n. 5, p. 905-980, 2021. https://doi.org/10.1039/d0np00066c

Sadeghi, A., Hesan, A.R., Askari, H., Qomi, N.D., Farsi, M., Hervan, E.M. Biocontrol of *Rhizoctonia solani* damping-off of sugar beet with native *Streptomyces* strains under field conditions. **Biocontrol Science and Technology**, vol. 19, n. 9, p. 985-991, 2009;

Sales, R. S. A. **Controle biológico de *Rhizoctonia solani*, agente causal do tombamento do tomateiro, com *Trichoderma* spp**. Dissertação de Mestrado, INPA, Manaus - AM, 2011;

Salha, G. B., Abderrabba, M., Labidi, J. A status review of terpenes and their separation methods. **Rev. Chem. Eng.**, 2019;

Santen, J. A., Jacob, G., Singh, A. L., Aniebok, V., Balunas, M. J., Bunsko, D., ..., Linington, R. G. The natural products atlas: an open access knowledge base for

microbial natural products discovery. **ACS central science**, vol. 5, n. 11, p. 1824-1833, 2019. https://doi.org/10.1021/acscentsci.9b00806;

Santiago, T. R., Lopes, C. A., Caetano-Anollés, G., Mizubuti, E. S. G. Phylotype and sequevar variability of *Ralstonia solanacearum* in Brazil, an ancient centre of diversity of the pathogen. **Plant Pathology**, vol 66, p. 383-392, 2017. https://doi.org/10.1111/ppa.12586

Santos, D. K. N., Vilamil, E. S., Demosthenes, L. C. R., Costa Neto, P. Q. Incidence of diseases caused by fungi in guarana plants in rural properties of Urucará, Amazonas. **Brazilian Journal of Development**, v.8, n.4, p. 27281-27295, 2022;

Santos, T. M. C. **Seleção de microorganismos antagônicos a *Rhizocthonia solani* (KHUN) e *Cylindrocladium scoparium* (Morgan), agentes causais de tombamento em *Eucalyptus***. Dissertação de mestrado, ESALQ - USP, Piracicaba - SP, 142p, 2011;

Saputra, R., Arwiyanto, T., Wibowo, A. Biological control of *Ralstonia solanacearum* causes bacterial wilt disease with Pseudomonas putida and *Streptomyce*s spp. on some tomato varieties. **IOP Conf Ser Earth. Environ. Sci.**, vol. 515, n. 12007, 2020. https://doi.org/10.1088/1755-1315/515/1/012007;

Savci, S. Investigation of effect of chemical fertilizers on environment. **APCBEE Procedia**, vol. 1, p. 287-292, 2012a. https://doi.org/10.1016/j.apcbee.2012.03.047;
Savci, S. An agricultural pollutant: Chemical fertilizer. **International Journal of Environmental Science and Development**, vol. 3, n. 1, p. 73-80, 2012b. https://doi.org/10.7763/IJESD.2012.V3.191;

Schiesari, L. **Defensivos agrícolas - Como evitar danos à saúde e ao meio ambiente**. Série Boas Práticas, vol. 8, 2012;

Sehrawat, A., Sindhu, S., Glick, B. R. Hydrogen cyanide production by soil bacteria: Biological control of pests and promotion of plant growth in sustainable agriculture. **Pedosphere**, vol. 32, n. 1, p. 15–38, 2022. https://doi.org/10.1016/S1002-0160(21)60058-9;

Senapati, M., Tiwari, A., Sharma, N., Chandra, P., Bashyal, B. M., Ellur, R. K., Bhowmick, P. K., Bollinedi, H., Vinod, K. K., Singh, A. K., Krishnan S. G. *Rhizoctonia solani* Kühn Pathophysiology: Status and Prospects of Sheath Blight Disease Management in Rice. **Frontiers in Plant Science**, vol. 13, 881116, 2022. https://doi.org/10.3389/fpls.2022.881116;

Shahini, E., Skuraj, E., Sallaku, F., Shahini, S. The supply shock in organic fertilizers for agriculture caused by the effect of Russia-Ukraine war. **Scientific Horizons**, vol. 25, n. 2, p. 97-103, 2022. https://doi.org/10.48077/scihor.25(2).2022.97-103;

Sharma, A., Chetani, R. A Review on the Effect of Organic and Chemical Fertilizers on Plants. **International Journal for Research in Applied Science & Engineering Technology**, vol. 5, n. 2, 2017;

Shen, B. A new golden age of natural products drug discovery. **Cell**, vol. 163, p.1297-1300, 2015;

Shuqin, J., Fang, Z. Zero Growth of Chemical Fertilizer and Pesticide Use: China's Objectives, Progress and Challenges. **Journal of Resources and Ecology**, vol. 9, n. 1, p. 50–58, 2018. https://doi.org/10.5814/j.issn.1674-764x.2018.01.006;

Silva, M. S. M. Avaliação do potencial produtivo de dois cultivares de tomate visando diminuir a incidência de murcha bacteriana, no Iranduba–Amazonas. Dissertação de mestrado, UFAM, Manaus-AM, 2011;

Souri, M. K. Aminochelate fertilizers: the new approach to the old problem, a review. **Open Agriculture**, vol. 1, p. 118-123, 2016. https://doi.org/10.1515/opag-2016-0016;

Sridhar, A., Balakrishnan, A., Jacob, M. M., Sillanpää, M., Dayanandan, N. Global impact of COVID-19 on agriculture: role of sustainable agriculture and digital farming. **Environmental Science and Pollution Research**, vol. 30, p. 42509–42525, 2023. https://doi.org/10.1007/s11356-022-19358-w;

Stamenković, S., Beškoski, V., Karabegović, I., Lazić, M., Nikolić, N. Microbial fertilizers: A comprehensive review of current findings and future perspectives. **Spanish Journal of Agricultural Research**, vol. 16, n. 1, e09R01, 2018. https://doi.org/10.5424/sjar/2018161-12117;

Süssmuth, R. D., Mainz, A. Nonribosomal peptide synthesis—principles and prospects. **Angewandte Chemie International Edition**, vol. 56, n. 14, p. 3770-3821, 2017. https://doi.org/10.1002/anie.201609079;

Tan, H. M., Cao, L. X., He, Z. F., Su, G. J., Lin, B., Zhou, S. N. Isolation of Endophytic Actinomycetes from Different Cultivars of Tomato and their Activities Against *Ralstonia solanacearum in vitro*. **World Journal of Microbiology and Biotechnology**, vol. 22, 1275–1280, 2006. https://doi.org/10.1007/s11274-006-9172-y;

Tan, H., Zhou, S., Deng, Z., He, M., Cao, L. Ribosomal-sequence-directed selection for endophytic streptomycete strains antagonistic to *Ralstonia solanacearum* to control tomato bacterial wilt. **Biological Control**, vol. 59, n. 2, p. 245-254, 2011. https://doi.org/10.1016/j.biocontrol.2011.07.018;

Timmusk S, Paalme V, Pavlicek T, Bergquist J, Vangala A, Danila, T., Nevo, E. Bacterial Distribution in the Rhizosphere of Wild Barley under Contrasting Microclimates. **PLoS ONE**, vol. 6 n. 3, e17968, 2011. https://doi.org/10.1371/journal.pone.0017968;

Timmusk, S., Behers, L., Muthoni, J., Muraya, A., Aronsson, A-C. Perspectives and Challenges of Microbial Application for Crop Improvement. **Frontiers in Plant Science**, vol. 8, n. 49, 2017. https://doi.org/10.3389/fpls.2017.00049;

Vailleau, F., Genin, S. *Ralstonia solanacearum*: An arsenal of virulence strategies and prospects for resistance. **Annual Review of Phytopathology**, 61, 25-47, 2023.

Vasconcelos, M. A., Veiga, J. A. P., Silva, J. L. G., Guimarães, D. F., Brito, A. L., Santos, Y. L. F., Lopes, M., Lima, A. L., Oliveira, E. T. Climate Change and Its Impact on the Agricultural Calendar of Riverine Farmers in Médio Juruá, Amazonas State, Brazil. **Atmosphere**, vol. 13, n. 2018, 2022. https://doi.org/10.3390/atmos13122018;

Vurukonda, S. S. K. P., Fotopoulos, V., Saeid, A. Production of a Rich Fertilizer Base for Plants from Waste Organic Residues by Microbial Formulation Technology. **Microorganisms**. vol. 12, n. 3, 541, 2024. https://doi.org/10.3390/microorganisms12030541;

Wang, Y., Zhu, Y. Zhang, S., Wang, Y. What could promote farmers to replace chemical fertilizers with organic fertilizers. **Journal of Cleaner Production**, vol. 199, p. 882-890, 2018. https://doi.org/10.1016/j.jclepro.2018.07.222;

Wicker, E., Lefeuvre, P., Cambiaire, J., Lemaire, C., Poussier, S., Prior, P. Contrasting recombination patterns and demographic histories of the plant pathogen *Ralstonia solanacearum* inferred from MLSA. **International Society for Microbial Ecology Journal**, vol. 6, p. 961-974, 2012;

Yamada, Y., Kuzuyama, T., Komatsu, M., Shin-Ya, K., Omura, S., Cane, D. E., Ikeda, H. Terpene synthases are widely distributed in bacteria. **Proceedings of the National Academy of Sciences**, vol. 112, n. 3, p. 857-862, 2015. https://doi.org/10.1073/pnas.1422108112;

Yuliar, Nion, Y. A., Toyota, K. Recent trends in control methods for bacterial wilt disease caused by *Ralstonia solanacearum*. **Microbes Environments**, vol. 30, p. 1-11, 2015. https://doi.org/10.1264/jsme2.ME14144

Zaroubi, L., Ozugergin, I., Mastronardi, K., Imfeld, A., Law, C., Gélinas, Y., Piekny, A., Findlay, B. L. The ubiquitous soil terpene geosmin acts as a warning chemical. Applied and Environmental Microbiology, vol. 88, n. 7, e00093-22, 2022. https://doi.org/10.1128/aem.00093-22;

Zengler, K., Zaramela, L. The social network of microorganisms – how auxotrophies shape complex communities. **Nature Reviews Microbiology**, 2018;

Zhao, B., Lin, X., Lei, L., Lamb, D. C., Kelly, S. L., Waterman, M. R., Cane, D. E. Biosynthesis of the sesquiterpene antibiotic albaflavenone in *Streptomyces coelicolor* A3 (2). **Journal of Biological Chemistry**, vol. 283, n. 13, p. 8183-8189, 2008.

Zhao J, Han L, Yu M, Cao P, Li D, Guo X, Liu Y, Wang X, Xiang W. Characterization of *Streptomyces sporangiiformans* sp. nov., a Novel Soil Actinomycete with Antibacterial Activity against *Ralstonia solanacearum*. **Microorganisms**, vol. 7, n. 9, 2019. https://doi.org/10.3390/microorganisms7090360;

Zhuang, X., Gao, C., Peng, C., Wang, Z., Zhao, J., Shen, Y., Liu, C. Characterization of a novel endophytic actinomycete, *Streptomyces physcomitrii* sp. nov., and its biocontrol potential against *Ralstonia solanacearum* on Tomato. **Microorganisms**, vol. 8, n. 12, 2020. https://doi.org/ 10.3390/microorganisms8122025;

Zörb, C., Senbayram, M., Peiter, E. Potassium in agriculture – Status and perspectives. **Journal of Plant Physiology**, vol. 171, p. 656-669, 2014. http://dx.doi.org/10.1016/j.jplph.2013.08.008;

Capítulo 2

ARTIGO 1 - PUBLICADO (https://doi.org/10.3390/microorganisms12071364)
Formatação de acordo com as normas da revista Microorganisms

Amazonian Bacteria from River Sediments as a Biocontrol Solution against *Ralstonia solanacearum*

Abstract: Bacterial wilt, caused by *Ralstonia solanacearum*, is one of the main challenges for sustainable tomato production in the Amazon region. This study evaluated the potential of bacteria isolated from sediments of the Solimões and Negro rivers for the biocontrol of this disease. From 36 bacteria selected through in vitro antibiosis, three promising isolates were identified: *Priestia aryabhattai* RN 11, *Streptomyces* sp. RN 24, and *Kitasatospora* sp. SOL 195, which inhibited the growth of the phytopathogen by 100%, 87.62%, and 100%, respectively. These isolates also demonstrated the ability to produce extracellular enzymes and plant growth-promoting compounds, such as indole-3-acetic acid (IAA), siderophore, and ammonia. In plant assays, during both dry and rainy seasons, *P. aryabhattai* RN 11 reduced disease incidence by 40% and 90%, respectively, while promoting the growth of infected plants. *Streptomyces* sp. RN 24 and *Kitasatospora* sp. SOL 195 exhibited high survival rates (85–90%) and pathogen suppression in the soil (>90%), demonstrating their potential as biocontrol agents. This study highlights the potential of Amazonian bacteria as biocontrol agents against bacterial wilt, contributing to the development of sustainable management strategies for this important disease.

Keywords: actinomycetes; phylogenomic identification; dDDH; ANI; bioactive metabolites; bioprospecting

1. Introduction

Bacterial wilt, caused by the *Ralstonia solanacearum* species complex (RSSC), is one of the most devastating diseases affecting tomato production worldwide [1–3]. In Brazil, losses due to this disease are particularly significant in the northern region, such as in the state of Amazonas, where losses of 40–80% are reported in the production of vegetables, including tomato, bell pepper, scarlet eggplant, chili pepper, and eggplant, as well as in banana (*Musa paradisiaca*) cultivation. In addition to solanaceous crops and banana, beach daisy (*Melanthera discoidea*) and miracle tree (*Moringa oleifera*) have also been reported as hosts in the state [4–7].

The RSSC, previously classified into phylotypes (I, II, III, IV), has recently been reclassified into three species: *R. solanacearum* (phylotype II), *R. pseudosolanacearum* (phylotypes I and III), and *R. syzygii* (phylotype IV) [8]. Studies suggest Brazil as a possible center of diversity for phylotype II, although phylotype I strains are also found in the country [7,9–15].

Controlling bacterial wilt is challenging due to the versatile lifestyle of *R. solanacearum*, which allows its adaptation to different ecological niches, such as soil, water, and plants (non-host plant rhizosphere and host xylem), and the ability to survive in the soil for long periods [10,16–18]. The pathogen infects plants through the roots, invades the xylem, and spreads throughout the aerial portion via the vascular system, multiplying intensely and producing exopolysaccharides (EPS) that obstruct the vessels, causing wilting symptoms and, eventually plant death [19,20].

Current strategies for controlling bacterial wilt in tomato include the genetic improvement of resistant cultivars, such as the Yoshimatsu cultivar, developed to adapt to the climatic conditions of the Amazon region [21–23]. However, this cultivar still faces challenges in consumer acceptance due to characteristics such as fruit size and cracking when ripe. Furthermore, the use of chemicals like validamycin A and validoxylamine to induce plant resistance is no longer recommended for application in tomato plants [24–26]. Although a mixture of lipopeptides produced by *Bacillus amyloliquefaciens* has shown potential for controlling the phytopathogen, no options are currently available on the market [27].

In this context, the formulation of biodefensives using bacteria capable of suppressing the phytopathogen through multiple mechanisms, such as competition, resistance induction, and the production of antibiotics, siderophores, and/or cell wall-degrading enzymes, has emerged as a promising approach for promoting sustainable agriculture [28–38]. Therefore, the objective of this study was to explore Amazonian microbial genetic resources, specifically bacteria isolated from sediments of the Negro and Solimões rivers, as a potential source for the biocontrol of *R. solanacearum*, focusing on the development of environmentally sustainable strategies for the control of bacterial wilt in tomato plants.

2. Materials and Methods

2.1. Bacterial Isolates

Thirty-six bacterial isolates obtained from sediments of the Solimões and Negro Rivers were used, which are preserved in the Laboratory of Genomics and Applied Microbiology of the Legal Amazon (GENAGRO) at Embrapa—CPAA. The strains were maintained in LB media for non-filamentous bacteria, incubated for 24 h at 28 °C, and ISP2 for actinobacteria, incubated for 7 days at 28 °C. Access to the genetic heritage was authorized by SISGEN No. A39C76B.

2.2. In Vitro Antimicrobial Activity against Ralstonia solanacearum

The antagonism of 36 bacterial isolates from the Negro and Solimões rivers (Table S1) against *R. solanacearum* was evaluated in vitro using paired culture tests adapted from Velho-Pereira and Kamat [36]. In Petri dishes containing LB medium (non-filamentous bacteria) and ISP2 (actinobacteria), a 7 cm vertical streak of the antagonists was made and incubated for 48 h for LB plates and seven days for ISP2 plates at 28 °C. Subsequently, a 3 cm streak of *R. solanacearum* was made at a distance of 1 cm from the incubated antagonist for 24 h. All assays were performed in triplicate. At the end of the assays, with the aid of a caliper, the length and width of the pathogen were measured to calculate the growth area. The determination of phytopathogen inhibition was performed according to the following formula:

$$PASDAAS\ (\%)\ = \frac{AWG}{TGA} x100$$

Where PASDAAS represents the percentage of specific antibiotic activity of the area score, AWG the area without growth, and TGA the total growth area of the pathogen. To calculate AWG, the area present in the treatment plate was subtracted by the TGA of the control plate.

2.3. Biocontrol Evaluation under Greenhouse Conditions

In planta evaluation in a greenhouse was performed in 1-L pots containing Vivatto plus® (São Paulo, Brazil) substrate based on a completely randomized design (CRD) with 20 replicates for each treatment using the San Marzano cultivar

(Isla®, Porto Alegre, Brazil), which is susceptible to bacterial wilt caused by R. solanacearum. In the preliminary study, isolates RN 11, RN 24, and SOL 195 were tested in September (32 ± 6.5 °C and humidity of 52 ± 8%), and the final test was performed with the best-performing isolate in December (28 ± 1.5 °C and humidity of 71 ± 10%) 2023. Tomato seedlings grew in a seedbed for 30 days, but on the 23rd day, the seedlings referring to the treatments with the isolates were inoculated with 5 mL of cell suspension (10^{10} CFU mL^{-1}) of each isolate, while in the negative and positive controls, 5 mL of distilled water was added. On the 30th day, the seedlings were transplanted, and after 5 days, small incisions were made in the roots at a distance of 2 cm from the collar of all seedlings for infection with 5 mL of *R. solanacearum* suspension (10^{10} CFU mL^{-1}) in the positive control and treatments. In the negative control, 5 mL of sterile distilled water was added. The calculation of the percentage of disease incidence is given by

$$Disease\ incidence\ (\%) = \frac{DP}{TP} \times 100$$

Where DP is the number of diseased plants and TP is the total number of plants in the experiment. Tomato plants were considered to be diseased when they presented at least one of the symptoms: wilted leaves and wilted branches. Symptom monitoring was performed daily for 30 days, and to avoid misinterpretation of the seedlings' condition, the evaluation was performed 1 h after irrigation. Survival was calculated using the following formula:

$$Survivor\ (\%)\ = \frac{LP}{TP} x100$$

Where LP is the number of live plants and TP is the total number of plants in the experiment. To evaluate the effect of the biocontrol agent on aspects related to plant development in the presence of the pathogen, the following were measured: height (cm), stem diameter (cm), root length (cm), as well as shoot dry weight (g) and root dry weight (g). The seedlings were measured using a measuring tape and caliper.

2.4. Analysis of R. solanacearum Suppression in Soil

At the end of the in planta tests, soil samples were collected from all treatments and controls. In a test tube, 1 g of soil was resuspended in 10 mL of sterile distilled water and shaken. From this tube, serial dilution was performed up to a concentration of 10^{-8}. In triplicate, 100 µL of the 10^{-1}, 10^{-5}, and 10^{-8} concentrations was plated on Petri dishes containing LB medium and incubated at 28 °C for 7 days, with daily monitoring of colony appearance. Bacterial colonies with colorimetry similar to that of the phytopathogen were inoculated on CPG (casamino acid-peptone-glucose) and TTC (triphenyl tetrazolium chloride) media for confirmation, where the formation of opaque white colonies on CPG medium turned dark pink on TTC medium, which was considered indicative of the presence of *R. solanacearum* [37,38]. Suppression was calculated using the following formula:

$$Suppression\ (\%) = 100 - \left(\frac{n°of\ R.\ solanacearum\ colonies\ in\ treatment}{n°\ of\ R.\ solanacearum\ colonies\ in\ PC} \times 100\right)$$

2.5. DNA Extraction, Sequencing, and Genome Assembly

Only the isolates selected for in planta tests were identified. Isolate RN 11 was cultured in LB medium for 24 h, while isolates RN 24 and SOL 195 were cultured in ISP2 medium for 96 h. The cultures were centrifuged, and the supernatants were discarded to obtain the cell mass. DNA was isolated using the

CTAB protocol [39]. The amount of DNA obtained was estimated by spectrophotometry (NanoDrop 2000, Thermo Scientific, Waltham, USA), while integrity was verified by electrophoresis on 0.8% (w/v) agarose gel. The Illumina platform (150 bp paired-end) was used for complete genome sequencing, with a minimum sequencing coverage of 100X. Genome De Novo assembly was performed using SPAdes assembler [40], kmer = 123, read correction algorithm was also performed in order to reduce the number of mismatches and short indels.

2.6. Phylogenomic Identification

The identification of isolates at the species level was performed based on the complete genome through comparison with type species using the TYGS platform (https://tygs.dsmz.de), accessed on 08-01-2024. From the most closely related species identified in TYGS, the dDDH calculation was obtained using the d2 formula with the aid of the GGDC platform (https://ggdc.dsmz.de/ggdc.php#), accessed on 10-01-2024 and the ANI (Average Nucleotide Identity) calculation was performed using the OAT software (https://www.ezbiocloud.net/tools/orthoani, accessed on 10-01-2024), where dDDH < 70% and ANI < 95% were used as indicative of a new species [41–43]. For the search of plasmids in the genomes, they were analyzed through Plasmidfinder, available on the Galaxy Europe platform (https://usegalaxy.eu/), accessed on 15-02-2024.

2.7. Production of Extracellular Enzymes

For extracellular enzymes, the assays were performed only for the isolates selected for biocontrol tests in specific media for amylase [44], cellulase [45], lipase [46], protease [47], and chitinase [48]. Starch (amylase), skimmed milk (protease), Tween 80 (lipase), chitin (chitinase), and carboxymethyl cellulose (cellulase) were used as substrates in the enzymatic tests. All assays were performed in triplicate with 5 mm discs of bacterial cultures and incubated for 48 h at 28 °C for subsequent measurement of halos (mm) with the aid of a caliper.

2.8. Production of In Vitro Growth Promotion Inducers

All the assays described below were performed with the selected isolates (RN 11, RN 24, and SOL 195) for the biocontrol tests in tomato plants.

2.8.1. Phosphate (P) and Zinc (Zn) Solubilization

The P solubilization was performed in Pikovskaya's medium [49], while Zn solubilization was performed according to Saravanan et al. [50] with modifications, where the medium was composed of 798 mL of distilled water, 200 mL of M95X solution (33.78 g L^{-1} Na_2HPO_4, 15 g L^{-1} KH_2PO_4, 2.5 g L^{-1} NaCl, and 5 g L^{-1} NH_4Cl), 2 mL of 1M $MgSO_4.7H_2O$ solution, 100 µL of 1 M $CaC_2.2H_2O$ solution, 15 g of agar, and 1 g of Zn source. ZnO and $ZnSO_4$ were used as Zn sources. All assays were performed in triplicate with 5 mm discs of isolate cultures and incubated for five days at 28 °C for subsequent measurement of halos with the aid of a caliper.

2.8.2. Siderophore

The assay was performed according to the modifications made by Thampi and Bhai [51] to the assay described by Schwyn and Neilands [52], where MGs-1 medium was used (20 g L^{-1} dextrose, 1 g L^{-1} KNO_3, 0.1 g L^{-1} NaCl, 0.1 g L^{-1}

$MgSO_4.7H_2O$, 0.5 g L^{-1} K_2HPO_4, 15 g L^{-1} agar, 900 mL of distilled water, and 100 mL of CAS). All assays were performed in triplicate with 5 mm discs of isolate cultures and incubated for five days at 28 °C for subsequent measurement of halos with the aid of a caliper.

2.8.3. Indole Acetic Acid (IAA)

Isolate RN 11 was cultured in LB medium, and isolates RN 24 and SOL 195 were cultured in ISP2 medium, both supplemented with tryptophan (150 mg L^{-1}), in triplicate under agitation at 150 rpm in the dark for 7 days. The cultures were centrifuged for 30 min at 4000 rpm to obtain the supernatant. The assay was performed with 1 mL of supernatant and 1 mL of Salkowski's solution. The reaction was incubated for 60 min in the dark for subsequent reading at 595 nm. In the blank, sterile culture medium was used instead of the supernatant. For quantification, a standard curve with IAA was made [51].

2.8.4. Ammonia

The isolates were cultured in triplicate in peptone-water medium (10 g L^{-1} peptone, 5 g L^{-1} NaCl) for 48 h at 30 °C. The culture was centrifuged for 30 min at 4000 rpm, and 10 mL of the supernatant was transferred to test tubes. Five hundred microliters of Nessler's solution was added, and the color change to yellow or brown was observed [53].

2.9. Statistical Analysis

The data were subjected to analysis of variance (ANOVA) using R 4.3.1. software, and when significant ($p < 0.05$), the Scott-Knott mean test was applied. The assumptions of ANOVA, such as normality and homoscedasticity of residuals, were verified using the Shapiro–Wilk and Levene's tests, respectively.

3. Results

3.1. In Vitro Antimicrobial Activity

Of the 36 isolates from sediments of the Solimões and Negro rivers evaluated against *R. solanacearum*, only three (RN 2, SOL 110, and SOL 229) did not show antibiotic activity. Twenty-nine isolates exhibited PASDAAS (percent area specific differential antibiotic activity score) between 7 and 36%, while isolates RN 11, RN 24, SOL 116, and SOL 195 stood out with the highest indices (Table S1). With the exception of SOL 116 (67% antibiotic activity), the others presented indices equal to or greater than 70% and were selected for biocontrol evaluation in the greenhouse. Isolates RN 11 and SOL 195 completely inhibited the growth of *R. solanacearum*, and RN 24 inhibited 87.62% (Figure S1).

3.2. Phylogenomic Identification

Phylogenomic analyses of isolates RN 11, RN 24, and SOL 195 were performed using ANI and dDDH. The results revealed that isolated RN 11 belongs to the species *Priestia aryabhattai*, with ANI and dDDH values of 98.61% and 88.3%, respectively. On the other hand, isolates RN 24 and SOL 195 presented ANI and dDDH values below the cutoff points for new species, and the most closely related type species were *Streptomyces ardesiascus* and *Kitasatospora aureofaciens*, respectively (Table 1). RN 24 exhibited 92.29% ANI and 46.8% dDDH with *S.*

ardesiascus, while SOL 195 presented 86.36% ANI and 31.1% dDDH with *K. aureofaciens*.

Table 1. Taxonomic identification of bacterial isolates tested in planta for biocontrol potential against *Ralstonia solanacearum*.

Isolate	Size (pb)	Scaffolds	Type Species	NCBI Accession	Specie	ANI (%)	dDDH2 (%)
RN 11	5.262.007	45	*Priestia aryabhattai*^{T}	NZ_CP024035	*Priestia aryabhattai*	98,61	88,3
RN 24	8.364.889	366	*Streptomyces ardesiascus*	BEWC01000001.1	*Streptomyces* sp nov	92,29	46,8
SOL195	9.091.611	397	*Kitasatospora aureofaciens*	CP020567.1	*Kitasatospora* sp nov	86,36	31,1

3.3. Production of Growth Promotion Inducers and Enzymes

The selected isolates were evaluated for the production of extracellular enzymes and plant growth inducers (Table 2). *Kitasatospora* sp. SOL 195 stood out in the production of amylase (20 ± 1.2 mm) and chitinase (18.2 ± 0.9 mm), while *P. aryabhattai* RN 11 showed the highest production of lipase (26 ± 1.5 mm) and was the only one to produce protease (17.7 ± 1.1 mm). *Streptomyces* sp. RN 24 exhibited the highest production of cellulase (31 ± 1.8 mm) (Figure S2).

Table 2. Plant growth inducers and enzymes produced in vitro by *Priestia aryabhattai* RN 11, *Streptomyces* sp. RN 24 and *Kitasatospora* sp. SOL 195.

Assay	***P. aryabhattai* RN 11**	***Streptomyces* sp. RN 24**	***Kitasatospora* sp. SOL 195**
Siderophore (mm)	12 ± 1.6	0	5 ± 0.8
IAA (µg/mL)	26.1 ± 2	42.1 ± 1.8	47.8 ± 2.3
Ammonia	+	++	+++
P (mm)	0	0	0
ZnO (mm)	11 ± 1.5	0	0
$ZnSO_4$ (mm)	15 ± 2	0	0

+ weak reaction; ++ medium reaction; +++ strong reaction; mean of triplicate.

All isolates produced indole-3-acetic acid (IAA) and ammonia, with the strain *Kitasatospora* sp. SOL 195 presents the highest levels for both compounds and RN 11 the lowest. *Streptomyces* sp. RN 24 did not produce P and Zn solubilizers or siderophores under the tested conditions (Table 2). *P. aryabhattai* RN 11 was the only one to solubilize Zn (both sources) and showed the highest production of siderophores.

3.4. Biological Control under Greenhouse Conditions

In the Amazonian dry period (September), plants inoculated with *P. aryabhattai* RN 11 showed symptoms after 10 days, while in plants inoculated only with the pathogen (positive control), symptoms were observed 24 h post-inoculation. Plants inoculated with the microbial agents *Streptomyces* sp. RN 24 and *Kitasatospora* sp. SOL 195 demonstrated symptoms from the third and sixth day, respectively. In this period, when the survival index was evaluated, the positive control presented an index of 65%, while this value increased to 85% in the treatment with SOL 195 and 90% with RN 11 and RN 24. Treatment with RN 11 reduced disease incidence by 40%, followed by RN 24 and SOL 195, which reduced incidence by 20% and 5%, respectively. In addition, all isolates suppressed the pathogen in the soil with indices > 90% (Figure 1).

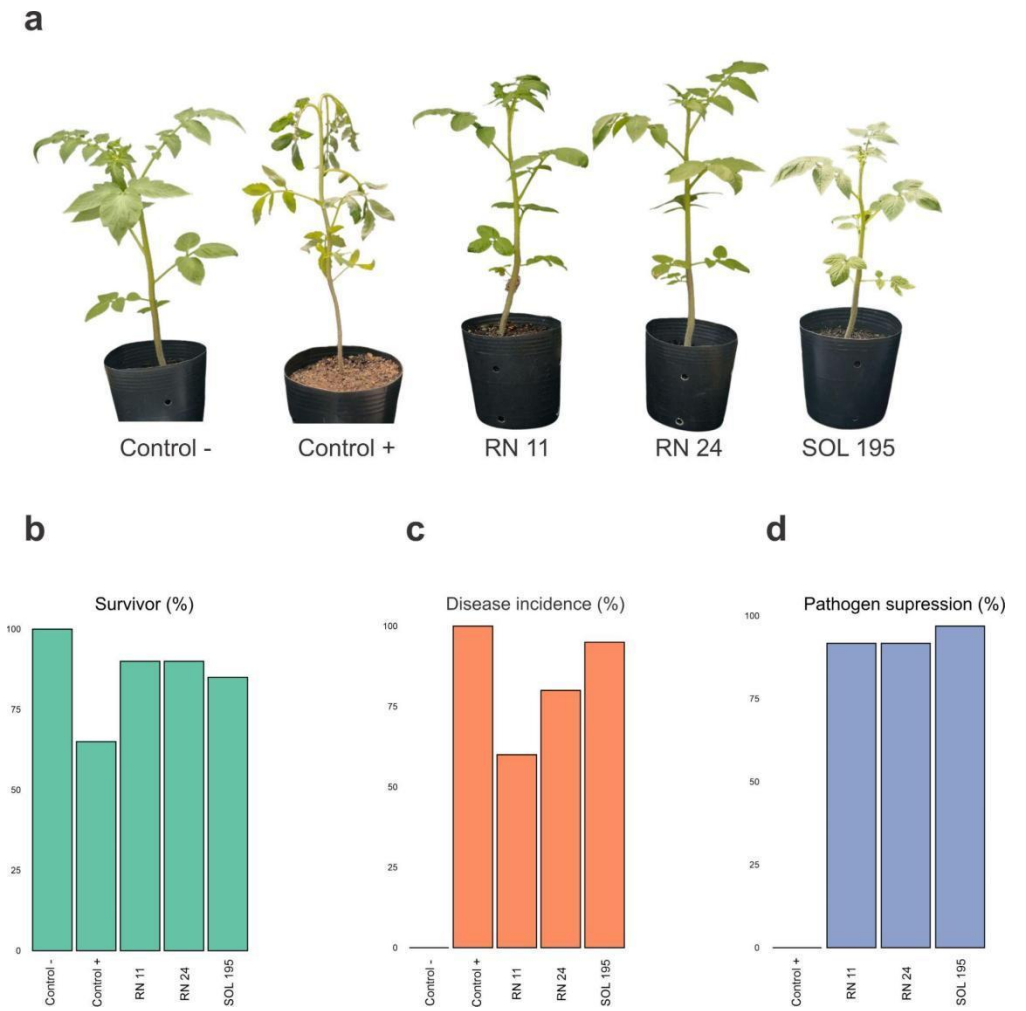

Figure 1. (a) Appearance of seedlings from control groups and those treated with *Priestia aryabhattai* RN 11, *Streptomyces* sp. RN 24, and *Kitasatospora* sp. SOL 195. (b) Survival indices, (c) disease incidence, and (d) suppression of *Ralstonia solanacearum* in the soil using the microbial agents as biological controllers during the Amazonian dry period.

Still in the dry period, in addition to the control of *R. solanacearum*, isolates RN 11, RN 24, and SOL 195 were also evaluated for their positive effect on parameters related to growth promotion under conditions of infection with *R. solanacearum* (Table 3). The results obtained demonstrated that treatment with RN 24 provided a significant increase in plant height compared to the positive control (PC), reaching values similar to the negative control (NC). Regarding stem diameter and leaf size, all treatments, except for those still in the dry period, in addition to the control of *R. solanacearum*, isolates RN 11, RN 24, and SOL 195 were also evaluated for their positive effect on parameters related to growth promotion under conditions of infection with *R. solanacearum* (Table 3). The results obtained demonstrated that treatment with RN 24 provided a significant increase in plant height compared to the positive control (PC), reaching values similar to the negative control (NC). Regarding stem diameter and leaf size, all treatments, except for SOL 195, were significantly superior to PC, with emphasis on RN 11, which did not differ statistically from NC for these parameters. Regarding root growth, all treatments showed significant differences compared to PC, with RN 11 being equal to NC. In addition, all treatments with biocontrol agents resulted in a higher number of

branches when compared to PC. Isolates RN 11 and RN 24 stood out in the parameters of shoot dry weight (ADW) and root dry weight (RDW), presenting values significantly higher than PC, although they did not differ from each other.

Table 3. Growth promotion of tomato cv. San Marzano infected with *Ralstonia solanacearum* using agents *Priestia aryabhattai* RN 11, *Streptomyces* sp. RN 24, and *Kitasatospora* sp. SOL 195 during summer.

Test	Height (cm)	Stem Diameter (cm)	Root (cm)	Branch (unit)	Leaf (cm)	ADW (g)	RDW (g)
NC	51.96 ± 5.9 a	0.40 a	14.77 ± 2.1 a	8 ± 1 b	7.05 ± 0.17 a	0.618 ± 0.06 a	0.116 ± 0.02 c
PC	42.64 ± 7.2 b	0.20 c	10.69 ± 1.7 c	6 ± 1 c	3.77 ± 0.22 c	0.309 ± 0.02 c	0.094 ± 0.01 d
RN 11	46.21 ± 3.1 b	0.36 ± 0.05 a	15.46 ± 2.8 a	8 ± 1 b	7.04 ± 0.12 a	0.525 ± 0.03 b	0.151 ± 0.01 b
RN 24	50.81 ± 3.2 a	0.32 ± 0.07 b	13.37 ± 1.9 b	8 ± 1 b	6.81 ± 0.35 b	0.509 ± 0.02 b	0.142 ± 0.01 b
SOL 195	44.85 ± 3.3 b	0.25 ± 0.05 c	13.96 ± 1.6 b	7 ± 1 b	3.74 ± 0.36 c	0.332 ± 0.01 c	0.116 ± 0.01 c

ADW—air dry weight (total); RDW—root dry weight (total); NC—negative control without pathogen; PC—positive control with pathogen. Means followed by the same letter do not differ by Scott-Knott test at 5% probability.

Based on the best performance obtained in the dry period, the strain *P. aryabhattai* RN 11 stood out from the other isolates based on disease incidence and survival of infected plants, being selected for evaluation in the rainy period. The disease incidence in the positive control in the rainy period was 80% with a mortality rate of 65%. Compared to the previous period, there was a 20% reduction in incidence and a 10% increase in mortality. The same is observed in the treatment with RN 11, which presents a high incidence (60%) in the dry period and low in the rainy period (10%); however, both the mortality rate (10%) and pathogen suppression (>90%) in the soil are similar in both periods (Figure S3).

In the rainy period, the growth promotion parameters are significantly influenced by the treatment with RN 11 compared to the positive control (PC) in all aspects evaluated (Table 4). The height of plants treated with RN 11 (42.04 cm) was significantly higher than both PC (34.19 ± 2.5 cm) and negative control (NC) (37.69 ± 3.1 cm). Stem diameter was also positively affected by RN 11 (0.38 ± 0.03 cm), being statistically superior to PC (0.22 ± 0.02 cm) and NC (0.3 ± 0.09 cm). Regarding root length, treatment with RN 11 (29.04 ± 3.6 cm) did not differ significantly from NC (27 ± 4.3 cm), but both were superior to PC (19.73 ± 3.6 cm). The number of branches was higher in plants treated with RN 11 (8 units) compared to PC and NC (both with 7 units). Leaf size did not differ between RN 11 (5.77 ± 0.5 cm) and NC (5.71 ± 0.4 cm), both being significantly superior to PC (4.77 ± 0.9 cm). Shoot dry weight (ADW) was significantly higher in the treatment with RN 11 (1.32 ± 0.23 g) compared to PC (0.94 ± 0.11 g) and NC (1.1 ± 0.28 g). Root dry weight (RDW) was statistically similar between RN 11 (0.26 ± 0.07 g) and NC (0.266 ± 0.08 g), both being superior to PC (0.126 ± 0.03 g). These results indicate that *P. aryabhattai* RN 11 can act not only as an efficient biocontrol agent but also as a growth promoter in tomato plants grown in the Amazon during the rainy period, significantly improving various plant development parameters compared to the positive control infected with *R. solanacearum*.

Table 4. Growth promotion effect with *Priestia aryabhattai* RN 11 on tomato cv. San Marzano infected with *Ralstonia solanacearum* in the rainy period.

Test	Height (cm)	Stem Diameter (cm)	Root (cm)	Branch (unit)	Leaf (cm)	ADW (g)	RDW (g)
NC	37.69 ± 3.1 b	0.3 ± 0.09 b	27 ± 4.3 a	7 ± 1 b	5.71 ± 0.4 a	1.1 ± 0.28 b	0.266 ± 0.08 a
PC	34.19 ± 2.5 c	0.22 ± 0.02 c	19.73 ± 3.6 b	7 ± 1 b	4.77 ± 0.9 b	0.94 ± 0.11 b	0.126 ± 0.03 b
RN 11	42.04 ± 4.8 a	0.38 ± 0.03 a	29.04 ± 3.6 a	8 ± 1 a	5.77 ± 0.5 a	1.32 ± 0.23 a	0.26 ± 0.07 a

ADW—air dry weight (total); RDW—root dry weight (total); NC—negative control without pathogen; PC—positive control with pathogen. Means followed by the same letter do not differ by Scott-Knott test at 5% probability.

4. Discussion

The dynamics of rivers and their tributaries promote the exchange of organic matter and microbial agents capable of producing diverse secondary metabolites, creating a unique dynamic in these ecosystems that favors the emergence and diversification of microbial lineages, enhancing the discovery of new biological solutions for challenges in health, agriculture, and industry [54–60]. In this context, Amazonian rivers have proven to be a rich source of microbial biodiversity with the capacity to produce new antimicrobial agents with biotechnological potential, filling the gaps in current knowledge about the diversity and potential of these microorganisms [61–63].

Exploring the microbial diversity of two Amazonian rivers with distinct characteristics, the Negro River and the Solimões River, provides a comprehensive view of the biotechnological potential of aquatic microorganisms in the region. The Negro River is considered the largest blackwater river in the world and is characterized by its high acidity (pH < 5.0), high concentration of humic compounds, low sediment load (clay), and low concentration of chemical elements (mainly cations), which is why it has low electrical conductivity [64–66]. In contrast, the Solimões River is classified as a whitewater river, with a pH of 5–7, rich in Ca^{2+} and HCO_3, and a high amount of suspended material and dissolved salts, resulting in a greater diversity of microorganisms [67].

The microbial diversity of the Amazonian aquatic environment, exemplified in this study by the exploration of microorganisms from the sediments of the Solimões and Negro rivers, reveals that this ecosystem can be an important source for the development of new inputs for the biological control of *R. solanacearum*, a serious problem for agriculture, especially related to vegetable production in northern Brazil. The results obtained in this study fill a gap in knowledge about the potential of Amazonian aquatic microorganisms for controlling this economically important phytopathogen.

The antibiotic activity identified against *R. solanacearum*, based on the Percent Area Specific Differential Antibiotic Activity Score (PASDAAS), showed variation among the selected isolates RN11, RN 24, and SOL195, with inhibition ranging from 87.55 to 100%. This variation in antimicrobial efficacy reflects what has been observed in previous studies documenting the metabolic diversity of aquatic microorganisms and their ability to produce bioactive compounds [55,59,68–70]. The observed differences in the antibiotic activity of the isolates may be related to the diversity of secondary metabolites produced by each strain and the cultivation conditions used in the assays. Additional studies are needed to elucidate the specific compounds responsible for antibiotic activity and to optimize the production conditions of these metabolites.

The results obtained confirm the identification of RN 11 as *P. aryabhattai*, because the ANI and dDDH values were above the cutoff points. On the other hand, the ANI and dDDH values below the cutoff points for RN 24 and SOL 195 provide strong evidence that these isolates represent new species within the genera *Streptomyces* and *Kitasatospora*, respectively. The ANI and dDDH metrics have been widely employed to delimit bacterial species, offering a robust and reliable alternative to conventional methods. The established cutoff points for species delimitation correspond to 95–96% for ANI and 70% for dDDH [41–43]. The discovery of new species of *Streptomyces* and *Kitasatospora* from Amazonian river sediments highlights the importance of this ecosystem as a source of unexplored microbial diversity. Future studies may investigate the biotechnological potential of these new species and their distribution and ecological role in the aquatic environments of the region.

Actinobacteria, such as those of the genera *Streptomyces* and *Kitasatospora*, are known for their range of molecules with antimicrobial, antitumor, and immunosuppressive properties, with emphasis on aspects related to biocontrol and growth promotion in agriculture [35,71–81]. The results of this study provide promising perspectives for the bioprospecting of new secondary metabolites from the RN 24 and SOL 195 strains, contributing to the expansion of the diversity of known bioactive compounds and to the advancement in the discovery of molecules with biotechnological applications.

In addition to secondary metabolites, the production of extracellular enzymes and plant growth inducers by bacteria is an important mechanism for promoting plant growth [82]. Extracellular enzymes, such as amylases, cellulases, chitinase, lipases, and proteases, play fundamental roles in the degradation of complex polymers, making nutrients available to plants and contributing to the suppression of phytopathogens [83]. In this study, the isolates showed different enzymatic production profiles, with emphasis on *Kitasatospora* sp. SOL 195 in the production of amylase and chitinase, *P. aryabhattai* RN 11 in the production of lipase and protease, and *Streptomyces* sp. RN 24 in the production of cellulase. This functional diversity can be exploited for the development of microbial inoculants with multiple enzymatic activities, aiming at promoting plant growth and protection against phytopathogens.

The production of protease by *Bacillus subtilis* B315 was used as evidence of antagonistic potential against *R. solanacearum*, as the enzyme assists in resistance to the phytopathogen by degrading the extracellular polymeric substances (EPS) and the biofilm produced by the pathogen [84]. Considering that *Bacillus* and *Priestia* are closely related genera, there is a possibility that *P. aryabhattai* RN 11 uses this mechanism, differentiating itself from *Streptomyces* sp. RN 24 and *Kitasatospora* sp. SOL 195, which did not demonstrate protease production. Additional studies are needed to elucidate the specific role of the protease produced by *P. aryabhattai* RN 11 in the suppression of *R. solanacearum* and to investigate other antagonism mechanisms that may be involved.

In addition to enzymes, the isolates also produced plant growth inducers, such as IAA, ammonia, and siderophores. IAA is an important plant hormone involved in regulating plant growth and development, while ammonia contributes to nitrogen nutrition [85]. Siderophores, in turn, are iron-chelating compounds that facilitate the absorption of this micronutrient by plants, especially in soils with low iron availability [86]. The production of these plant growth inducers by the isolates suggests their potential for promoting plant growth, in addition to their biocontrol

activity against *R. solanacearum*. Future studies may evaluate the effect of these isolates on the growth and development of different agricultural crops, as well as investigate the molecular mechanisms involved in the plant-microorganism interaction.

The ability to solubilize trace nutrients, such as P and Zn, is another relevant mechanism for promoting plant growth. Although none of the isolates solubilized P under the tested conditions, RN 11 stood out in the solubilization of Zn from different sources (ZnO and $ZnSO_4$). The solubilization of Zn by actinomycetes can increase the availability of this micronutrient for plants, contributing to their growth and productivity [87]. The ability of *P. aryabhattai* RN 11 to solubilize Zn suggests its potential for application as a biofertilizer, especially in soils deficient in this micronutrient. Additional studies are needed to evaluate the effectiveness of RN 11 in promoting plant growth under field conditions and to investigate the mechanisms involved in Zn solubilization.

The strains RN 11, RN 24, and SOL 195, when evaluated for the biological control of *R. solanacearum* under greenhouse conditions, demonstrated efficacy in suppressing the phytopathogen in the soil > 90%, but only RN 11 showed a high survival rate associated with reduced disease incidence and a significant growth promotion effect compared to the positive control. These results highlight the potential of *P. aryabhattai* RN 11 as an effective biocontrol agent against *R. solanacearum*, capable of suppressing the pathogen in the soil, reducing disease incidence, and promoting plant growth. Future studies may investigate the efficacy of RN 11 under field conditions, as well as evaluate its compatibility with other integrated disease management practices.

Agricultural production losses caused by *R. solanacearum* vary due to several factors such as the level of resistance of the cultivar used, climate, soil, and genetic variation of the *R. solanacearum* strains present in the crop [26]. In this complex context of interactions influenced by various factors, we evaluated the biological control of *R. solanacearum* in the two main climatic seasons that occur in the Amazon region (summer and rainy period). As observed in the obtained results, the isolate RN 11 showed a 40% reduction in the incidence of tomato disease in summer and 90% in the rainy season, exemplifying what was exposed by Yuliar et al. [26], but it is important to note that the climatic effect did not interfere with the suppression of the phytopathogen in the soil or with the survival rate promoted by biological control.

The analysis of the results under different climatic conditions provides insight into the robustness of *P. aryabhattai* RN 11 as a biocontrol agent, demonstrating its efficacy in different environmental contexts. This characteristic is highly desirable for the implementation of large-scale biocontrol strategies, as climatic conditions can vary significantly between different regions and times of the year.

The results obtained with *P. aryabhattai* RN 11 are superior to those observed with the use of improved mutants of *Bacillus amyloliquefaciens* for the biocontrol of *R. solanacearum*. In the study by Yadav et al. [88], conducted under climatic conditions similar to those of the rainy period in the present work, the mutants of *B. amyloliquefaciens* DSBA-11 (MNTG-21, MUV-19, and MHNO2-20) provided a 50–73% reduction in disease incidence and 60–88% survival. These values are lower than those obtained with the RN 11 strain, demonstrating its greater potential in controlling *R. solanacearum*, even when compared to a species already consolidated as a biological control agent for various phytopathogens, such as B. amyloliquefaciens [31,89–95].

The fact that *P. aryabhattai* RN 11 presents superior results to improved mutants of *B. amyloliquefaciens* further highlights the potential of this strain, as it has not undergone genetic modifications to enhance its biocontrol efficiency. These findings open up promising perspectives for the development of microbial inoculants based on *P. aryabhattai* RN 11, with the potential to surpass the efficacy of products already on the market.

Priestia aryabhattai, previously known as *Bacillus aryabhattai* [96], was isolated as an endophytic bacterium and has evidence in the literature regarding its potential for the biocontrol of phytopathogens such as *Fusarium oxysporum* [97] and *Ralstonia syzygii* [98], a species that is part of the *R. solanacearum* species complex that causes bacterial wilt [8].

The results of the present study corroborate and expand the knowledge about the potential of *P. aryabhattai* as a biocontrol agent, demonstrating its efficacy against *R. solanacearum* under different climatic conditions and highlighting its potential for application in the integrated management of bacterial wilt in tomato. Furthermore, this is the first report of a P. aryabhattai strain isolated from Amazonian river sediments, revealing the importance of this ecosystem as a source of microorganisms with biotechnological potential.

As promising perspectives, these studies open up a range of opportunities for future research aimed at exploring the biotechnological potential of bacteria isolated from Amazonian river sediments. Among the most relevant possibilities is the development of microbial inoculants based on *P. aryabhattai* RN 11 for the integrated management of bacterial wilt in tomato. To this end, additional studies are needed to evaluate the efficacy of this isolate under field conditions, as well as to optimize the formulations and application methods of the inoculant.

Another promising line of research is the investigation of the molecular mechanisms involved in the interaction between *P. aryabhattai* RN 11 and the host plant, as well as in the suppression of *R. solanacearum*. The elucidation of these mechanisms, through omic approaches (genomics, transcriptomics, proteomics, and metabolomics), may provide insights for the improvement of biological control strategies and plant growth promotion mediated by this microorganism.

Moreover, the discovery of new species of *Streptomyces* sp. (RN 24) and *Kitasatospora* sp. (SOL 195) opens up perspectives for the bioprospecting of bioactive secondary metabolites from these actinomycetes. Future studies may focus on the isolation, structural characterization, and evaluation of the biological activities of the compounds produced by these new species, aiming at the identification of new antimicrobial, antitumor, and immunosuppressive agents, among others.

5. Conclusion

The present study revealed the biotechnological potential of bacteria isolated from sediments of the Amazonian rivers Negro and Solimões as biocontrol agents against *R. solanacearum* and plant growth promoters. Among the evaluated isolates, *P. aryabhattai* RN 11 stood out for its efficacy in suppressing the phytopathogen in the soil, reducing the incidence of bacterial wilt, and promoting the growth of tomato plants under different climatic conditions. These results open up promising perspectives for the development of microbial inoculants based on *P. aryabhattai* RN 11, aiming at the integrated management of bacterial wilt in tomato.

Furthermore, the discovery of possible new species of *Streptomyces* (RN 24) and *Kitasatospora* (SOL 195) highlights the importance of the microbial biodiversity of Amazonian rivers as a source of new bioactive compounds and

biocontrol agents. These findings emphasize the need for future studies to explore the biotechnological potential of these new species, as well as to investigate the efficacy of the isolates under field conditions and elucidate the molecular mechanisms involved in the plant–microorganism interaction.

References

1. Assefa, W.; Tesfaye, B.; Dessalegn, L. Influence of Inter-Intra Row Spacing on Yield Losses of Tomato Cultivars. *Ethiop. J. Agric. Sci.* **2015**, *25*, 15–28.
2. Elnaggar, S.; Mohamed, A.M.; Bakeer, A.; Osman, T.A. Current Status of Bacterial Wilt (*Ralstonia solanacearum*) Disease in Major Tomato (*Solanum lycopersicum* L.) Growing Areas in Egypt. *Arch. Agric. Environ. Sci.* **2018**, *3*, 399–406. https://doi.org/10.26832/24566632.2018.0304012.
3. Kurabachew, H.; Ayana, G. Bacterial Wilt Caused by *Ralstonia solanacearum* in Ethiopia: StatusaAnd Management Approaches: A Review. *Int. J. Phytopathol.* **2016**, *5*, 107–119. https://doi.org/10.33687/phytopath.005.03.1829.
4. Coelho Netto, R.A.; Noda, H.; Boher, B. Melanthera discoidea: Um novo hospedeiro de *Ralstonia solanacearum*. *Fitopatol. Bras.* **2001**, *26*, 781. https://doi.org/10.1590/S0100-41582001000400020.
5. Coelho Netto, R.A.; Pereira, B.G.; Noda, H.; Boher, B. Caracterização de isolados de *Ralstonia solanacearum* obtidos de tomateiros em várzea e em terra firme, no Estado do Amazonas. *Fitopatol. Bras.* **2003**, *28*, 362–366. https://doi.org/10.1590/S0100-41582003000400003.
6. Coelho Netto, R.A.; Pereira, B.G.; Noda, H.; Boher, B. Murcha bacteriana no estado do Amazonas, Brasil. *Fitopatol. Bras.* **2004**, *29*, 17–23. https://doi.org/10.1590/S0100-41582004000100004.
7. Lopes, C.A.; Rossato, M. History and Status of Selected Hosts of the *Ralstonia solanacearum* Species Complex Causing Bacterial Wilt in Brazil. *Front. Microbiol.* **2018**, *9*, 1228. https://doi.org/10.3389/fmicb.2018.01228.
8. Vailleau, F.; Genin, S. *Ralstonia solanacearum*: An Arsenal of Virulence Strategies and Prospects for Resistance. *Annu. Rev. Phytopathol.* **2023**, *61*, 25–47. https://doi.org/10.1146/annurev-phyto-021622-104551.
9. Cellier, G.; Prior, P. Deciphering Phenotypic Diversity of *Ralstonia solanacearum* Strains Pathogenic to Potato. *Phytopathology* **2010**, *100*, 1250–1261. https://doi.org/10.1094/PHYTO-02-10-0059.
10. Lebeau, A.; Daunay, M.C.; Frary, A.; Palloix, A.; Wang, J.F.; Dintinger, J.; Chiroleu, F.; Wicker, E.; Prior, P. Bacterial Wilt Resistance in Tomato, Pepper, and Eggplant: Genetic Resources Respond to Diverse Strains in the *Ralstonia solanacearum* Species Complex. *Phytopathology* **2011**, *101*, 154–165. https://doi.org/10.1094/PHYTO-02-10-0048.
11. Romo, J.P.; Osorio, J.G.M.; Yepes, M.S. Identification of New Hosts for *Ralstonia solanacearum* (Smith) Race 2 from Colombia. *Rev. Protección Veg.* **2012**, *27*, 151–161.
12. Wicker, E.; Lefeuvre, P.; De Cambiaire, J.-C.; Lemaire, C.; Poussier, S.; Prior, P. Contrasting Recombination Patterns and Demographic Histories of the Plant Pathogen *Ralstonia solanacearum* Inferred from MLSA. *ISME J.* **2012**, *6*, 961–974. https://doi.org/10.1038/ismej.2011.160.
13. Santiago, T.R.; Lopes, C.A.; Caetano-Anollés, G.; Mizubuti, E.S.G. Phylotype and Sequevar Variability of *Ralstonia solanacearum* in Brazil, an Ancient Centre of Diversity of the Pathogen. *Plant Pathol.* **2017**, *66*, 383–392. https://doi.org/10.1111/ppa.12586.
14. Bergsma-Vlami, M.; Van De Bilt, J.L.J.; Tjou-Tam-Sin, N.N.A.; Westenberg, M.; Meekes, E.T.M.; Teunissen, H.A.S.; Van Vaerenbergh, J. Phylogenetic

Assignment of *Ralstonia pseudosolanacearum* (*Ralstonia solanacearum* Phylotype I) Isolated from *Rosa* spp. *Plant Dis.* **2018**, *102*, 2258–2267. https://doi.org/10.1094/PDIS-09-17-1345-RE.
15. Rodrigues, F.; Ana Carolina, P.N.; Daniel Diego, C.C.; Mylla Crysthyan, R. Induction of Tolerance to Bacterial Wilt in Hybrids of Tomatoes by Application of Gibberellin. *Rev. Cienc. Agrovet.* **2018**, *17*, 54–60. https://doi.org/10.5965/223811711712018054.
16. Huet, G. Breeding for Resistances to *Ralstonia solanacearum*. *Front. Plant Sci.* **2014**, *5*, 715. https://doi.org/10.3389/fpls.2014.00715.
17. Kaari, M.; Joseph, J.; Manikkam, R.; Sreenivasan, A.; Venugopal, G. Biological Control of *Streptomyces* sp. UT4A49 to Suppress Tomato Bacterial Wilt Disease and Its Metabolite Profiling. *J. King Saud Univ.-Sci.* **2022**, *34*, 101688. https://doi.org/10.1016/j.jksus.2021.101688.
18. Kaari, M.; Joseph, J.; Manikkam, R.; Sreenivasan, A.; Venugopal, G.; Alexander, B.; Krishnan, S. Anti-Biofilm Activity and Biocontrol Potential of *Streptomyces* Cultures Against *Ralstonia solanacearum* on Tomato Plants. *Indian J. Microbiol.* **2022**, *62*, 32–39. https://doi.org/10.1007/s12088-021-00963-1.
19. Genin, S.; Denny, T.P. Pathogenomics of the *Ralstonia solanacearum* Species Complex. *Annu. Rev. Phytopathol.* **2012**, *50*, 67–89. https://doi.org/10.1146/annurev-phyto-081211-173000.
20. Saputra, R.; Arwiyanto, T.; Wibowo, A. Biological Control of *Ralstonia solanacearum* Causes of Bacterial Wilt Disease with Pseudomonas Putida and *Streptomyces* spp. on Some Tomato Varieties. *IOP Conf. Ser. Earth Environ. Sci.* **2020**, *515*, 012007. https://doi.org/10.1088/1755-1315/515/1/012007.
21. Noda, H.; Pahlen, A.V.; Der Silva Filho, D.F. Avaliação Da Resistência de Progênies de Tomate à Murcha Bacteriana Em Solo Naturalmente Infestado Por *Pseudomonas solanacearum* (Smith) Dows. *Rev. Bras. Genética* **1986**, *9*, 55–66.
22. Costa, K.D.D.S.; Santos, P.R.; Santos, A.M.M.; Silva, A.M.F.; Chagas, J.T.B.; Carvalho Filho, J.L.S.; Pereira, J.W.D.L.; Silva, M.D.O.; Silva, J.R.; Menezes, D. Genetic Control of Tomato Resistance to *Ralstonia solanacearum*. *Euphytica* **2019**, *215*, 136. https://doi.org/10.1007/s10681-019-2458-6.
23. Marques, M.J.; Vizú, J.D.F.; Silva Filho, D.F.; Ticona-Benavente, C.A. Tomato Progenies Selection in Rondônia, Brazil. *Hortic. Bras.* **2019**, *37*, 106–111. https://doi.org/10.1590/s0102-053620190117.
24. Ishikawa, R.; Fujimori, K.; Matsuura, K. Antibacterial Activity of Validamycin A against Pseudomonas Solanacearum and Its Efficacy against Tomato Bacterial Wilt. *Jpn. J. Phytopathol.* **1996**, *62*, 478–482.
25. Sing'ombe Ombiro, G.; Sawai, T.; Noutoshi, Y.; Nishina, Y.; Matsui, H.; Yamamoto, M.; Toyoda, K.; Ichinose, Y. Specific Growth Inhibitors of *Ralstonia Solanacearum*, *Xanthomonas Oryzae* Pv. *Oryzae, X. Campestris* Pv. *Campestris*, and *Clavibacter Michiganensis* Subsp. *Michiganensis*. *Microbiol. Res.* **2018**, *215*, 29–35. https://doi.org/10.1016/j.micres.2018.06.005.
26. Yuliar; Nion, Y.A.; Toyota, K. Recent Trends in Control Methods for Bacterial Wilt Diseases Caused by *Ralstonia Solanacearum*. *Microbes Environ.* **2015**, *30*, 1–11. https://doi.org/10.1264/jsme2.ME14144.
27. Chen, M.; Wang, J.; Liu, B.; Zhu, Y.; Xiao, R.; Yang, W.; Ge, C.; Chen, Z. Biocontrol of Tomato Bacterial Wilt by the New Strain *Bacillus Velezensis* FJAT-46737 and Its Lipopeptides. *BMC Microbiol.* **2020**, *20*, 160. https://doi.org/10.1186/s12866-020-01851-2.
28. Boukaew, S.; Chuenchit, S.; Petcharat, V. Evaluation of *Streptomyces* spp. for Biological Control of Sclerotium Root and Stem Rot and Ralstonia Wilt of Chili

Pepper. *BioControl* **2011**, *56*, 365–374. https://doi.org/10.1007/s10526-010-9336-4.
29. Elsayed, T.R.; Jacquiod, S.; Nour, E.H.; Sørensen, S.J.; Smalla, K. Biocontrol of Bacterial Wilt Disease Through Complex Interaction between Tomato Plant, Antagonists, the Indigenous Rhizosphere Microbiota, and *Ralstonia solanacearum*. *Front. Microbiol.* **2020**, *10*, 2835. https://doi.org/10.3389/fmicb.2019.02835.
30. Ling, L.; Han, X.; Li, X.; Zhang, X.; Wang, H.; Zhang, L.; Cao, P.; Wu, Y.; Wang, X.; Zhao, J.; et al. A Streptomyces Sp. NEAU-HV9: Isolation, Identification, and Potential as a Biocontrol Agent against *Ralstonia solanacearum* of Tomato Plants. *Microorganisms* **2020**, *8*, 351. https://doi.org/10.3390/microorganisms8030351.
31. Raza, W.; Ling, N.; Yang, L.; Huang, Q.; Shen, Q. Response of Tomato Wilt Pathogen Ralstonia Solanacearum to the Volatile Organic Compounds Produced by a Biocontrol Strain *Bacillus amyloliquefaciens* SQR-9. *Sci. Rep.* **2016**, *6*, 24856. https://doi.org/10.1038/srep24856.
32. Tan, H.M.; Cao, L.X.; He, Z.F.; Su, G.J.; Lin, B.; Zhou, S.N. Isolation of Endophytic Actinomycetes from Different Cultivars of Tomato and Their Activities against *Ralstonia Solanacearum* in Vitro. *World J. Microbiol. Biotechnol.* **2006**, *22*, 1275–1280. https://doi.org/10.1007/s11274-006-9172-y.
33. Tan, H.; Zhou, S.; Deng, Z.; He, M.; Cao, L. Ribosomal-Sequence-Directed Selection for Endophytic Streptomycete Strains Antagonistic to *Ralstonia solanacearum* to Control Tomato Bacterial Wilt. *Biol. Control* **2011**, *59*, 245–254. https://doi.org/10.1016/j.biocontrol.2011.07.018.
34. Zhao, J.; Han, L.; Yu, M.; Cao, P.; Li, D.; Guo, X.; Liu, Y.; Wang, X.; Xiang, W. Characterization of *Streptomyces sporangiiformans* sp. nov., a Novel Soil Actinomycete with Antibacterial Activity against *Ralstonia solanacearum*. *Microorganisms* **2019**, *7*, 360. https://doi.org/10.3390/microorganisms7090360.
35. Zhuang, X.; Gao, C.; Peng, C.; Wang, Z.; Zhao, J.; Shen, Y.; Liu, C. Characterization of a Novel Endophytic Actinomycete, *Streptomyces physcomitrii* sp. nov., and Its Biocontrol Potential against *Ralstonia solanacearum* on Tomato. *Microorganisms* **2020**, *8*, 2025. https://doi.org/10.3390/microorganisms8122025.
36. Velho-Pereira, S.; Kamat, N.M. Antimicrobial Screening of Actinobacteria Using a Modified Cross-Streak Method. *Indian J. Pharm. Sci.* **2011**, *73*, 223–228. https://doi.org/10.4103/0250-474X.91566.
37. Agarwal, H.; Dowarah, B.; Baruah, P.M.; Bordoloi, K.S.; Krishnatreya, D.B.; Agarwala, N. Endophytes from *Gnetum Gnemon* L. Can Protect Seedlings against the Infection of Phytopathogenic Bacterium *Ralstonia solanacearum* as Well as Promote Plant Growth in Tomato. *Microbiol. Res.* **2020**, *238*, 126503. https://doi.org/10.1016/j.micres.2020.126503.
38. Kim, S.G.; Hur, O.-S.; Ro, N.-Y.; Ko, H.-C.; Rhee, J.-H.; Sung, J.S.; Ryu, K.-Y.; Lee, S.-Y.; Baek, H.J. Evaluation of Resistance to *Ralstonia solanacearum* in Tomato Genetic Resources at Seedling Stage. *Plant Pathol. J.* **2016**, *32*, 58–64. https://doi.org/10.5423/PPJ.NT.06.2015.0121.
39. Doyle, J.J.; Doyle, J.L. A Rapid DNA Isolation Procedure for Small Quantities of Fresh Leaf Tissue. *Phytochem. Bull.* **1987**, *19*, 11–15.
40. Prjibelski, A.; Antipov, D.; Meleshko, D.; Lapidus, A.; Korobeynikov, A. Using SPAdes De Novo Assembler. *Curr. Protoc. Bioinform.* **2020**, *70*, e102. https://doi.org/10.1002/cpbi.102.
41. Lee, I.; Kim, Y.O.; Park, S.C.; Chun, J. OrthoANI: An Improved Algorithm and Software for Calculating Average Nucleotide Identity. *Int. J. Syst. Evol. Microbiol.* **2016**, *66*, 1100–1103. https://doi.org/10.1099/ijsem.0.000760.

42. Meier-Kolthoff, J.P.; Auch, A.F.; Klenk, H.-P.; Öker, M.G. Genome Sequence-Based Species Delimitation with Confidence Intervals and Improved Distance Functions. *BMC Bioinform.* **2013**, *14*, 60. https://doi.org/10.1186/1471-2105-14-60.
43. Meier-Kolthoff, J.P.; Carbasse, J.S.; Peinado-Olarte, R.L.; Göker, M. TYGS and LPSN: A Database Tandem for Fast and Reliable Genome-Based Classification and Nomenclature of Prokaryotes. *Nucleic Acids Res.* **2022**, *50*, D801–D807. https://doi.org/10.1093/nar/gkab902.
44. Abd-Elhalem, B.T.; El-Sawy, M.; Gamal, R.F.; Abou-Taleb, K.A. Production of Amylases from Bacillus Amyloliquefaciens under Submerged Fermentation Using Some Agro-Industrial by-Products. *Ann. Agric. Sci.* **2015**, *60*, 193–202. https://doi.org/10.1016/j.aoas.2015.06.001.
45. Kasana, R.; Salwan, R.; Dhar, H.; Dutt, S.; Gulati, A. A Rapid and Easy Method for the Detection of Microbial Cellulases on Agar Plates Using Gram's Iodine. *Curr. Microbiol.* **2008**, *57*, 503–507. https://doi.org/10.1007/s00284-008-9276-8.
46. Haba, E.; Bresco, O.; Ferrer, C.; Marqués, A.; Busquets, M.; Manresa, A. Isolation of Lipase-Secreting Bacteria by Deploying Used Frying Oil as Selective Substrate. *Enzym. Microb. Technol.* **2000**, *26*, 40–44. https://doi.org/10.1016/S0141-0229(99)00125-8.
47. Masi, C.; Gemechu, G.; Tafesse, M. Isolation, Screening, Characterization, and Identification of Alkaline Protease-Producing Bacteria from Leather Industry Effluent. *Ann. Microbiol.* **2021**, *71*, 24. https://doi.org/10.1186/s13213-021-01631-x.
48. Souza, C.; Burbano-Rosero, E.; Almeida, B.; Martins, G.; Albertini, L.; Rivera, I. Culture Medium for Isolating Chitinolytic Bacteria from Seawater and Plankton. *World J. Microbiol. Biotechnol.* **2009**, *25*, 2079–2082. https://doi.org/10.1007/s11274-009-0098-z.
49. Subba Rao, N.S. *Soil Microorganisms and Plant Growth*; Oxford and IBH Publishing Co.: Delhi, India, 1977.
50. Saravanan, V.S.; Subramoniam, S.R.; Raj, S.A. Assessing in Vitro Solubilization Potential of Different Zinc Solubilizing Bacterial (Zsb) Isolates. *Braz. J. Microbiol.* **2004**, *35*, 121–125. https://doi.org/10.1590/S1517-83822004000100020.
51. Thampi, A.; Bhai, R.S. Rhizosphere Actinobacteria for Combating *Phytophthora Capsici* and *Sclerotium Rolfsii*, the Major Soil Borne Pathogens of Black Pepper (*Piper nigrum* L.). *Biol. Control* **2017**, *109*, 1–13. https://doi.org/10.1016/j.biocontrol.2017.03.006.
52. Schwyn, B.; Neilands, J.B. Universal Chemical Assay for the Detection and Determination of Siderophores. *Anal. Biochem.* **1987**, *160*, 47–56. https://doi.org/10.1016/0003-2697(87)90612-9.
53. Cappucino, J.C.; Sherman, N. *Microbiology: A Laboratory Manual*; Benjamin/Cumming Pub. Co.: New York, NY, USA, 1992.
54. Weisse, T. Biodiversity of Freshwater Microorganisms—Achievements, Problems, and Perspectives. *Pol. J. Ecol.* **2006**, *54*, 633–652.
55. Barreto, E.S.; Torres, A.R.; Barreto, M.R.; Vasconcelos, A.T.R.; Astolfi-Filho, S.; Hungria, M. Diversity in Antifungal Activity of Strains of *Chromobacterium violaceum* from the Brazilian Amazon. *J. Ind. Microbiol. Biotechnol.* **2008**, *35*, 783–790. https://doi.org/10.1007/s10295-008-0331-z.
56. Farjalla, V.F. Are the Mixing Zones between Aquatic Ecosystems Hot Spots of Bacterial Production in the Amazon River System? *Hydrobiologia* **2014**, *728*, 153–165. https://doi.org/10.1007/s10750-014-1814-8.
57. Toyama, D.; Santos-Júnior, C.D.; Kishi, L.T.; Oliveira, T.C.S.; Garcia, J.W.; Sarmento, H.; Miranda, F.P.; Henrique-Silva, F. A Snapshot on Prokaryotic

Diversity of the Solimões River Basin (Amazon, Brazil). *Genet. Mol. Res.* **2017**, *16*, gmr16029567. https://doi.org/10.4238/gmr16029567.
58. Doherty, M.; Yager, P.L.; Moran, M.A.; Coles, V.J.; Fortunato, C.S.; Krusche, A.V.; Medeiros, P.M.; Payet, J.P.; Richey, J.E.; Satinsky, B.M.; et al. Bacterial Biogeography across the Amazon River-Ocean Continuum. *Front. Microbiol.* **2017**, *8*, 882. https://doi.org/10.3389/fmicb.2017.00882.
59. Santos-Júnior, C.D.; Sarmento, H.; Miranda, F.P.; Henrique-Silva, F.; Logares, R. Uncovering the Genomic Potential of the Amazon River Microbiome to Degrade Rainforest Organic Matter. *Microbiome* **2020**, *8*, 151. https://doi.org/10.1186/s40168-020-00930-w.
60. Gomes, R.P.; Oliveira, T.R.; Gama, A.R.; Alves, K.R.; Santos, R.K.; Vieira, J.D.G.; Pires, D.J.; Carneiro, L.C. Phenotypic Plasticity and Characterization of Chromobacterium Isolates from Aquatic Environment. *Res. Soc. Dev.* **2022**, *11*, e28111536821. https://doi.org/10.33448/rsd-v11i15.36821.
61. Motta, A.S.; Cladera-Olivera, F.; Brandelli, A. Screening for Antimicrobial Activity among Bacteria Isolated from the Amazon Basin. *Braz. J. Microbiol.* **2004**, *35*, 307–310. https://doi.org/10.1590/S1517-83822004000300007.
62. Oliveira-Longatti, S.M.; Marra, L.M.; Lima Soares, B.; Bomfeti, C.A.; Silva, K.; Avelar Ferreira, P.A.; Souza Moreira, F.M. Bacteria Isolated from Soils of the Western Amazon and from Rehabilitated Bauxite-Mining Areas Have Potential as Plant Growth Promoters. *World J. Microbiol. Biotechnol.* **2014**, *30*, 1239–1250. https://doi.org/10.1007/s11274-013-1547-2.
63. Pereira, J.O.; Souza, A.Q.L.; Souza, A.D.L.; Castro França, S.; Oliveira, L.A. Overview on Biodiversity, Chemistry, and Biotechnological Potential of Microorganisms from the Brazilian Amazon. In *Diversity and Benefits of Microorganisms from the Tropics*; De Azevedo, J.L., Quecine, M.C., Eds.; Springer International Publishing: Cham, Switzerland, 2017; pp. 71–103. https://doi.org/10.1007/978-3-319-55804-2_5.
64. Do Nascimento Monte, C.; Correa Saldanha, E.; Costa, I.; Shinaigger Rocha Do Nascimento, T.; Santos Pereira, M.; Farias Batista, L.; Costa Pinheiro, D. The Physical-Chemical Characteristics of Surface Waters in the Management of Quality in Clearwater Rivers in the Brazilian Amazon. *Water Policy* **2021**, *23*, 1303–1313. https://doi.org/10.2166/wp.2021.258.
65. Pinheiro, L.A.; Borges, J.T. Avaliação hidroquímica qualitativa das aguas do baixo Rio Negro. *RUnPetro* **2013**, *1*, 26–32.
66. Silva, H.A.; Araújo, N.B.; Santos, N.L.; Bolson, A.; Gontijo, E.S.J.; Junior, E.S.[Concentração Letal (CL50) e Potencial de Substâncias Húmicas Aquáticas Do Rio Negro (Brasil) Na Redução de Toxicidade Aguda de Íons Cu^{2+}. *Sci. Amazon.* **2020**, *9*, 63–75.
67. Queiroz, M.M.A.; Horbe, A.M.C.; Seyler, P.; Moura, C.A.V. Hidroquímica do rio Solimões na região entre Manacapuru e Alvarães: Amazonas-Brasil. *Acta Amaz.* **2009**, *39*, 943–952. https://doi.org/10.1590/S0044-59672009000400022.
68. Feliatra, F.; Mardalisa, M.; Setiadi, J.; Lukistyowaty, I.; Hutasoit, A.Y. Potential of Secondary Metabolite from Marine Heterotrophic Bacteria against Pathogenic Bacteria in Aquaculture. *J. Phys. Conf. Ser.* **2020**, *1655*, 012044. https://doi.org/10.1088/1742-6596/1655/1/012044.
69. Setiaji, J.; Feliatra, F.; Teruna, H.Y.; Lukistyowati, I.; Suharman, I.; Muchlisin, Z.A.; Johan, T.I. Antibacterial Activity in Secondary Metabolite Extracts of Heterotrophic Bacteria against *Vibrio Alginolyticus*, *Aeromonas Hydrophila*, and *Pseudomonas aeruginosa*. *F1000Research* **2020**, *9*, 1491. https://doi.org/10.12688/f1000research.26215.1.
70. Viju, N.; Punitha, S.M.J.; Satheesh, S. In Vitro and In Silico Antifouling Activity Analysis of Secondary Metabolites Extracted from the Marine

Bacterium *Vibrio Alginolyticus*. *Thalassas* **2024**, *40*, 225–235. https://doi.org/10.1007/s41208-023-00642-6.
71. Srinivas, V.; Gopalakrishnan, S.; Kamidi, J.P.; Chander, G. Effect of plant growth-promoting *Streptomyces* sp. on plant growth and yield of tomato and chilli. *Andhra Pradesh J. Agril. Sci.* **2020**, *6*, 65–70.
72. Javed, Z.; Tripathi, G.D.; Mishra, M.; Dashora, K. Actinomycetes—The Microbial Machinery for the Organic-Cycling, Plant Growth, and Sustainable Soil Health. *Biocatal. Agric. Biotechnol.* **2021**, *31*, 101893. https://doi.org/10.1016/j.bcab.2020.101893.
73. Dede, A.; Güven, K.; Şahin, N. Isolation, Plant Growth-Promoting Traits, Antagonistic Effects on Clinical and Plant Pathogenic Organisms and Identification of Actinomycetes from Olive Rhizosphere. *Microb. Pathog.* **2020**, *143*, 104134. https://doi.org/10.1016/j.micpath.2020.104134.
74. Chaurasia, A.; Meena, B.R.; Tripathi, A.N.; Pandey, K.K.; Rai, A.B.; Singh, B. Actinomycetes: An Unexplored Microorganisms for Plant Growth Promotion and Biocontrol in Vegetable Crops. *World J. Microbiol. Biotechnol.* **2018**, *34*, 132. https://doi.org/10.1007/s11274-018-2517-5.
75. Cunha-Ferreira, I.C.; Vizzotto, C.S.; Freitas, M.A.M.; Peixoto, J.; Carvalho, L.S.; Tótola, M.R.; Thompson, F.L.; Krüger, R.H. Genomic and Physiological Characterization of *Kitasatospora* sp. Nov., an Actinobacterium with Potential for Biotechnological Application Isolated from Cerrado Soil. *Braz. J. Microbiol.* **2024**, *55*, 1099–1115. https://doi.org/10.1007/s42770-024-01324-y.
76. Li, C.; Pan, D.; Li, M.; Wang, Y.; Song, L.; Yu, D.; Zuo, Y.; Wang, K.; Liu, Y.; Wei, Z.; et al. Aerobactin-Mediated Iron Acquisition Enhances Biofilm Formation, Oxidative Stress Resistance, and Virulence of *Yersinia pseudotuberculosis*. *Front. Microbiol.* **2021**, *12*, 699913. https://doi.org/10.3389/fmicb.2021.699913.
77. Tu, C.K.; Wang, P.H.; Lee, M.H. Endophytic Bacterium Lysobacter Firmicutimachus Strain 5-7 Is a Promising Biocontrol Agent against Rice Seedling Disease Caused by *Pythium arrhenomanes* in Nursery Trays. *Plant Dis.* **2023**, *107*, 1075–1086. https://doi.org/10.1094/PDIS-05-22-1195-RE.
78. Haesler, F.; Hagn, A.; Frommberger, M.; Hertkorn, N.; Schmitt-Kopplin, P.; Munch, J.C.; Schloter, M. In Vitro Antagonism of an Actinobacterial *Kitasatospora* Isolate against the Plant Pathogen *Phytophthora citricola* as Elucidated with Ultrahigh Resolution Mass Spectrometry. *J. Microbiol. Methods* **2008**, *75*, 188–195. https://doi.org/10.1016/j.mimet.2008.05.028.
79. Pithakkit, S.; Petcharat, V.; Chuenchit, S.; Pornsuriya, C.; Sunpapao, A. Isolation of Antagonistic Actinomycetes Species from Rhizosphere as Effective Biocontrol against Oil Palm Fungal Diseases. *Agric. Technol. Biol. Sci. Walailak J. Sci. Tech.* **2015**, *12*, 481–490.
80. Shrivastava, S.; D'souza, S.F.; Desai, P.D. Production of Indole-3-Acetic Acid by Immobilized Actinomycete (*Kitasatospora* sp.) for Soil Applications. *Curr. Sci.* **2008**, *94*, 1595–1604.
81. Takahashi, Y. Genus Kitasatospora, Taxonomic Features and Diversity of Secondary Metabolites. *J. Antibiot.* **2017**, *70*, 506–513. https://doi.org/10.1038/ja.2017.8.
82. Viaene, T.; Langendries, S.; Beirinckx, S.; Maes, M.; Goormachtig, S. *Streptomyces* as a Plant's Best Friend? *FEMS Microbiol. Ecol.* **2016**, *92*, fiw119. https://doi.org/10.1093/femsec/fiw119.
83. Singh, D.P.; Patil, H.J.; Prabha, R.; Yandigeri, M.S.; Prasad, S.R. Chapter 2—Actinomycetes as Potential Plant Growth-Promoting Microbial Communities. In *Crop Improvement through Microbial Biotechnology*; Prasad, R., Gill, S.S.,

Tuteja, N., Eds.; Elsevier: Amsterdam, The Netherlands, 2018; pp. 27–38. https://doi.org/10.1016/B978-0-444-63987-5.00002-5.
84. Prihatiningsih, N.; Asnani, A.; Djatmiko, H.A. Extracellular Protease from Bacillus Subtilis B315 with Antagonistic Activity against Bacterial Wilt Pathogen (*Ralstonia solanacearum*) of Chili. *Biodiversitas* **2021**, *22*, 1291–1295. https://doi.org/10.13057/biodiv/d220327.
85. Spaepen, S.; Vanderleyden, J. Auxin and Plant-Microbe Interactions. *Cold Spring Harb. Perspect. Biol.* **2011**, *3*, a001438. https://doi.org/10.1101/cshperspect.a001438.
86. Ahmed, E.; Holmström, S.J.M. Siderophores in Environmental Research: Roles and Applications. *Microb. Biotechnol.* **2014**, *7*, 196–208. https://doi.org/10.1111/1751-7915.12117.
87. Mumtaz, M.Z.; Ahmad, M.; Jamil, M.; Hussain, T. Zinc Solubilizing *Bacillus* Spp. Potential Candidates for Biofortification in Maize. *Microbiol. Res.* **2017**, *202*, 51–60. https://doi.org/10.1016/j.micres.2017.06.001.
88. Yadav, D.K.; Devappa, V.; Kashyap, A.S.; Kumar, N.; Rana, V.S.; Sunita, K.; Singh, D. Boosting the Biocontrol Efficacy of *Bacillus amyloliquefaciens* DSBA-11 through Physical and Chemical Mutagens to Control Bacterial Wilt Disease of Tomato Caused by *Ralstonia solanacearum*. *Microorganisms* **2023**, *11*, 1790. https://doi.org/10.3390/microorganisms11071790.
89. Singh, D.; Kumar Yadav, D. Potential of *Bacillus amyloliquefaciens* for Biocontrol of Bacterial Wilt of Tomato Incited by *Ralstonia Solanacearum*. *J. Plant Pathol. Microbiol.* **2016**, *7*, 1000327. https://doi.org/10.4172/2157-7471.1000327.
90. Tan, S.; Gu, Y.; Yang, C.; Dong, Y.; Mei, X.; Shen, Q.; Xu, Y. *Bacillus amyloliquefaciens* T-5 May Prevent *Ralstonia Solanacearum* Infection through Competitive Exclusion. *Biol. Fertil. Soils* **2016**, *52*, 341–351. https://doi.org/10.1007/s00374-015-1079-z.
91. Kang, B.R.; Park, J.S.; Jung, W.-J. Antifungal Evaluation of Fengycin Isoforms Isolated from *Bacillus amyloliquefaciens* PPL against *Fusarium Oxysporum* f. Sp. Lycopersici. *Microb. Pathog.* **2020**, *149*, 104509. https://doi.org/10.1016/j.micpath.2020.104509.
92. Khan, N.; Bano, A.; Ali, S.; Babar, M.A. Crosstalk amongst Phytohormones from Planta and PGPR under Biotic and Abiotic Stresses. *Plant Growth Regul.* **2020**, *90*, 189–203. https://doi.org/10.1007/s10725-020-00571-x.
93. Khan, M.; Salman, M.; Ahmad Jan, S.; Khan Shinwari, Z. Biological Control of Fungal Phytopathogens: A Comprehensive Review Based on *Bacillus* Species. *MOJ Biol. Med.* **2021**, *6*, 90–92. https://doi.org/10.15406/mojbm.2021.06.00137.
94. Khan, A.R.; Mustafa, A.; Hyder, S.; Valipour, M.; Rizvi, Z.F.; Gondal, A.S.; Yousuf, Z.; Iqbal, R.; Daraz, U. *Bacillus* spp. as Bioagents: Uses and Application for Sustainable Agriculture. *Biology* **2022**, *11*, 1763. https://doi.org/10.3390/biology11121763.
95. Mousa, M.A.A.; Abo-Elyousr, K.A.M.; Abdel Alal, A.M.K.; Alshareef, N.O. Management Fusarium Wilt Disease in Tomato by Combinations of *Bacillus amyloliquefaciens* and Peppermint Oil. *Agronomy* **2021**, *11*, 2536. https://doi.org/10.3390/agronomy11122536.
96. Gupta, R.S.; Patel, S.; Saini, N.; Chen, S. Robust Demarcation of 17 Distinct *Bacillus* Species Clades, Proposed as Novel Bacillaceae Genera, by Phylogenomics and Comparative Genomic Analyses: Description of *Robertmurraya Kyonggiensis* sp. Nov. and Proposal for an Emended Genus *Bacillus* Limiting It Only to the Members of the Subtilis and Cereus Clades of Species. *Int. J. Syst. Evol. Microbiol.* **2020**, *70*, 5753–5798. https://doi.org/10.1099/ijsem.0.004475.

97. Akram, W.; Waqar, S.; Hanif, S.; Anjum, T.; Aftab, Z.-E.-H.; Li, G.; Ali, B.; Rizwana, H.; Hassan, A.; Rehman, A.; et al. Comparative Effect of Seed Coating and Biopriming of *Bacillus Aryabhattai* Z-48 on Seedling Growth, Growth Promotion, and Suppression of Fusarium Wilt Disease of Tomato Plants. *Microorganisms* **2024**, *12*, 792. https://doi.org/10.3390/microorganisms12040792.
98. Yanti, Y.; Hamid, H.; Reflin. Development of the PGPR and Cyanobacteria Consortium for Growth Promotion and Control *Ralstonia syzigii* subsp. *indonesiensis* of Tomato. *IOP Conf. Ser. Earth Environ. Sci.* **2021**, *709*, 012085. https://doi.org/10.1088/1755-1315/709/1/012085.

SUPPLEMENTARY MATERIAL

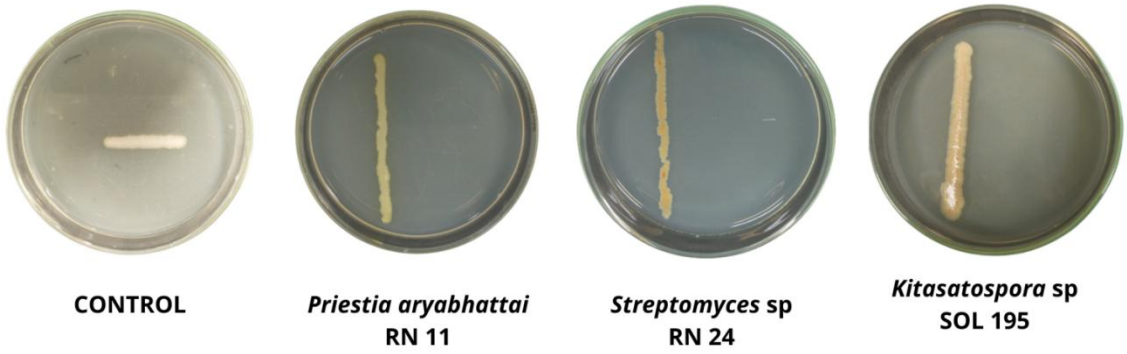

Figure S1. Antimicrobial assay against *Ralstonia solanacerum* using the bacterial strains *Priestia aryabhattai* RN 11, *Streptomyces* sp. RN 24, and *Kitasatospora* sp. SOL 195.

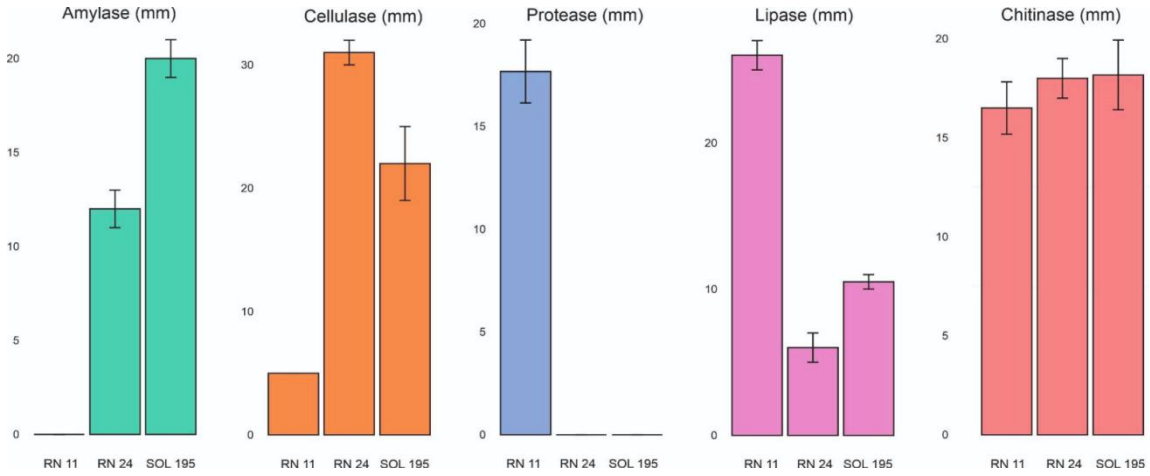

Figure S2. Comparative production of enzymes Amylase, Cellulase, Protease, Lipase, and Chitinase by the bacterial strains *Priestia aryabhattai* RN 11, *Streptomyces* sp. RN 24, and *Kitasatospora* sp. SOL 195.

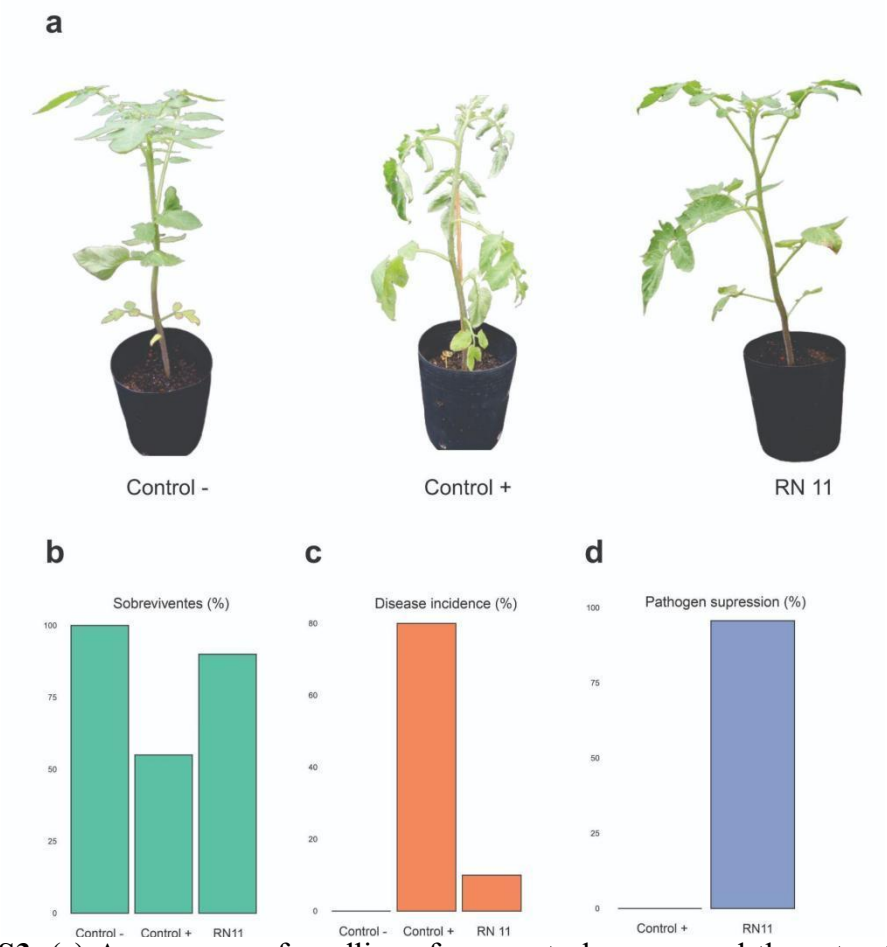

Figure S3. (a) Appearance of seedlings from control groups and those treated with Priestia aryabhattai RN 11. (b) Survival indices, (c) disease incidence, and (d) suppression of Ralstonia solanacearum in the soil using Priestia aryabhattai RN 11 as a biological control agent during the rainy period.

Table S1. Origin of each isolate and Its PASDAAS (percent area specific differential antibiotic activity score) against *Raltonia solanacearum* under *in vitro* condition.

Isolates	Geographic coordinates	City	PASDAAS (%)	Type of bacteria
SOL 92	3°21'31.5"S 68°11'59.8"W	Amaturá	21.31±1.03	Actinobacteria
SOL 105	3°51'58.1"S 63°49'03.9"W	Coari	19.15±2.07	Non-filamentous
SOL 134	3°54'56.1"S 62°38'44.2"W		10.59±0.65	Non-filamentous
SOL 229	4°04'21.9"S 63°08'18.7"W		0	Non-filamentous
SOL 65	3°50'05.2"S 62°04'05.2"W	Codajás	16.45±1.26	Actinobacteria
SOL 152	3°16'21.0"S 60°15'01.7"W	Iranduba	12.51±1.78	Non-filamentous
SOL 49	2°34'23.5"S 67°16'08.5"W	Jutaí	11.28±1.42	Actinobacteria
SOL 156	2°46'51.9"S 66°57'27.0"W		15.37±2.51	Non-filamentous
SOL 171			10.54±0.46	Non-filamentous
SOL 230	2°31'19.1"S 66°23'35.0"W		14.37±2.51	Actinobacteria
SOL 110	3°39'59.8"S 64°12'38.2"W	Tefé	0	Non-filamentous
SOL 116			67±2.04	Actinobacteria
SOL 126	3°24'48.9"S 64°32'05.4"W		21.04±1.58	Actinobacteria
SOL 53	2°51'56.9"S 67°46'30.4"W	Tonantins	17.76±2.48	Actinobacteria
SOL 194	2°51'33.7"S 65°11'39.6"W	Uarini	15.67±1.46	Actinobacteria
SOL 195	2°59'08.8"S 65°07'55.2"W		100	Actinobacteria
SOL 196			8.12±0.24	Non-filamentous
SOL 199			10.34±1.53	Actinobacteria
RN 1	3°09'13"S 59°54'42"W	Manaus	13.2±0.82	Non-filamentous
RN 2			0	Non-filamentous
RN 3			16.04±0.81	Non-filamentous
RN 4			14.3±0.26	Non-filamentous
RN 5			9.43±0.41	Non-filamentous
RN 6			8.32±1.14	Non-filamentous
RN 7			7.35±0.92	Non-filamentous
RN 8			13.88±1.59	Actinobacteria
RN 9			10.54±0.46	Actinobacteria
RN 10			9.09±0.98	Non-filamentous
RN11			100	Non-filamentous
RN 12			0	Non-filamentous
RN 14			29.27±0.79	Non-filamentous
RN 15			21.94±1.68	Non-filamentous
RN 16			10.8±0.74	Non-filamentous
RN 22			36±1.37	Non-filamentous
RN 23			7.28±1.22	Non-filamentous
RN 24			87.55±0.8	Actinobacteria
RN 32			11.13±0.92	Actinobacteria

Capítulo 3

ARTIGO 2 - DRAFT
Formatação de acordo com as normas da revista Biocatalysis and Agricultural Biotechnology

Unveiling the genomic potential and agricultural applications of the amazonian strain *Alcaligenes nematophilus* SOL 109

Abstract

Alcaligenes species are poorly explored in terms of their biotechnological potential and agricultural applications. In this study, the SOL 109 strain isolated from Solimões River sediments was extensively investigated. The results of the phylogenomic inferences, dDDH, and ANI analyses indicate that SOL 109 belongs to the *Alcaligenes nematophilus* species. A comparative analysis of the biosynthetic gene clusters (BGCs) composition revealed that in *Alcaligenes*, the BGCs for ectoine and terpene are shared by all species of the genus, whereas clusters for butyrolactone production and Ni-siderophores were detected only in specific lineages, demonstrating the need for characterization of *Alcaligenes* spp. to better understand their chemodiversity. Furthermore, the BGC of hydrogen cyanide-like metabolites was detected in SOL 109 and may be related to biocontrol and growth promotion. Genes related to antibiotics and heavy metal resistance were also identified in the SOL 109 genome. The analysis of metabolites present in SOL 109 extracts allowed the annotation of metabolites such as Cyclo(Tyr-Pro), NRPS with antimicrobial properties, and 13-docosenamide, a polyamide that stimulates nitrogen metabolism. In dual-culture assays, SOL 109 produced a high inhibition percentage of the phytopathogens *Colletotrichum siamense, Corynespora cassiicola*, and *Rhizoctonia solani,* ranging from 74.81-93%, whereas for *Ralstonia solanacearum*, the inhibition was only 7%. In addition, SOL 109 promoted the growth and control of *R. solani* in tomato plants. This work demonstrated the agricultural application of *A. nematophilus* and provides insights into *Alcaligenes* secondary metabolism via genome mining. We report for the first time the occurrence of these species in Brazil.

Key-words: Biosynthetic gene clusters, Resistance genes, Biocontrol, Growth promotion, Amazonian microorganisms.

1. Introdução

The genus *Alcaligenes* was first described in 1919 by Castellani and Chalmers, but its name was accepted only in 1980 (Skerman et al., 1980). It belongs to the family Alcaligenaceae and the order Burkholderiales. *Alcaligenes* strains are found in various environments, such as water, soil, industrial processes, insects, nematodes, and human clinical samples (Basharat et al., 2018; Liu et al., 2016; Machado et al., 2023; Pedrosa-Silva and Venancio, 2023; Qiu et al., 2017; Singha et al., 2017).

Although 26 species have been reported, only five are characterized as "taxa with a validly published and correct name" according to the LPSN (https://lpsn.dsmz.de/genus/alcaligenes): *Alcaligenes aquatilis* (Van Trappen et al., 2005), *Alcaligenes endophyticus* (Lu et al., 2017), *Alcaligenes faecalis* (Castellani and Chalmers, 1919), *Alcaligenes pakistanensis* (Abbas et al., 2015), and *Alcaligenes nematophilus*. The latter species was recently described in an isolate of the nematode *Oscheius tipulae* collected in Tails, Tunisia (Machado et al., 2023).

The genome of the described *Alcaligenes* spp. has an estimated size of 3.68–4.44 Mbp and possesses a GC content between 55.4% and 57.6%. Prophages were identified in *A. faecalis* Mc250, *A. eutrophus* A5, *A. ammoniaoxydans* HO-1, and *A. aquatilis* FA (Du et al., 2023; Duran et al., 2019; Faelen et al., 1993; Felestrino et al., 2020; Wu et al., 2021). The genome of *A. nematophilus* A-TC2 was reported to have a size of 4.25 Mbp and a GC content of 56.41%, but the presence of prophages has not been reported (Machado et al., 2023).

Alcaligenes species have been used in various bioremediation processes because they can degrade a range of xenobiotics, polycyclic aromatic hydrocarbons (PAHs), pesticides, dyes, 4-hydroxyacetophenone, and phenols. Some species can also oxidize and remove ammonia from nitrogen-rich wastewater (Adebusoye et al., 2007; Basharat et al., 2018; Hopper and Kaderbhai, 1999; Kiyohara et al., 1982; Kumar et al., 2013; Regar et al., 2016; Rehfuss and Urban, 2005; Sagarkar et al., 2014; Singha et al., 2017; Wu et al., 2021).

Alcaligenes strains can also be used to control phytopathogens through mechanisms such as resistance induction, lytic enzyme production, secondary metabolite production, and the production of volatile organic compounds (Felestrino et al., 2020; Kakar et al., 2018; Sayyed et al., 2010). Plant diseases controlled by *Alcaligenes* include collar rot caused by *Sclerotium rolfsii*, *Fusarium* wilt, brown rot caused by *Monilinia* spp., damping-off caused by *Rhizoctonia solani*, and clubroot caused by the parasite *Plasmodiophora brassicae* (Honda et al., 1999; Jia et al., 2022; Lahlali et al., 2020; Patel et al., 2020; Ray et al., 2023). In addition to biological control, the genus has also been reported as a growth promoter in economically important plant species, such as *Zea mays* (Dixit et al., 2020), *Oryza sativa* (Fatema et al., 2024; Fatima et al., 2020), and *Brassica oleracea* (Jia et al., 2022).

The metabolic arsenal of *Alcaligenes* species primarily includes molecules with antibiotic activity, such as kalimantan A, B, and C, as well as thomasin, which exhibit antibacterial activity against various microorganisms (Tokunaga et al., 1996). The production of antimicrobials, such as the antibiotic tunicamycin, which is effective against multidrug-resistant strains of bacteria, and the antifungal octadecyl 3-(3, 5-di-tert-butyl-4-hydroxyphenyl), which has shown promising activity, has been reported (El-Sayed et al., 2024; Kapley et al., 2016). *Alcaligenes* species are also producers of lipopolysaccharides and compounds such as phenylacetic acid, p-hydroxyphenylacetylamide, and cyclo-(Gly-L-Pro), which have immunostimulatory properties (Wu et al., 2012). Although there are few chemical studies on secondary metabolism in *Alcaligenes*, the genus has a repository of more than 110 genomes deposited in public databases, which can be utilized to explore the potential of biosynthetic pathways and their correlations with secondary metabolites.

2. Material and methods

2.1. Strain

Alcaligenes nematophilus SOL109 was isolated from sediments collected from the bottom of the Solimões River (3°39'59.8"S 64°12'38.2"W) and is part of the working collection of the Laboratory of Genomics and Applied Microbiology of the Legal Amazon (GENAGRO) at Embrapa Amazônia Ocidental. Isolation was performed using 10 mg of sediment in 900 µL of sterile distilled water, which was diluted to 10^{-5}, with the 10^{-1} and 10^{-5} dilutions inoculated in LB medium (10 g L^{-1} tryptone, 5 g L^{-1} yeast extract, 10 g L^{-1} NaCl). Cultivation and maintenance were carried out in LB medium at 28 °C. Access to genetic heritage was authorized under SISGEN No. A39C76B.

2.2. Morphological and biochemical characterization

Tests were performed for Gram staining, urease (Stuart et al., 1945), indole (MacWilliams, 2009), citrate, catalase (Davis and Pezzlo, 2023), and hydrogen sulfide (H_2S) (Kahn, 1925). For sugar assimilation (Mac Faddin, 2000), 1% solutions of arabinose, cellobiose, dextrose, fructose, galactose, lactose, maltose, mannitol, mannose, sucrose, and trehalose were used. Colony colorimetry was described according to the Web Safe Colors table available at https://html-color-codes.info/web-safe-colors. For extracellular enzymes, assays were performed in media suitable for amylase (Abd-Elhalem et al., 2015), cellulase (Kasana et al., 2008), lipase (Haba et al., 2000), protease (Masi et al., 2021), and chitinase (Souza et al., 2009). All tests were performed in triplicate using 5-mm disks containing the SOL 109 culture, which were incubated for 48 hours at 28 °C until the halos (mm) were measured with the aid of a caliper.

2.3. DNA extraction, sequencing and genome assembly

The bacteria were grown overnight in LB medium, centrifuged, and the supernatant was discarded to obtain cell mass. DNA was isolated using the CTAB protocol (Doyle and Doyle, 1987). The amount of DNA obtained was estimated by spectrophotometry (NanoDrop 2000, Thermo Scientific), and the integrity was checked by electrophoresis on a 0.8% (w/v) agarose gel. The Illumina platform (150 bp paired-end) was used for complete genome sequencing with a minimum sequencing coverage of 100X. The genome de novo assembly was performed using SPAdes assembler (Prjibelski et al., 2020) with kmer = 123. A read correction algorithm was also applied to reduce the number of mismatches and short indel.

2.4. Phylogenomic identification

The identification of the SOL 109 lineage at the species level was carried out based on the complete genome using the TYGS platform (https://tygs.dsmz.de) for comparison with previously characterized type species. From the most related species identified in TYGS, dDDH was calculated using the d2 formula based on GGDC (https://ggdc.dsmz.de/ggdc.php#) and ANI using the OAT software, where dDDH >70% and ANI >95% indicate the presence of the same species (Lee et al., 2016; Meier-Kolthoff et al., 2013). The genomes were analyzed using Plasmidfinder (available on the Galaxy Europe platform (https://usegalaxy.eu/), to identify plasmids.

2.5 Genomic mining and comparative genomics

The genome was annotated using the RAST platform (https://rast.nmpdr.org/rast.cgi). To identify the gene clusters responsible for the production of secondary metabolites, *Alcaligenes* genomes were submitted to the antiSMASH platform (https://antismash.secondarymetabolites.org/). The resulting gbks files were analyzed using BiG-SCAPE (https://bigscape-corason.secondarymetabolites.org/) to build molecular networks and dendrograms based on BGCs. Antibiotic resistance genes were identified using CARD (https://card.mcmaster.ca) in RDI (Resistance Gene Identifier) analysis. Comparative heatmaps were generated using the Pheatmap package in R. The comparison of orthologous genes between strain SOL 109 and the type species was performed using Orthoveen 3 (https://orthovenn3.bioinfotoolkits.net), whereas cluster synteny was performed using the clinker tool available on the CAGECAT web service (https://cagecat.bioinformatics.nl).

2.6. Annotation of chemical compounds

Alcaligenes nematophilus SOL 109 was grown in LB medium for 48h at 30 °C and shaken at 200 rpm. At the end of the incubation period, the culture was centrifuged for 30 min at 5000 rpm to obtain the supernatant. Extraction was performed in a manifold system using Strata C18-E® 200 mg 3 mL cartridges (Phenomenex) with high-performance liquid chromatography (HPLC) grade methanol. The collected samples were dried in a desiccator with silica to determine the final mass of each extract. Subsequently, the samples were solubilized in 1 mL of HPLC grade MeOH and centrifuged at 13,000 rpm for 10 min. The supernatant was transferred to 1.5 mL vials, and each sample was subsequently analyzed in a high-performance liquid chromatography system coupled to high resolution mass spectrometry (HPLC-HRMS).

The equipment comprises a Nexera X2 liquid chromatograph (Shimadzu, Kyoto, Japan) with diode array detector (DAD)-SPD M20A coupled to a spectrometer with quadrupole-time-of-flight (QTOF), MicroTOF-QII (Bruker Daltonics, Bremen, Germany), equipped with an electrospray source (ESI), operating in positive ionization mode, with an ion transfer time of 70 µs and prepulse of 5 µs.

The mass range selected was *m/z* 50-1200, AutoMS mode, with collision energy ranging from 20-65 eV according to *m/z* 50-700, and with the energy constant at 65 eV for mass values above m/z 700. A maximum of five precursor ions were acquired per cycle. The operating parameters of the equipment were the following: capillary 4500 V, nebulizer gas (nitrogen) 4 bar, drying gas (nitrogen) 9 L min-1, source temperature 200 °C. For internal calibration of the system, 10 nM sodium formate solution in isopropanol/water (1:1 v/v) was used.

For chromatographic separation, a Kinetex C18 analytical column (100 × 2.1 mm, 2.6 µm) (Phenomenex, Torrance, CA, USA), maintained at 50 °C, was used with a flow rate of 0.35 mL min^{-1}. The mobile phase (A) consisted of deionized water, while phase (B) consisted of ACN, both HPLC grade and containing 20 mM of formic acid as an additive. Initially, 15% isocratic elution of (B) was applied for 2 min, with subsequent gradient elution from 15% to 95% of (B) during 2-15 min and a repeated 95% isocratic elution of (B) for 15-21 min. For sample injection, a volume of 10 µL was used. Mass spectra were visualized using DataAnalysis 4.2 software (Bruker Daltomics). The annotation of molecules was performed through manual interpretation of the MS/MS spectra and comparison with data available in the literature.

2.7. Dual culture assays

2.7.1 Inhibition of phytopathogenic fungal mycelial growth

The tests against *Corynespora cassiicola* (INPA 2671), *Colletotrichum siamense* (Col2N), and *Rhizoctonia solani* (INPA 2942) were performed in triplicate on BDA medium. For co-cultivation with fungi, a mycelial disk was placed in the center of the plate, and 4-cm strips of the bacterial cultures were placed 2 cm away from the disk. The plates were incubated at 30 °C and evaluated every 5 days for 15 days, and the percentage of inhibition of mycelial growth was measured according to the following formula: $Inhibition\ (\%) = \frac{Control\ diameter - Test\ diameter}{Control\ diameter} x100$ (Thampi and Bhai, 2017).

2.7.2. Antibiotic activity against *Ralstonia solanacearum*

The assay against *R. solanacearum* (V8) was adapted from the methodology described by Velho-Pereira and Kamat (2011). For this purpose, a 7-cm vertical streak of *A. nematophilus* SOL109 was inoculated on Petri dishes containing LB agar medium and incubated at 28 °C for 48 hours. After the incubation period, a 3-cm streak was inoculated perpendicular to SOL 109, using *R. solanacearum* as the inoculum, at a distance of 1 cm from the antagonist, and incubated for another 24 hours. All assays were performed in triplicate.

At the end of incubation, the length and width of the pathogen were measured using a caliper to calculate the growth area. The inhibition of the phytopathogen was determined using the following formula: $PASDAAS\,(\%) = \frac{AWG}{TSA} x100$, where PASDAAS represents the percentage of specific antibiotic activity in the area, AWG is the area without growth, and TSA is the total area of growth of the pathogen. To calculate the AWG, the area on the treatment plate was subtracted from the TSA of the control plate.

2.8. Growth-promoting traits

2.8.1. Solubilization of phosphate (P) and zinc (Zn)

P solubilization was carried out in Pikovskaya's medium (Subba Rao, 1977), while Zn solubilization was conducted according to Saravanan et al. (2004) with modifications. The medium for Zn solubilization was composed of 798 mL of distilled water, 200 mL of M95X solution (33.78 g L^{-1} Na_2HPO_4, 15 g L^{-1} KH_2PO_4, 2.5 g L^{-1} NaCl, and 5 g L^{-1} NH_4Cl), 2 mL of 1M $MgSO_4 \cdot 7H_2O$ solution, 100 µL of 1 M $CaCl_2.2H_2O$ solution, 15 g of agar, and 1 g of Zn source (ZnO and $ZnSO_4$). All tests were carried out in triplicate with 5-mm disks from the culture of the SOL 109 strain, which were incubated for five days at 28 °C for subsequent measurement of the halos (mm) using a caliper.

2.8.2. Siderophore

The culture of SOL 109 strain was carried out in nutrient medium for 48h at 30 °C and shaken at 150 rpm. At the end of the incubation period, the culture was centrifuged for 30 min at 5000 rpm to obtain the supernatant. Quantification was performed using 1 mL of extract and 1 mL of CAS solution (60.5 mg of Chrome Azurol S in 50 mL of distilled water, 10 mL of $FeCl_3$.$6H_2O$ 1 mM diluted with 10 mM HCl and 40 mL solution of 72.9 mg HDTMA). The samples were incubated at room temperature for 2 h and analyzed using a spectrophotometer at 630 nm. The tests were performed in triplicate. The percentage of siderophores is estimated by the formula $Siderophore\,(\%) = \frac{(B-A)}{B} x100$, where A is the absorbance of the sample and B is the absorbance of the blank (Ghazy and El-Nahrawy, 2021; Schwyn and Neilands, 1987).

2.8.3. Indole acetic acid (IAA)

The SOL 109 strain was grown in LB medium plus tryptophan (150 mg L^{-1}) in triplicate at 150 rpm in the dark for 7 days. The extracts were centrifuged for 30 min at 4000 rpm to obtain the supernatant. The test was performed with 1 mL of the supernatant and 1 mL of Salkoswski's solution (1.2 g of $FeCl_2.6H_2O$, 42.1 mL of H_2SO_4 and 57.89 mL of distilled water). The reaction was incubated for 60 min in the dark and then read at 595 nm. Sterile culture medium was used instead of the supernatant as a blank. The tests were performed in triplicate. For quantification, a standard curve was made with IAA (Sigma) at concentrations of 0.0156, 0.0312, 0.0625, 0.125, 0.25, 0.5, and 1 mg mL^{-1} (Thampi and Bhai, 2017).

2.8.4. Ammonia

The SOL 109 strain was grown in 10 mL peptone–water medium (10 g L^{-1} peptone, 5 g L^{-1} NaCl) for 48h at 30 °C. The extracts were centrifuged, and the supernatant was transferred to test tubes. 500 µL of Nessler's solution (2 g $HgCl_2$, 7 g KI, 4 g NaOH and 100 mL distilled water) was added, and the color changed to yellow or brown (Cappucino and Sherman, 1992).

2.8.5 Catalase

The SOL 109 strain was grown in LB medium for 24h at 28 °C. Subsequently, 1 mL of H_2O_2 (3%) was added to the cultures to observe the formation of bubbles. Visualization of an effervescent reaction was considered positive for catalase production.

2.9. Growth promotion of tomato

The evaluation of growth promotion under greenhouse conditions was performed using the Yoshimatsu cultivar obtained from the Germplasm Bank of the National Institute for Amazonian Research (INPA). For this purpose, 1-L pots were used in a completely randomized design with 3 treatments and 20 replicates each. The seedlings were grown for 30 days and transplanted into 1 L pots containing Vivatto plus® substrate. The seedlings from the group inoculated with *A. nematophilus* SOL 109 were inoculated with 5 mL of a bacterial cell solution (10^{10} CFU mL^{-1}). The growth promotion of the SOL 109 strain was also compared with commercial Plantafol® fertilizer (Nitrogen (N): 20%, phosphorus (P_2O_5): 20%, potassium (K_2): 20%, boron (B): 0.02%, iron (Fe): 0.1%, copper (Cu): 0.05%, and EDTA: 0.75%), to which 5 mL of solution (1.5 g L^{-1}) was added, whereas in the control group (without fertilizer or bioinoculant) 5 mL of sterile distilled water was added. After 30 days of inoculation, the height (cm), stem diameter (cm), root (cm), flowers (units), aerial dry weight (g), and root dry weight (g) were measured. The experiment was conducted in May – June (27.3±4 °C and humidity 77.2±6.8%) and September– October (30.4±1.3 °C and humidity 48±7.1%).

2.10. Biocontrol of Rhizoctonia solani in tomato

The *in plant* evaluation of the protective effect of inoculating the SOL 109 strain against *Rhizoctonia solani* was performed under greenhouse conditions in 1-L pots using a completely randomized design with 3 treatments and 20 replicates each. The substrate for the mycelial growth of *R. solani* was prepared in 2 kg polypropylene bags with 100 g of pasteurized rice grains and 100 mL of distilled water, which were autoclaved at 120 °C for 25 minutes. Soon after, ten 5 mm diameter discs of the phytopathogen culture were inoculated into each bag. and

incubated at room temperature under light for 10 days, turning the contents every two days.

To obtain seedlings, tomato seeds cv. Yoshimatsu were cultivated in seedbeds for 30 days. On the 23rd day, the biocontrol agent SOL 109 (5 mL at 10^{10} CFU mL^{-1}) was inoculated and distilled water (5 mL) was added to the positive and negative controls. On the 30th day, the seedlings were transplanted into 1L pots containing Vivatto plus® substrate and colonized with pasteurized rice (375:6; w/w). The negative control was inoculated with rice free of phytopathogens. The negative control was inoculated with rice free of phytopathogens. The calculation of the percentage of disease incidence is given by

$$Disease\ Incidence\ (\%) = \frac{DP}{TP} \times 100$$

Where DP is the number of diseased plants and TP is the total number of plants in the experiment. Tomato plants were considered to be diseased when they presented at least one of the symptoms: thinning and blackening of the stem, superficial necrosis on the neck, and seedlings falling over (Figure S1). Survival was calculated using the following formula:

$$Survivor\ (\%) = \frac{LP}{TP} \times 100$$

Where LP is the number of live plants and TP is the total number of plants in the experiment. To evaluate the effect of the biocontrol agent on aspects related to plant development in the presence of the pathogen, the following were measured: height (cm), stem diameter (cm), root length (cm), as well as shoot dry weight (g) and root dry weight (g). The seedlings were measured using a measuring tape and caliper. The experiment was conducted in April – May (26±4 °C and 82±10% humidity) and August (29±7 °C and 66±5% humidity).

2.11. Statistical analysis

The data were subjected to analysis of variance (ANOVA) using R 4.3.1. software, and when significant ($p < 0.05$), the Scott-Knott mean test was applied. The assumptions of ANOVA, such as normality and homoscedasticity of residuals, were verified using the Shapiro-Wilk and Levene's tests, respectively.

3. Results

3.1. Strain identification

Taxonomic identification of SOL 109 based on the complete genome revealed that the Amazonian isolate obtained from sediments of the Solimões River has dDDH values >70% in the formula d2 (92.6%) and d4 (83.0%), indicating that the strain belongs to the species *Alcaligenes nematophilus*. In addition, phylogenomic analysis revealed the formation of a monophyletic clade of the type species *A. nematophilus* A-TC2 with high pseudobootstrap support (Figure 1). The species most closely related to *A. nematophilus* were *A. faecalis* (dDDH d4: 66.5%) and *A. pakistanensis* (dDDH d4: 30.5%). In addition, an ANI value of 98.04% for SOL 109 in the presence of *A. nematophilus* A-TC2 corroborates the dDDH data because ANI values >95% are also indicative of known species (Figura S2).

Notably, *A. endophyticus* did not form a monophyletic clade with other *Alcaligenes* representatives in the whole genome-based phylogram, contrary to previous 16S rRNA-based phylogenies. It exhibited low dDDH values (<18%) with *Alcaligenes* sp. and was more related to *Eoetvosiella caeni* (dDDH: 21.7%), suggesting potential misclassification (Table S1 and S2).

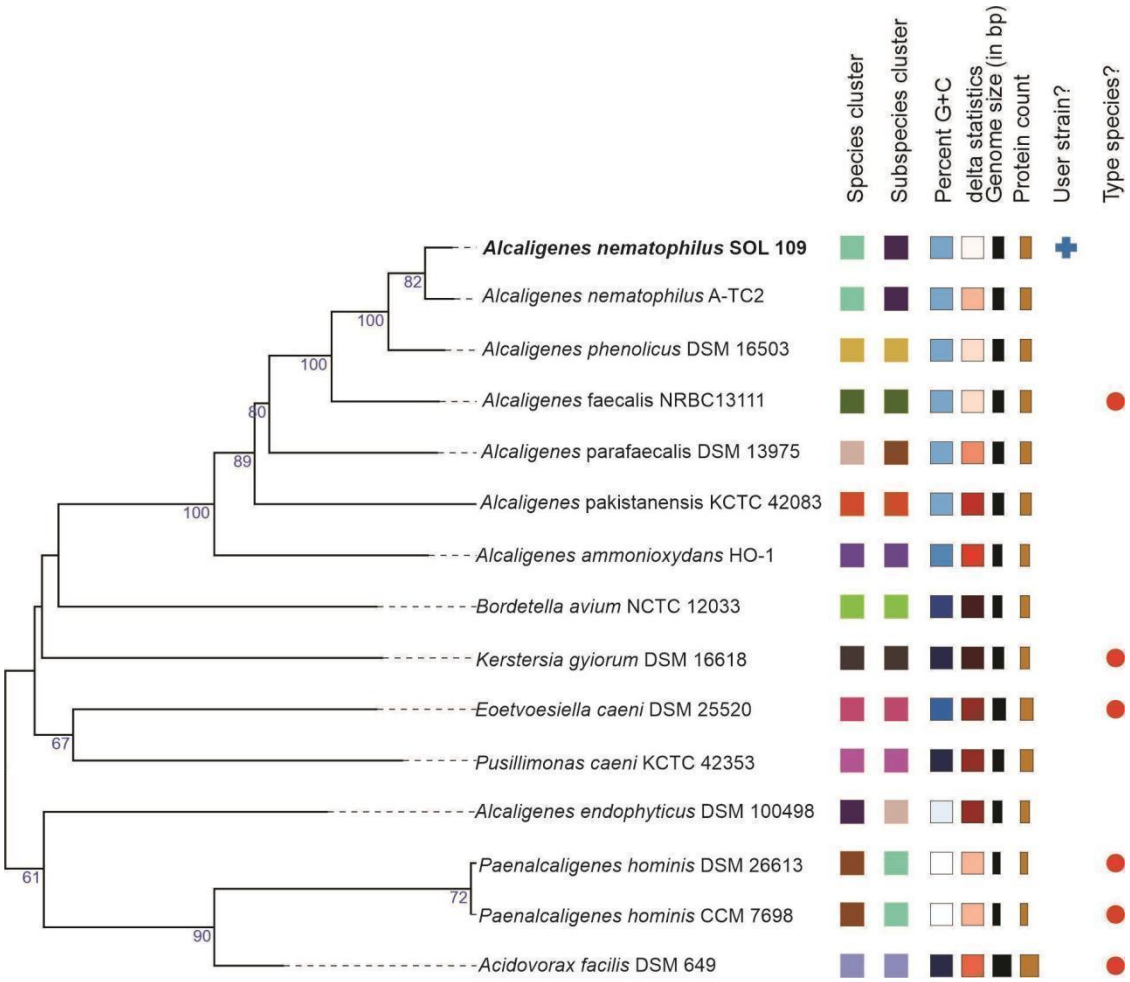

Figure 1. Phylogram generated by Type Strain Genome Server (TYGS) based on whole genome sequence of amazonian strain SOL 109 and closely related species genomes. The numbers in the branches indicated the pseudobootstrap.

3.2. Morphological and biochemical characterization

Alcaligenes nematophilus SOL 109 colonies exhibited an opaque yellow (#FFFFCC) with a smooth surface, flat elevation, wavy margin, and viscous texture. The cells were gram-negative coccobacilli. Biochemical assays revealed no amylase, lipase, protease, cellulase, H_2S production, or indole activity. Positive reactions were observed for the hydrolysis of urea and citrate. Chitinase activity was detected (11±1 mm halo). The strain demonstrated the ability to oxidize glucose and lactose but showed no response to mannitol, fructose, sucrose, cellobiose, galactose, maltose, mannose, trehalose, or arabinose.

3.3 Genome mining and comparative analysis

Detailed analysis of the *A. nematophilus* SOL 109 genome revealed several biosynthetic gene clusters (BGCs) of interest. Importantly, no plasmids were detected in the SOL 109 genome using the PlasmidFinder database, indicating that all identified BGCs were chromosomal-encoded. This genomic organization may contribute to the genetic stability of biosynthetic pathways.

Genomic mining of *A. nematophilus* SOL 109 identified biosynthetic gene clusters (BGCs) of betalactone (2), ectoine (1), NRPS (1), terpene (1), PKS I (1), and phosphonate-like (1) compounds. Intraspecific variation in BGC composition was observed between the *A. nematophilus* strains SOL 109 and A-TC2, with SOL 109 possessing a phosphonate-like cluster absent in A-TC2 (Figure 2). *A. nematophilus* SOL 109 and *A. faecalis* ZD02 shared identical BGC compositions. Unique clusters (singletons) of butyrolactone and Ni-siderophore classes were identified in *A. parafaecalis* and *A. phenolicus*, respectively. *A. ammonioxydans* and *A. endophyticus* exhibited unique variations in NRPS cluster gene composition, whereas *A. endophyticum* showed unique betalactone BGC variations. Ectoine- and terpene-related BGCs were conserved across all compared strains (Figures 2 and 3). Hydrogen cyanide biosynthesis clusters were exclusive to *A. pakistanensis*, *A. nematophilus*, and *A. faecalis* (Figures 2 and 4).

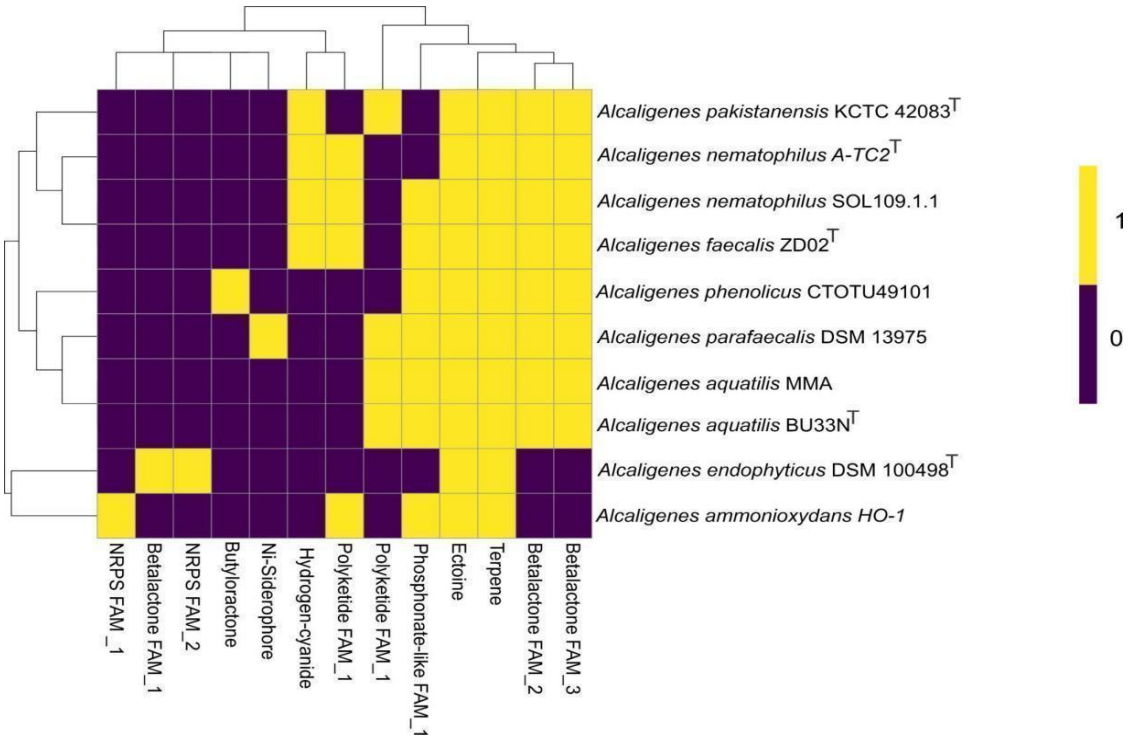

Figure 2. Comparative heatmap of the composition of biosynthetic gene clusters (BGCs) and biosynthetic gene cluster families (GCF) of *Alcaligenes nematophilus* SOL 109 and 9 other strains of the genus, including the type species. Purple indicates presence and yellow indicates absence.

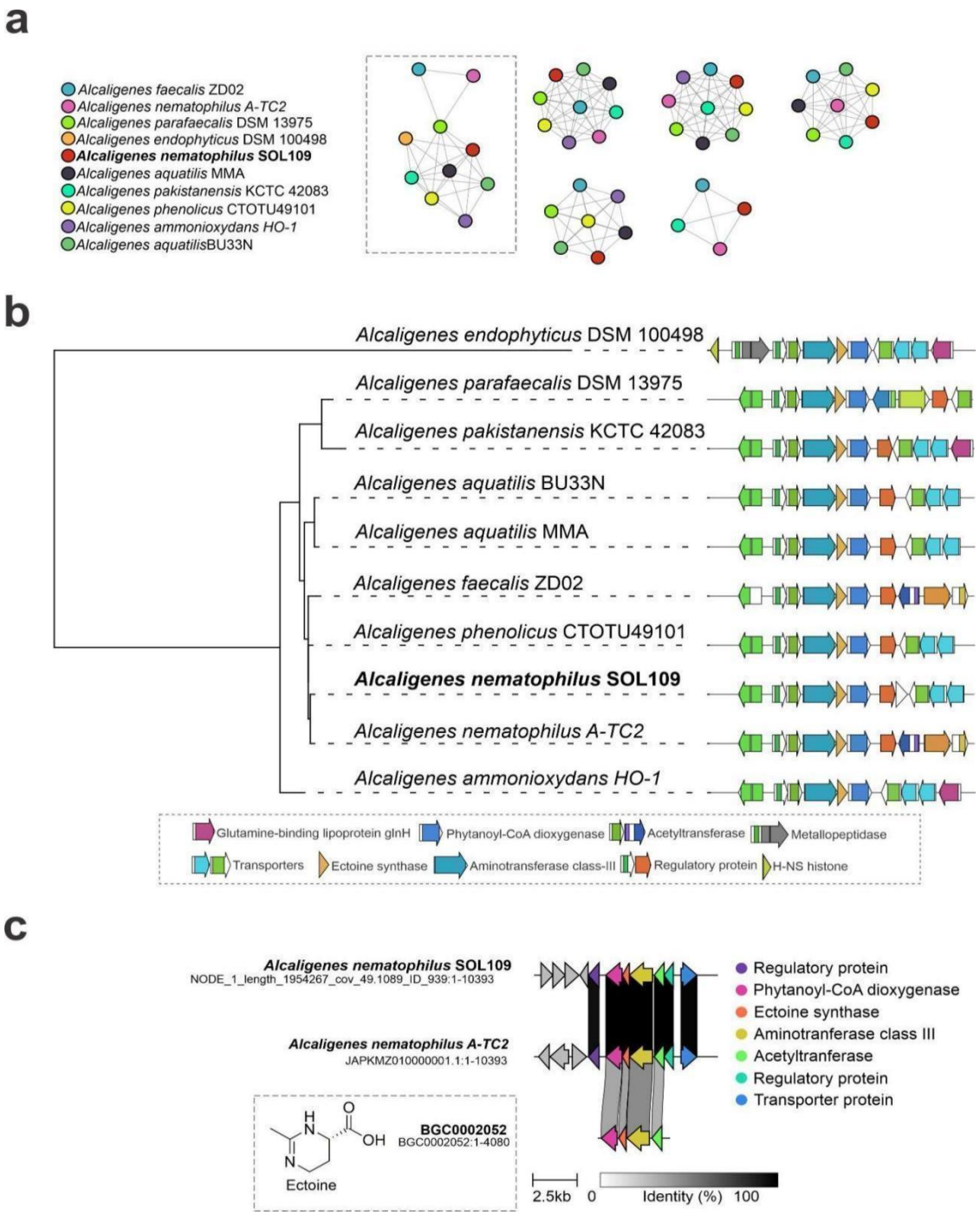

Figure 3. a. Molecular network of ectoine biosynthetic gene clusters (BGCs) from different species. **b.** Dendrogram based on the ectoine synthase gene and BGC composition. **c.** Synteny analysis of ectoine BGC from *Alcaligenes nematophilus* SOL 109 and A-TC2 compared with a well characterized ectoine BGC from *Streptomyces* sp.

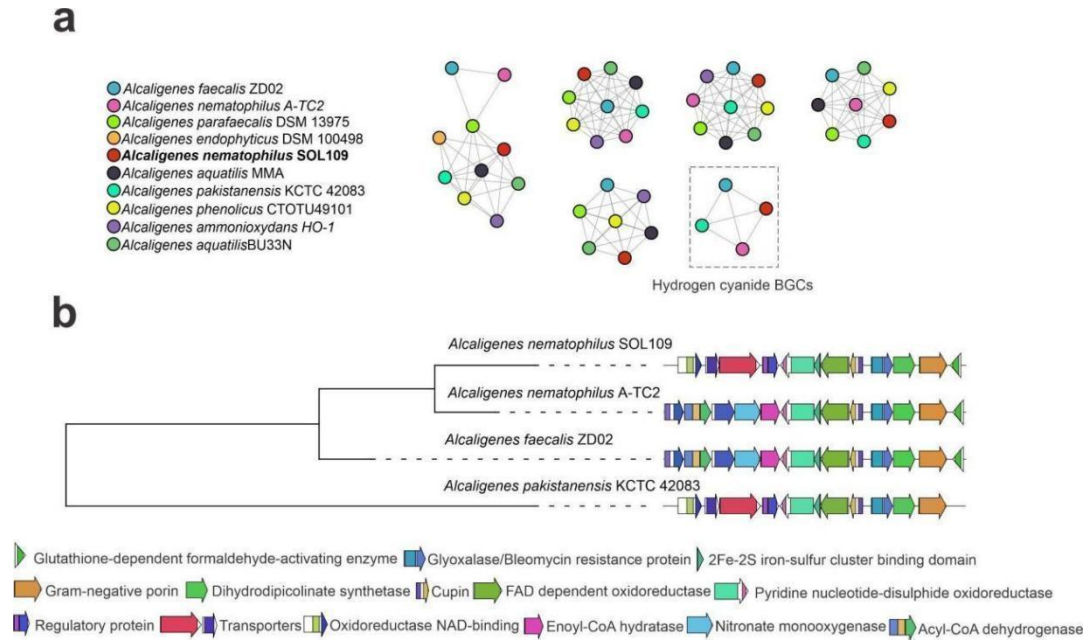

Figure 4. **a.** Molecular network of hydrogen cyanide biosynthetic gene clusters (BGCs) from different species of *Alcaligenes*. **b.** Dendrogram based on the gene core and BGC composition.

Orthologous gene comparisons revealed 3009 protein clusters shared among *A. nematophilus* SOL 109, *A. faecalis* ZD02, *A. pakistanensis*, and *A. nematophilus* A-TC2. Additionally, 153 clusters were shared exclusively between *A. nematophilus* strains A-TC2 and SOL 109. Three orthologous clusters containing seven proteins (two encoding DNA binding protein dnaC and five hypothetical proteins) were unique to SOL 109 (Figure S3).

Genome annotation of SOL 109 identified resistance genes for various heavy metals, including cadmium, zinc, cobalt, chromium, and copper (Table 1).

Table 1. Resistance and tolerance genes of *Alcaligenes nematophilus* SOL109 to heavy metals annotated by RAST

Gene	Annotation	Scaffold	Size (pb)	Amino acids (aa)
FieF	Divalent metal cation (Fe/Co/Zn/Cd) transporter	2	897	299
ChrA	Chromium resistance protein	2	1263	421
CutE	Copper resistance protein	2	1620	540
CopD	Copper resistance protein	1	1623	541

The antibiotic resistance genes qacG and adeF were present in all analyzed *Alcaligenes* strains (Figure 5), conferring resistance to fluoroquinolone, tetracyclines (adeF), and disinfectants/antiseptics (qacG). Notably, adeF gene copy numbers varied: *A. parafaecalis* DSM139 and *A. faecalis* ZD02 possessed two copies each, *A. nematophilus* SOL 109 had three copies, and *A. nematophilus* A-TC2 had only one copy.

Alcaligenes ammonioxydans and *A. faecalis* exhibited the most diverse antibiotic resistance profiles, uniquely possessing sul1, catB3, and qacEdelta1 genes conferring resistance to sulfonamides, phenicols, and disinfectants/antiseptics, respectively. *A. faecalis* additionally harbored resistance genes for macrolides (mphA, Mrx) and aminoglycoside (AAC.6.31, aadA3). *A. ammonioxydans* uniquely possessed genes conferring resistance to cephalosporins (OXA.21), tetracyclines

(tetA), and aminoglycosides (AAC.6.IIa). The gene encoding fluoroquinolone resistance QnrD1 is exclusive to *Alcaligenes aquatilis* MMA.

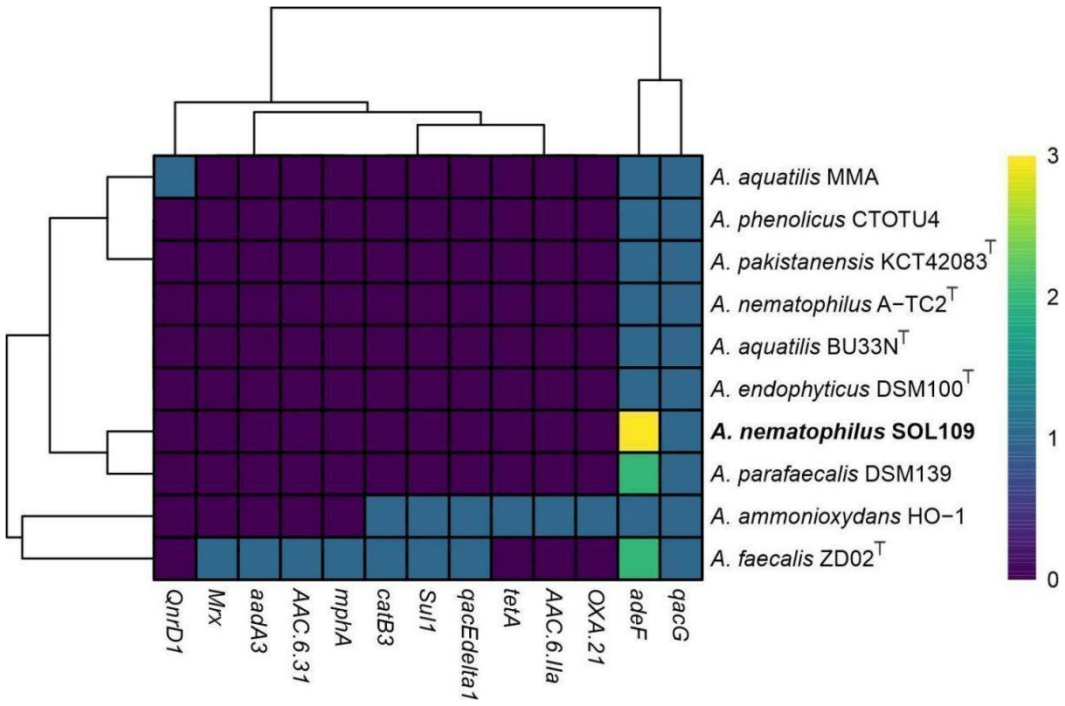

Figure 5. Comparative heatmap of resistance genes in 10 *Alcaligenes* strains.

3.4 Metabolites annotation

LC-MS analysis of *A. nematophilus* SOL 109 extracts led to the annotation of five distinct molecules (Table 2, Figure S4). Compound **1** was identified as an amino acid, compounds **2** and **3** as peptides, and compound **5** as a primary fatty amide. These metabolites were detected based on *m/z* pairs corresponding to protonated structures. These metabolites were detected based on *m/z* pairs corresponding to protonated structures.

Table 2. Molecules annotated in the extract of *Alcaligenes nematophilus* SOL 109 grown in LB medium.

n°	Molecule	Chemical formula	*m/z* $[M+H]^+$	Retention time (min)	Mass error (ppm)
1	Tryptophan	$C_{11}H_{12}N_2O_2$	205,0987	1.0	4,88
2	Cyclo(Tyr-Pro)	$C_{14}H_{16}N_2O_3$	261,1241	1.2	0,76
3	Val-Leu-Pro-Val-Pro	$C_{31}H_{53}N_7O_8$	652,4042	2.1	1,22
4	Ile-Pro-Ile	$C_{17}H_{31}N_3O_4$	342,2380	2.1	-3,51
5	13-docosenamide	$C_{22}H_{43}NO$	338,3426	13.4	0,88

3.5 In vitro antimicrobial activity and growth promotion traits

Alcaligenes nematophilus SOL 109 demonstrated significant antifungal activity against phytopathogens through antibiosis. The highest inhibition rates were observed against *Rhizoctonia solani* (93%), *Corynespora cassiicola* (77.4%), and *Colletotrichum siamense* (74.81%). In contrast, antibacterial activity against *Ralstonia solanacearum* was minimal, with only 7% inhibition (Figure S5).

Regarding plant growth promotion traits, SOL 109 exhibited the ability to solubilize zinc and iron sources and produce indole acetic acid and ammonia (Table 3). However, phosphate solubilization and catalase production were not observed.

Table 3. Production of plant growth inducers by *Alcaligenes nematophilus* SOL 109.

Nutrient solubilization (mm)			Siderophore (%)	IAA (µg mL^{-1})	NH_3	Catalase
P	ZnO	$ZnSO_4$				
0	11±2	11±2	25.7±2	18.4±1	++	-

- no reaction; + weak reaction; ++average reaction; +++ strong reaction; triplicate average.

3.6. Growth promotion under greenhouse condition

Growth promotion assays were conducted during two seasonal periods: the rainy season (May-June) and the summer (September-October). During the rainy season, plants inoculated with SOL 109 showed no significant differences in height or diameter compared to control and Plantafol treatments. Root size in SOL 109 was comparable to that of Plantafol but smaller than the control. The aerial and root dry weights were lower than those of the fertilizer and control treatments. Notably, SOL 109 inoculation stimulated flowering, with an average of 2 flowers per plant, whereas no flowering occurred in the control or fertilizer treatments (Figure 6; Table 4).

In the summer, SOL-109-inoculated plants exhibited significantly greater heights and root diameters than control and Plantafol-treated plants. The stem diameter was larger in the control treatment. The aerial and root dry weights surpassed those of the control and Plantafol treatments. Consistent with the rainy season results, flowering was observed only in SOL 109-inoculated plants. These summer data strongly support the growth-promoting capabilities of SOL 109.

Table 4. Growth promotion using *Alcaligenes nematophilus* SOL 109 in tomato seedlings cv. Yoshimatsu in Amazonas rainy and summer seasons.

May-June (rainy)						
Test	Height (cm)	Root (cm)	Stem diameter (cm)	Flower (unit)	ADW (g)	RDW (g)
Control	70.88±7.8 a	15.4±2.71 a	0.41±0.07 a	0 b	17.14	2.24
Plantafol	67.75±7 a	12.92±2.2 b	0.45±0.11 a	0 b	16.35	2.83
SOL 109	66.62±7 a	13.72±2.1 b	0.41±0.08 a	2±1 a	13.38	2.13
September-October (summer)						
Test	Height (cm)	Root (cm)	Stem diameter (cm)	Flower (unit)	ADW (g)	RDW (g)
Control	58.04±6.3 b	14.1±2.57 b	0.32±0.07 a	0 b	20.13	3.8
Plantafol	57.86±5.4 b	14.4±3.15 b	0.16±0.05 b	0 b	15.68	3.8
SOL 109	71.01±9.3 a	20.6±6.03 a	0.17±0.07 b	1±1 a	22.92	4.3

ADW - Air summer weight (total); RDW - Root dry weight (total); *Means followed by the same letter do not differ from each other using the Scott-Knott test at 5% probability.

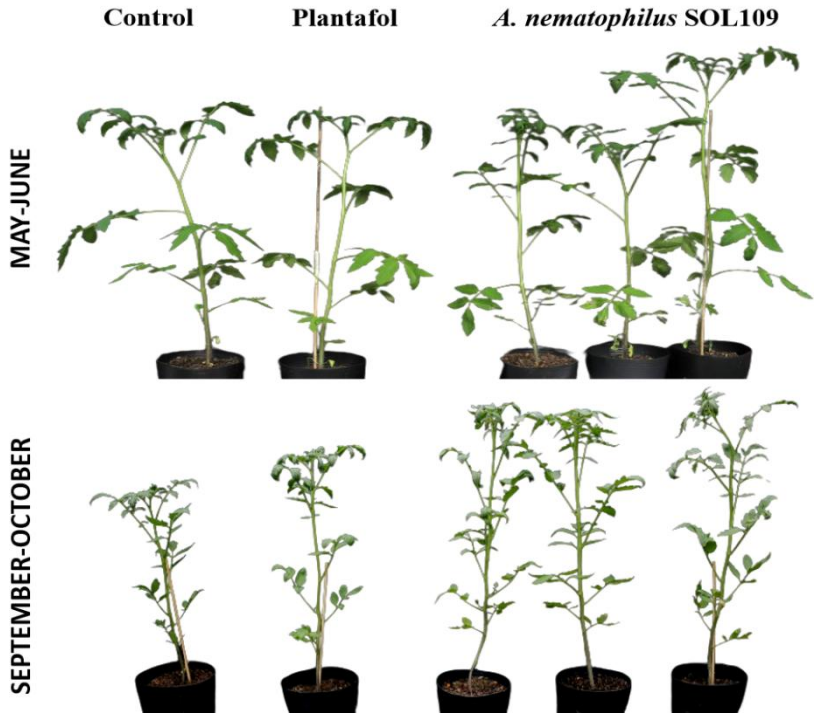

Figure 6. Aspect of tomato seedlings cv. Yoshimatsu during the growth promotion experiment in the rainy (May-June) and summer (September-October) seasons comparing the effect of the application of chemical fertilizer (plantafol) and *Alcaligenes nematophillus* SOL 109 in relation to the control group.

3.7. Biological control under greenhouse condition

The biological control efficacy against *Rhizoctonia solani* was evaluated during April-May (rainy season) and August (summer). In the rainy season, SOL 109 inoculation delayed symptom onset by three days compared with pathogen-only treatments. The disease incidence was reduced by 70% and the plant survival rate increased to 95% compared with the survival rate of 50% in pathogen-only treatments (Figure 7).

The summer season results differed slightly, with 25% of *R. solani*-inoculated seedlings remaining asymptomatic, whereas overall survival decreasing to 65%. In contrast, SOL 109-treated seedlings maintained 100% survival.

Regarding plant development under pathogen pressure, rainy season data revealed significantly higher plant heights and diameters in SOL 109-treated plants than in the positive and negative controls. Root size did not differ significantly between treatments. Aerial and root dry weights were higher following SOL 109 treatment. The summer data revealed greater plant heights in the SOL 109 group than in the positive control group but lower heights in the negative control. No significant differences in root size were observed between treatments. The plant diameter after SOL 109 treatment was comparable to the positive control. The aerial and root dry weights were highest in SOL 109-inoculated plants. These results collectively demonstrate the efficacy of *A. nematophilus* SOL 109 in controlling *R. solani* and promoting plant development under pathogen stress (Table 5; Figure 8).

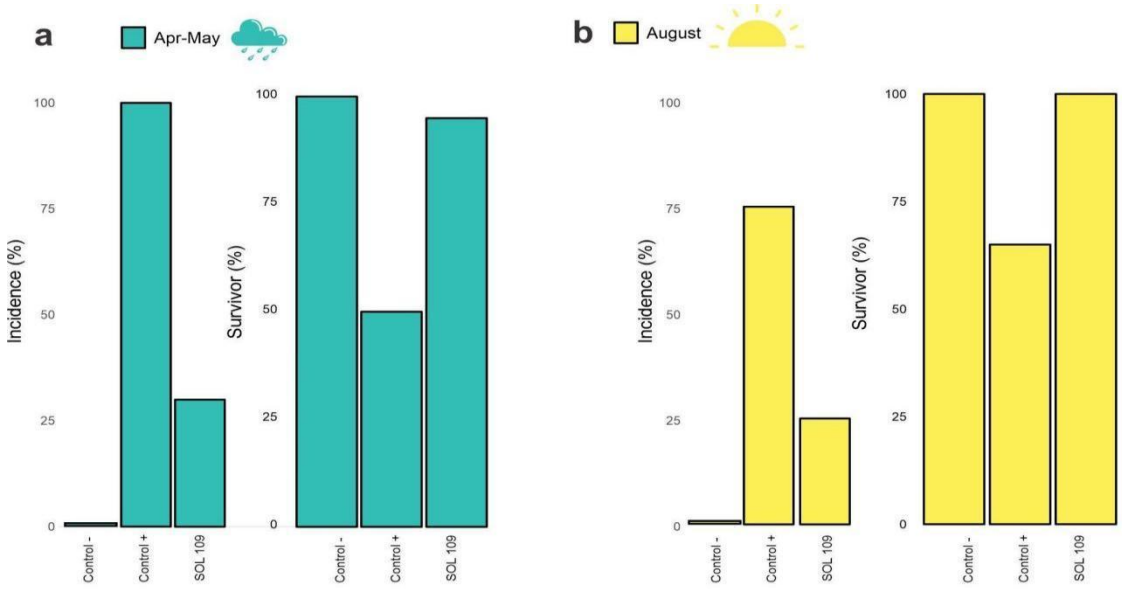

Figure 7. Incidence and survival rate in the (a) rainy and (b) summer seasons of Amazonas in biocontrol experiments of *Rhizoctonia solani* in tomato seedlings cv. Yoshimatsu using *Alcaligenes nematophilus* SOL 109 as a biocontrol agent.

Table 5. Biocontrol of *Rhizoctonia solani* using *Alcaligenes nematophilus* SOL 109 on tomato seedlings cv. Yoshimatsu in Amazonas rainy and summer seasons.

April-May (rainy)					
Test	Height (cm)	Root (cm)	Stem diameter (cm)	ADW (g)	RDW (g)
Negative	40.05±4.1 b	8.72±1.51 a	0.15±0.05 c	4.39	1.04
Positive	28.39±7.32 c	7.73±1.48 a	0.2±0.06 b	2.15	0.45
SOL 109	44.4±2.98 a	9.18±2.63 a	0.28±0.05 a	5.97	1.07
August (summer)					
Test	Height (cm)	Root (cm)	Stem diameter (cm)	ADW (g)	RDW (g)
Negative	54.5±5.5 a	12.7±1.1 a	0.37±0.07 a	12.97	2.12
Positive	38.7±6.7 c	8.7±2.1 b	0.3±0.06 b	8.99	1.46
SOL 109	47.4±9.3 b	10.1±3 b	0.3±0.07 b	7.77	1.73

ADW - Air dry weight (total); RDW - Root dry weight (total); Means followed by the same letter do not differ from each other using the Scott-Knott test at 5% probability.

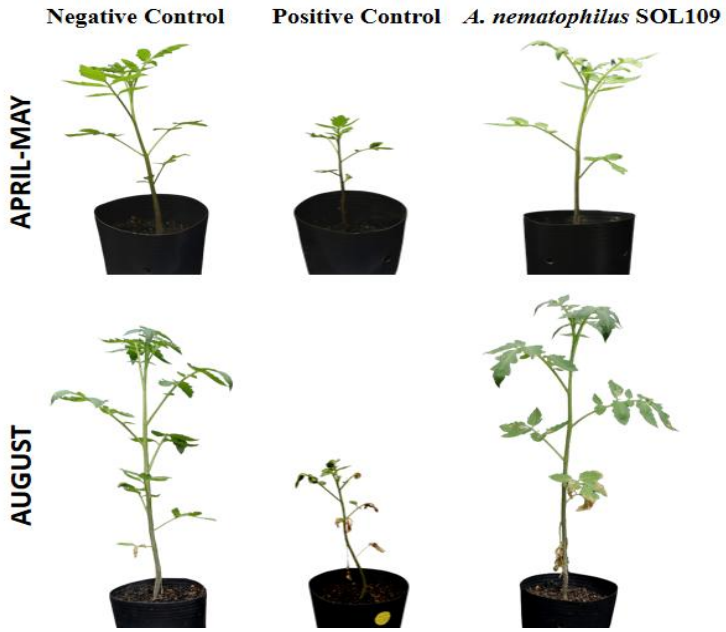

Figure 8. Appearance of tomato seedlings cv. Yoshimatsu infected with *Rhizoctonia solani* and treated with *Alcaligenes nematophilus* SOL 109 compared to negative and positive controls in the periods of April-May (rainy) and August (summer).

4. Discussion

Control methods based on microorganisms have emerged as a sustainable alternative to chemical methods and have become a growing priority in modern agriculture. This shift is driven by concerns about environmental impacts, pathogen resistance to synthetic pesticides, and food safety. In this context, we conducted a comprehensive characterization of SOL 109 strain isolated from sediments of the Solimões River, focusing on its taxonomy, genomics, secondary metabolism, and capacity for plant growth promotion and biological control of *Rhizoctonia solani* in tomato plants.

Using phylogenetic inference, dDDH, and ANI data, we confidently identified the strain at the species level as *Alcaligenes nematophilus*. This species was recently proposed in Tunisia, with its etymology (nemato + suff. -philus) referring to the *phylum nematoda*, as the type strain was isolated from the nematode *Oscheius tipulae* (Machado et al., 2023). Interestingly, our phylogenetic analysis revealed some discrepancies within the *Alcaligenes* genus. While *A. endophyticus* forms a monophyletic clade in the 16S region-based phylogram (Lu et al., 2017), it does not cluster with other *Alcaligenes* species when the complete genome is considered (Figure 1). Our dDDH analysis suggests that *A. endophyticum* might be misclassified given its closer proximity to bacteria from other genera (Table S2).

Based on the taxonomic data, we analyzed the biochemical and morphological characteristics of the SOL 109 strain, comparing it with the type species *A. nematophilus* A-TC2. Our results not only confirm the phenotypic characteristics described by Machado et al. (2023) but also provide new insights into the species metabolism. We observed the production of amylase, lipase, cellulase, and urea, as well as the ability to utilize various sugar sources (arabinose, cellobiose, fructose, glucose, lactose, maltose, and trehalose). This study is the first report of *A. nematophilus* in Brazil, as well as the first to provide a genomic overview and potential agricultural applications for this species.

The genomic analysis of *A. nematophilus* SOL 109 revealed a promising profile for bioremediation applications, particularly in the context of heavy metal contamination. We identified genes associated with the bioremediation of cadmium, copper, chromium, and zinc, suggesting that SOL 109 has potential as a metal bioremediator. These findings align with previous research on the *Alcaligenes* genus, which has demonstrated significant capabilities in heavy metal bioremediation (Diels et al., 1995; Ibrahim et al., 2022; McEntee et al., 1986; Sodhi et al., 2021, 2020). For instance, Sodhi et al. (2023) identified similar heavy metal resistance genes in *Alcaligenes aqualitis* strain MMA. However, unlike *A. aqualitis* MMA, which also showed resistance to the antibiotic amoxicillin, our analysis did not detect similar antibiotic resistance genes in SOL 109. This difference could be advantageous in certain bioremediation applications where antibiotic resistance is a concern.

The antibiotic resistance profile of SOL 109 further distinguishes it from other *Alcaligenes* species. Unlike *A. ammonioxydans* and *A. faecalis*, which possess genes for various antibiotic classes and can pose risks to immunocompromised patients (Huang, 2020), SOL 109 only presented resistance genes for fluoroquinolone-like antibiotics, antiseptics, and disinfectants (benzalkonium chloride and ethidium bromide). These resistance traits are shared by all *Alcaligenes* species (Figure 5), suggesting that SOL 109 could be safely used as both a bioremediator and a bioinoculant without the risk of being categorized as an opportunistic pathogen like *A. faecalis.*

Our comparative analysis of biosynthetic gene clusters (BGCs) among *Alcaligenes* species revealed both conserved and unique features. All ten analyzed strains, including all valid type species, shared clusters for terpene and ectoine production. However, the presence of non-shared clusters indicates untapped chemodiversity within the genus, warranting further exploration. Among the shared BGCs, those related to ectoine and hydroxyectoine production are particularly noteworthy. These osmolytes are abundant in nature and act as biofunctional stabilizers, protecting macromolecules and cells under high salt stress. Interestingly, both molecules have been reported to inhibit the formation of β-amyloid peptide, the main constituent of senile plaques in Alzheimer's disease (Calderón et al., 2004; Kanapathipillai et al., 2005; Liu et al., 2021; Pastor et al., 2010). Their water retention properties have also led to applications in skin care products (Eiberweiser et al., 2015; Graf et al., 2008; Saum and Müller, 2008).

A notable finding in our BGC analysis was the presence of clusters for hydrogen cyanide (HCN) production in only four of the ten studied *Alcaligenes* strains, including the *A. nematophilus* strains. HCN has been associated with plant growth promotion, fungicidal, and insecticidal activities, particularly in *Pseudomonas* species active against *Galleria mellonella* (Flury et al., 2017; Sehrawat et al., 2022). This discovery in *A. nematophilus* SOL 109 suggests potential applications in biocontrol and plant growth promotion.

Indeed, our physiological characterization of SOL 109 revealed several traits indicative of plant growth promotion capabilities, including the production of ammonia, indole acetic acid (IAA), siderophores, and the ability to solubilize zinc sources. These characteristics are consistent with other plant growth-promoting strains within the *Alcaligenes* genus. For example, *Alcaligenes* sp. AF7 has been shown to increase root size, stem size, and biomass in rice plants (Fatima et al., 2020), while *A. faecalis* NBRI NB2.5 promoted growth in maize plants (Dixit et al., 2020). Our greenhouse experiments confirmed that *A. nematophilus* SOL 109 can

indeed promote tomato plant growth, extending these findings to a new crop and species within the genus.

Our experiments also revealed a seasonal dependency in the growth promotion effects of SOL 109. Significant promotion of tomato plant growth occurred only during the September–October period (summer season), characterized by higher temperatures (average 3.05°C higher) and lower humidity (10% lower) compared to the May–June period (rainy season). Under these summer conditions, SOL 109 outperformed commercial fertilizer in promoting shoot growth and root length (Table 4). This seasonal variation in efficacy can be attributed to the influence of environmental factors on plant-microbe interactions, with the summer conditions potentially favoring these beneficial associations (Barman and Jha, 2021; Dunfield and Germida, 2003; Pramanick et al., 2023).

The biocontrol capabilities of SOL 109 against *R. solani* were consistent across seasons. In both *in vitro* and greenhouse conditions, during rainy and summer seasons, SOL 109 reduced disease incidence by up to 75% and achieved a 100% plant survival rate in the summer season. These results demonstrate the ability of the strain to suppress the pathogen in contaminated soil and mitigate plant symptoms under biotic stress caused by *R. solani*. Within the *Alcaligenes* genus, *A. faecalis* has been the most reported species for biocontrol, effective against various pathogens including *Sclerotium rolfsii*, *Fusarium graminearum*, *Pyricularia oryzae* (syn. *Magnaporthe oryzae*), *Botrytis cinerea*, and others (Ray et al., 2023; Kakar et al., 2018; Lahlali et al., 2020; Sriram and Poornachanddra, 2013; Honda et al., 1998). Our findings with *A. nematophilus* SOL 109 expand the biocontrol potential within the genus, particularly for managing *R. solani* in tomato plants.

The observed seasonal plasticity in the efficacy of *A. nematophilus* SOL 109 for growth promotion and biocontrol is particularly intriguing. While growth promotion was more pronounced in summer, biocontrol showed enhanced effectiveness during the rainy season. This functional versatility suggests an ability to adapt to environmental conditions and pathogen pressure, potentially allocating resources to protective or growth-promoting functions as needed. The consistent stimulation of flowering by SOL 109 across both seasons indicates a possible effect on plant hormonal balance, which could be an interesting area for future research.

Our metabolomic analysis of *A. nematophilus* SOL 109 revealed several noteworthy secondary metabolites, including erucamide (13-docosenamide), Cyclo(Tyr-Pro), Val-Leu-Pro-Val-Pro, and Ile-Pro-Ile. This represents the first annotation of these metabolites within the *Alcaligenes* genus, expanding our understanding of its metabolic capabilities. Erucamide, in particular, has been shown to repress virulence genes in *Ralstonia pseudosolanacearum* and reduce bacterial wilt symptoms in mulberry seedlings (Li et al.,2024). The non-ribosomal peptides (NRPs) Val-Leu-Pro-Val-Pro and Ile-Pro-Ile have demonstrated pharmacological properties, acting as antihypertensives and inhibitors of dipeptidyl peptidase IV and HIV entry into certain cell types (Abiram and Kolandaivel, 2007; FitzGerald and Meisel, 2000; Liu et al., 2007; Nongonierma et al., 2018; Tulipano et al., 2011). The NRP Cyclo(Tyr-Pro) has shown bactericidal activity against *Bacillus subtilis* and *Staphylococcus aureus* when encapsulated in liposomes, though its effects on plant pathogens remain unexplored (Kilian et al., 2011).

In conclusion, the comprehensive characterization of *Alcaligenes nematophilus* SOL 109 reveals a multifaceted microorganism with significant potential for agricultural and environmental applications. Its unique combination of plant growth-promoting traits, biocontrol capabilities, potential for heavy metal

bioremediation, and production of bioactive metabolites positions it as a promising candidate for sustainable agricultural practices and environmental management.

5. Conclusion

A. nematophilus SOL 109 represents a promising tool in the microbial toolbox for sustainable agriculture and environmental management. Its diverse capabilities align well with the growing need for environmentally friendly alternatives to chemical pesticides and fertilizers, as well as solutions for environmental contamination.The ability of the strain to promote plant growth, control pathogens, and potentially remediate heavy metals, coupled with its seasonal adaptability, positions it as a versatile candidate for various agricultural and environmental applications.

The unique metabolite profile and genomic features of *A. nematophilus* SOL 109 open up new avenues for research and development in areas such as plant protection, bioremediation, and potentially human health. As we continue to unravel the complexities of plant-microbe interactions and microbial ecology, strains like SOL 109 may play pivotal roles in shaping the future of agriculture and environmental stewardship.

Further research, including extensive field trials and mechanistic studies, will be crucial to fully harness the potential of *A. nematophilus* SOL 109. This strain exemplifies the untapped potential of microbial resources in addressing global challenges related to sustainable food production and environmental conservation. As such, it underscores the importance of continued exploration and characterization of beneficial microorganisms for developing innovative, eco-friendly solutions in agriculture and environmental management.

References

Abbas, S., Ahmed, I., Iida, T., Lee, Y.-J., Busse, H.-J., Fujiwara, T., Ohkuma, M., 2015. A heavy-metal tolerant novel bacterium, *Alcaligenes pakistanensis* sp. nov., isolated from industrial effluent in Pakistan. Antonie Van Leeuwenhoek 108, 859–870. https://doi.org/10.1007/s10482-015-0540-1

Abd-Elhalem, B.T., El-Sawy, M., Gamal, R.F., Abou-Taleb, K.A., 2015. Production of amylases from *Bacillus amyloliquefaciens* under submerged fermentation using some agro-industrial by-products. Annals of Agricultural Sciences 60, 193–202. https://doi.org/10.1016/j.aoas.2015.06.001

Abiram, A., Kolandaivel, P., 2007. Conformational study of palindromic tripeptides (GPG, IPI and KPK) in HIV-1 protease-A density functional theory study. Biochimica et Biophysica Acta - Proteins and Proteomics 1774, 382–391. https://doi.org/10.1016/j.bbapap.2006.12.010

Adebusoye, S.A., Ilori, M.O., Amund, O.O., Teniola, O.D., Olatope, S.O., 2007. Microbial degradation of petroleum hydrocarbons in a polluted tropical stream. World J Microbiol Biotechnol 23, 1149–1159. https://doi.org/10.1007/s11274-007-9345-3

Barman, D., Jha, D.K., 2021. Metallotolerant Microorganisms and Microbe-Assisted Phytoremediation for a Sustainable Clean Environment, in: Singh, R.P., Manchanda, G., Bhattacharjee, K., Panosyan, H. (Eds.), Microbes in Microbial Communities: Ecological and Applied Perspectives. Springer, Singapore, pp. 307–336. https://doi.org/10.1007/978-981-16-5617-0_15

Basharat, Z., Yasmin, A., He, T., Tong, Y., 2018. Genome sequencing and analysis

of *Alcaligenes faecalis* subsp. *phenolicus* MB207. Sci Rep 8, 3616. https://doi.org/10.1038/s41598-018-21919-4

Calderón, M.I., Vargas, C., Rojo, F., Iglesias-Guerra, F., Csonka, L.N., Ventosa, A., Nieto, J.J., 2004. Complex regulation of the synthesis of the compatible solute ectoine in the halophilic bacterium *Chromohalobacter salexigens* DSM 3043T. Microbiology 150, 3051–3063. https://doi.org/10.1099/mic.0.27122-0

Cappucino, J.C., Sherman, N., 1992. Microbiology: A Laboratory Manual. Benjamin / Cumming Pub. Co., New York.

Castellani, A., Chalmers, A.J., 1919. Manual of Tropical Medicine, 3rd ed. William Wood and Co., New York.

Davis, R., Pezzlo, M., 2023. 3.17 Biochemical Tests for the Identification of Aerobic Bacteria, in: Clinical Microbiology Procedures Handbook. ASM Press Washington, DC.

Diels, L., Dong, Q., Lelie, D., Baeyens, W., Mergeay, M., 1995. Theczc operon of *Alcaligenes eutrophus* CH34: from resistance mechanism to the removal of heavy metals. Journal of Industrial Microbiology 14, 142–153. https://doi.org/10.1007/BF01569896

Dixit, V.K., Misra, S., Mishra, S.K., Tewari, S.K., Joshi, N., Chauhan, P.S., 2020. Characterization of plant growth-promoting alkalotolerant *Alcaligenes* and *Bacillus* strains for mitigating the alkaline stress in *Zea mays*. Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology 113, 889–905. https://doi.org/10.1007/s10482-020-01399-1

Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19, 11–15.

Du, Y., Cheng, Q., Qian, M., Liu, Y., Wang, F., Ma, J., Zhang, X., Lin, H., 2023. Biodegradation of sulfametoxydiazine by *Alcaligenes aquatillis* FA: Performance, degradation pathways, and mechanisms. Journal of Hazardous Materials 452, 131186. https://doi.org/10.1016/j.jhazmat.2023.131186

Dunfield, K.E., Germida, J.J., 2003. Seasonal Changes in the Rhizosphere Microbial Communities Associated with Field-Grown Genetically Modified Canola (*Brassicanapus*). Appl Environ Microbiol 69, 7310–7318. https://doi.org/10.1128/AEM.69.12.7310-7318.2003

Durán, R.E., Méndez, V., Rodríguez-Castro, L., Barra-Sanhueza, B., Salvà-Serra, F., Moore, E.R.B., Castro-Nallar, E., Seeger, M., 2019. Genomic and Physiological Traits of the Marine Bacterium *Alcaligenes aquatilis* QD168 Isolated From Quintero Bay, Central Chile, Reveal a Robust Adaptive Response to Environmental Stressors. Front. Microbiol. 10, 528. https://doi.org/10.3389/fmicb.2019.00528

Eiberweiser, A., Nazet, A., Kruchinin, S.E., Fedotova, M.V., Buchner, R., 2015. Hydration and Ion Binding of the Osmolyte Ectoine. J Phys Chem B 119, 15203–15211. https://doi.org/10.1021/acs.jpcb.5b09276

El-Sayed, S.E., Abdelaziz, N.A., El-Housseiny, G.S., Aboshanab, K.M., 2024. Nanosponge hydrogel of octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propanoate of *Alcaligenes faecalis*. Applied Microbiology and Biotechnology 108, 1–25. https://doi.org/10.1007/s00253-023-12819-3

Faelen, M., Merlin, C., Geuskens, M., Mergeay, M., Toussaint, A., 1993. Characterization of a temperate phage hosted by *Alcaligenes eutrophus* strain A5. Research in Microbiology 144, 627–631. https://doi.org/10.1016/0923-2508(93)90065-A

Fatema, K., Mahmud, N.U., Gupta, D.R., Siddiqui, M.N., Sakif, T.I., Sarker, A., Sharpe, A.G., Islam, T., 2024. Enhancing rice growth and yield with weed endophytic bacteria *Alcaligenes faecalis* and *Metabacillus indicus* under reduced chemical fertilization. PLOS ONE 19, e0296547. https://doi.org/10.1371/journal.pone.0296547

Fatima, T., Mishra, I., Verma, R., Arora, N.K., 2020. Mechanisms of halotolerant plant growth promoting *Alcaligenes* sp. involved in salt tolerance and enhancement of the growth of rice under salinity stress. 3 Biotech 10, 361. https://doi.org/10.1007/s13205-020-02348-5

Felestrino, É.B., Sanchez, A.B., Caneschi, W.L., Lemes, C.G.D.C., Assis, R.D.A.B., Cordeiro, I.F., Fonseca, N.P., Villa, M.M., Vieira, I.T., Kamino, L.H.Y., Carmo, F.F., Silva, A.M., Thomas, A.M., Patané, J.S.L., Ferreira, F.C., Freitas, L.G., Varani, A.D.M., Ferro, J.A., Silva, R.S., Almeida, N.F., Garcia, C.C.M., Setubal, J.C., Moreira, L.M., 2020. Complete genome sequence and analysis of *Alcaligenes faecalis* strain Mc250, a new potential plant bioinoculant. PLoS ONE 15, e0241546. https://doi.org/10.1371/journal.pone.0241546

FitzGerald, R.J., Meisel, H., 2000. Milk protein-derived peptide inhibitors of angiotensin-I-converting enzyme. Br J Nutr 84, 33–37. https://doi.org/10.1017/S0007114500002221

Flury, P., Vesga, P., Péchy-Tarr, M., Aellen, N., Dennert, F., Hofer, N., Kupferschmied, K.P., Kupferschmied, P., Metla, Z., Ma, Z., Siegfried, S., Weert, S., Bloemberg, G., Höfte, M., Keel, C.J., Maurhofer, M., 2017. Antimicrobial and Insecticidal: Cyclic Lipopeptides and Hydrogen Cyanide Produced by Plant-Beneficial *Pseudomonas* Strains CHA0, CMR12a, and PCL1391 Contribute to Insect Killing. Front Microbiol 8, 100. https://doi.org/10.3389/fmicb.2017.00100

Ghazy, N., El-Nahrawy, S., 2021. Siderophore production by *Bacillus subtilis* MF497446 and *Pseudomonas koreensis* MG209738 and their efficacy in controlling *Cephalosporium maydis* in maize plant. Arch Microbiol 203, 1195–1209. https://doi.org/10.1007/s00203-020-02113-5

Graf, R., Anzali, S., Buenger, J., Pfluecker, F., Driller, H., 2008. The multifunctional role of ectoine as a natural cell protectant. Clin Dermatol 26, 326–333. https://doi.org/10.1016/j.clindermatol.2008.01.002

Haba, E., Bresco, O., Ferrer, C., Marqués, A., Busquets, M., Manresa, A., 2000. Isolation of lipase-secreting bacteria by deploying used frying oil as selective substrate. Enzyme and Microbial Technology 26, 40–44. https://doi.org/10.1016/S0141-0229(99)00125-8

Honda, N., Hirai, M., Ano, T., Shoda, M., 1999. Control of Tomato Damping-off Caused by *Rhizoctonia solani* by the Heterotrophic Nitrifier *Alcaligenes faecalis* and Its Product, Hydroxylamine. Jpn. J. Phytopathol. 65, 153–162. https://doi.org/10.3186/jjphytopath.65.153

Honda, N., Hirai, M., Ano, T., Shoda, M., 1998. Antifungal effect of a heterotrophic nitrifier *Alcaligenes faecalis*. Biotechnology Letters 20, 703–705. https://doi.org/10.1023/A:1005382810088

Hopper, D.J., Kaderbhai, M.A., 1999. 2,4h-Dihydroxyacetophenone dioxygenase (EC 1.13.11.41) from *Alcaligenes* sp. 4HAP : a novel enzyme with an atypical dioxygenase sequence.

Huang, C., 2020. Extensively drug-resistant *Alcaligenes faecalis* infection. BMC Infectious Diseases 20. https://doi.org/10.1186/s12879-020-05557-8

Ibrahim, U.B., Yahaya, S., Yusuf, I., Kawo, A.H., 2022. Optimization and Simulation of Process Parameters in Biosorption of Heavy Metals by *Alcaligenes faecalis* Strain UBI (MT107249) Isolated from Soil of Local Mining Area in North-West Nigeria. Soil and Sediment Contamination: An International Journal 31, 438–455. https://doi.org/10.1080/15320383.2021.1963211

Jia, R., Chen, J., Hu, L., Liu, X., Xiao, K., Wang, Y., 2022. *Alcaligenes faecalis* Juj3 alleviates *Plasmodiophora brassicae* stress to cabbage via promoting growth and inducing resistance. Front. Sustain. Food Syst. 6. https://doi.org/10.3389/fsufs.2022.942409

Kahn, M.C., 1925. Hydrogen sulphide production by anaerobic spore-bearing bacteria. J Bacteriol 10, 439–447. https://doi.org/10.1128/jb.10.5.439-447.1925

Kakar, K.U., Nawaz, Z., Cui, Z., Almoneafy, A.A., Ullah, R., Shu, Q.-Y., 2018. Rhizosphere-associated *Alcaligenes* and *Bacillus* strains that induce resistance against blast and sheath blight diseases, enhance plant growth and improve mineral content in rice. J Appl Microbiol 124, 779–796. https://doi.org/10.1111/jam.13678

Kanapathipillai, M., Lentzen, G., Sierks, M., Park, C.B., 2005. Ectoine and hydroxyectoine inhibit aggregation and neurotoxicity of Alzheimer's β-amyloid. FEBS Letters 579, 4775–4780. https://doi.org/10.1016/j.febslet.2005.07.057

Kapley, A., Tanksale, H., Sagarkar, S., Prasad, A.R., Kumar, R.A., Sharma, N., Qureshi, A., Purohit, H.J., 2016. Antimicrobial activity of *Alcaligenes* sp. HPC 1271 against multidrug resistant bacteria. Funct Integr Genomics 16, 57–65. https://doi.org/10.1007/s10142-015-0466-8

Kasana, R., Salwan, R., Dhar, H., Dutt, S., Gulati, A., 2008. A Rapid and Easy Method for the Detection of Microbial Cellulases on Agar Plates Using Gram's Iodine. Current Microbiology 57, 503–507. https://doi.org/10.1007/s00284-008-9276-8

Kilian, G., Tshanga, S.S., Oidu, B., Milne, P.J., 2011. Antimicrobial activity of liposome encapsulated cyclo(L-tyrosyl- L-prolyl). Pharmazie 66, 421–423. https://doi.org/10.1691/ph.2011.0352

Kiyohara, H., Nagao, K., Kouno, K., Yano, K., 1982. Phenanthrene-degrading phenotype of *Alcaligenes faecalis* AFK2. Appl Environ Microbiol 43, 458–461. https://doi.org/10.1128/aem.43.2.458-461.1982

Kumar, A., Bhunia, B., Dasgupta, D., Mandal, T., Dey, A., Datta, S., Bhattacharya, P., 2013. Optimization of culture condition for growth and phenol degradation by Alcaligenes faecalis JF339228 using Taguchi Methodology. Desalination and Water Treatment 51, 3153–3163. https://doi.org/10.1080/19443994.2012.749021

Lahlali, R., Aksissou, W., Lyousfi, N., Ezrari, S., Blenzar, A., Tahiri, A., Ennahli, S., Hrustić, J., MacLean, D., Amiri, S., 2020. Biocontrol activity and putative mechanism of *Bacillus amyloliquefaciens* (SF14 and SP10), *Alcaligenes faecalis* ACBC1, and *Pantoea agglomerans* ACBP1 against brown rot disease of fruit. Microbial Pathogenesis 139, 103914. https://doi.org/10.1016/j.micpath.2019.103914

Lee, I., Kim, Y.O., Park, S.C., Chun, J., 2016. OrthoANI: An improved algorithm and software for calculating average nucleotide identity. International Journal of Systematic and Evolutionary Microbiology 66, 1100–1103.

https://doi.org/10.1099/ijsem.0.000760
Li, P., Wang, S., Liu, M., Dai, X., Shi, H., Zhou, W., Sheng, S., Wu, F., 2024. Antibacterial Activity and Mechanism of Three Root Exudates from Mulberry Seedlings against Ralstonia pseudosolanacearum. Plants 13, 482. https://doi.org/10.3390/plants13040482
Liu, D., Sun, H., Zhang, L., Li, S., Qin, Z., 2007. High-Level Expression of Milk-Derived Antihypertensive Peptide in *Escherichia coli* and Its Bioactivity. J. Agric. Food Chem. 55, 5109–5112. https://doi.org/10.1021/jf0703248
Liu, M., Liu, H., Shi, M., Jiang, M., Li, L., Zheng, Y., 2021. Microbial production of ectoine and hydroxyectoine as high-value chemicals. Microb Cell Fact 20, 76. https://doi.org/10.1186/s12934-021-01567-6
Liu, X., Huang, D., Wu, J., Yu, C., Zhou, R., Liu, C., Zhang, W., Yao, J., Cheng, M., Guo, S., 2016. The Genome Sequence of *Alcaligenes faecalis* NBIB-017 Contains Genes with Potentially High Activities against *Erwinia carotovora*. Genome Announc 4, e00222-16. https://doi.org/10.1128/genomeA.00222-16
MacFaddin, J.F., 2000. Biochemical tests for identification of medical bacteria, 3rd ed. Lippincott Williams & Wilkins, Philadelphia.
Machado, R.A.R., Loulou, A., Bhat, A.H., Mastore, M., Terrettaz, C., Brivio, M.F., Kallel, S., 2023. *Acinetobacter nematophilus* sp. nov., *Alcaligenes nematophilus* sp. nov., *Enterobacter nematophilus* sp. nov., and *Kaistia nematophila* sp. nov., Isolated from Soil-Borne Nematodes and Proposal for the Elevation of *Alcaligenes faecalis* subsp. *faecalis*, *Alcaligenes faecalis* subsp. *parafaecalis*, and *Alcaligenes faecalis* subsp. *phenolicus* to the Species Level. Taxonomy 3, 148–168. https://doi.org/10.3390/taxonomy3010012
MacWilliams, M.P., 2009. Indole test protocol [WWW Document]. URL https://asm.org/getattachment/200d3f34-c75e-4072-a7e6-df912c792f62/indole-test-protocol-3202.pdf (accessed 5.15.24).
Masi, C., Gemechu, G., Tafesse, M., 2021. Isolation, screening, characterization, and identification of alkaline protease-producing bacteria from leather industry effluent. Ann Microbiol 71, 24. https://doi.org/10.1186/s13213-021-01631-x
McEntee, J.D., Woodrow, J.R., Quirk, A.V., 1986. Investigation of cadmium resistance in an *Alcaligenes* sp. Appl Environ Microbiol 51, 515–520. https://doi.org/10.1128/aem.51.3.515-520.1986
Meier-Kolthoff, J.P., Auch, A.F., Klenk, H.-P., ¨ Oker, M.G., 2013. Genome sequence-based species delimitation with confidence intervals and improved distance functions, BMC Bioinformatics.
Nongonierma, A.B., Dellafiora, L., Paolella, S., Galaverna, G., Cozzini, P., FitzGerald, R.J., 2018. *In Silico* Approaches Applied to the Study of Peptide Analogs of Ile-Pro-Ile in Relation to Their Dipeptidyl Peptidase IV Inhibitory Properties. Front. Endocrinol. 9, 329. https://doi.org/10.3389/fendo.2018.00329
Pastor, J.M., Salvador, M., Argandoña, M., Bernal, V., Reina-Bueno, M., Csonka, L.N., Iborra, J.L., Vargas, C., Nieto, J.J., Cánovas, M., 2010. Ectoines in cell stress protection: Uses and biotechnological production. Biotechnology Advances 28, 782–801. https://doi.org/10.1016/j.biotechadv.2010.06.005
Patel, S., Vrinda Thaker, Kuvad, R., Saraf, M., 2020. Role of lipopolysaccaride extracted from Alcaligenes faecalis as elicitor for the induction of plant defense against fusarium wilt. J Plant Pathol 102, 351–357. https://doi.org/10.1007/s42161-019-00425-0
Pedrosa-Silva, F., Venancio, T.M., 2023. Comparative Genomics Reveals Novel

Species and Insights into the Biotechnological Potential, Virulence, and Resistance of *Alcaligenes*. Genes 14, 1783. https://doi.org/10.3390/genes14091783

Pramanick, B., Bera, A., Saha, P., Barman, A., Paramanick, B., Maitra, S., Hossain, A., 2023. Soil Microflora and Their Interaction with Plants Under Changing Climatic Scenarios, in: Mathur, P., Kapoor, R., Roy, S. (Eds.), Microbial Symbionts and Plant Health: Trends and Applications for Changing Climate. Springer Nature, Singapore, pp. 19–40. https://doi.org/10.1007/978-981-99-0030-5_2

Prjibelski, A., Antipov, D., Meleshko, D., Lapidus, A., Korobeynikov, A., 2020. Using SPAdes De Novo Assembler. Current Protocols in Bioinformatics 70. https://doi.org/10.1002/cpbi.102

Qiu, J., Zhang, J., Zhang, Y., Wang, Y., Tong, L., Hong, Q., He, J., 2017. Biodegradation of Picolinic Acid by a Newly Isolated Bacterium *Alcaligenes faecalis* Strain JQ135. Curr Microbiol 74, 508–514. https://doi.org/10.1007/s00284-017-1205-2

Ray, S., Singh, P., Singh, J., Singh, S., Sarma, B.K., Singh, H.B., 2023. Killed fungal pathogen triggers antifungal metabolites in *Alcaligenes faecalis* for plant defense. Physiological and Molecular Plant Pathology 125, 101996. https://doi.org/10.1016/j.pmpp.2023.101996

Regar, R.K., Gaur, V.K., Mishra, G., Jadhao, S., Kamthan, M., Manickam, N., 2016. Draft Genome Sequence of *Alcaligenes faecalis* Strain IITR89, an Indole-Oxidizing Bacterium. Genome Announc 4, e00067-16. https://doi.org/10.1128/genomeA.00067-16

Rehfuss, M., Urban, J., 2005. *Alcaligenes faecalis* subsp. *phenolicus* subsp. nov. a phenol-degrading, denitrifying bacterium isolated from a graywater bioprocessor. Systematic and Applied Microbiology 28, 421–429. https://doi.org/10.1016/j.syapm.2005.03.003

Sagarkar, S., Bhardwaj, P., Yadav, T.C., Qureshi, A., Khardenavis, A., Purohit, H.J., Kapley, A., 2014. Draft Genome Sequence of Atrazine-Utilizing Bacteria Isolated from Indian Agricultural Soil. Genome Announc 2, e01149-13. https://doi.org/10.1128/genomeA.01149-13

Saravanan, V.S., Subramoniam, S.R., Raj, S.A., 2004. Assessing in vitro solubilization potential of different zinc solubilizing bacterial (zsb) isolates. Braz. J. Microbiol. 35, 121–125. https://doi.org/10.1590/S1517-83822004000100020

Saum, S.H., Müller, V., 2008. Growth phase-dependent switch in osmolyte strategy in a moderate halophile: ectoine is a minor osmolyte but major stationary phase solute in *Halobacillus halophilus*. Environ Microbiol 10, 716–726. https://doi.org/10.1111/j.1462-2920.2007.01494.x

Sayyed, R.Z., Gangurde, N.S., Patel, P.R., Joshi, S.A., Chincholkar, S.B., 2010. Siderophore production by *Alcaligenes faecalis* and its application for growth promotion in *Arachis hypogaea*. Indian Journal of Biotechnology 9, 302–307.

Schwyn, B., Neilands, J.B., 1987. Universal chemical assay for the detection and determination of siderophores. Analytical Biochemistry 160, 47–56. https://doi.org/10.1016/0003-2697(87)90612-9

Sehrawat, A., Sindhu, S.S., Glick, B.R., 2022. Hydrogen cyanide production by soil bacteria: Biological control of pests and promotion of plant growth in sustainable agriculture. Pedosphere 32, 15-38. https://doi.org/10.1016/S1002-0160(21)60058-9

Singha, L.P., Kotoky, R., Pandey, P., 2017. Draft Genome Sequence of Alcaligenes faecalis BDB4, a Polyaromatic Hydrocarbon-Degrading Bacterium Isolated from Crude Oil-Contaminated Soil. Genome Announc 5, e01346-17. https://doi.org/10.1128/genomeA.01346-17

Skerman, V.B.D., McGowan, V., Sneath, P.H.A., 1980. Approved Lists of Bacterial Names. International Journal of Systematic Bacteriology 30, 225–420. http://dx.doi.org/10.1099/00207713-30-1-225

Sodhi, K.K., Kumar, M., Singh, D.K., 2021. Insight into the amoxicillin resistance, ecotoxicity, and remediation strategies. Journal of Water Process Engineering 39, 101858. https://doi.org/10.1016/j.jwpe.2020.101858

Sodhi, K.K., Kumar, M., Singh, D.K., 2020. Multi-metal resistance and potential of *Alcaligenes* sp. MMA for the removal of heavy metals. SN Applied Sciences 2. https://doi.org/10.1007/s42452-020-03583-4

Sodhi, K.K., Singh, C.K., Kumar, M., Singh, D.K., 2023. Whole-genome sequencing of Alcaligenes sp. strain MMA: insight into the antibiotic and heavy metal resistant genes. Front. Pharmacol. 14, 1144561. https://doi.org/10.3389/fphar.2023.1144561

Souza, C., Burbano-Rosero, E., Almeida, B., Martins, G., Albertini, L., Rivera, I., 2009. Culture medium for isolating chitinolytic bacteria from seawater and plankton. World Journal of Microbiology and Biotechnology 25, 2079–2082. https://doi.org/10.1007/s11274-009-0098-z

Stuart, C.A., Van Stratum, E., Rustigan, R., 1945. Further studies on urease production by *Proteus* and related organisms. Journal of Bacteriology 49, 437–444. https://doi.org/10.1128/jb.49.5.437-444

Subba Rao, N.S., 1977. Soil Microorganisms and Plant Growth. Oxford and IBH Publishing Co.

Thampi, A., Bhai, R.S., 2017. Rhizosphere actinobacteria for combating *Phytophthora capsici* and *Sclerotium rolfsii*, the major soil borne pathogens of black pepper (*Piper nigrum* L.). Biological Control 109, 1–13. https://doi.org/10.1016/j.biocontrol.2017.03.006

Tokunaga, T., Kamigiri, K., Orita, M., Nishikawa, T., Shimizu, M., Kaniwa, H., 1996. Kalimantacin A, B, and C, Novel Antibiotics Produced by *Alcaligenes* sp. YL-02632S. II. Physico-chemical Properties and Structure Elucidation. J. Antibiot. 49, 140–144. https://doi.org/10.7164/antibiotics.49.140

Tulipano, G., Sibilia, V., Caroli, A.M., Cocchi, D., 2011. Whey proteins as source of dipeptidyl dipeptidase IV (dipeptidyl peptidase-4) inhibitors. Peptides 32, 835–838. https://doi.org/10.1016/j.peptides.2011.01.002

Velho-Pereira, S., Kamat, N.M., 2011. Antimicrobial screening of actinobacteria using a modified cross-streak method. Indian Journal of Pharmaceutical Sciences 73, 223–228. https://doi.org/10.4103/0250-474X.91566

Wu, M., Hou, T., Liu, Y., Miao, L., Ai, G., Ma, L., Zhu, H., Zhu, Y., Gao, X., Herbold, C.W., Wagner, M., Li, D., Liu, Z., Liu, S., 2021. Novel *Alcaligenes ammonioxydans* sp. nov. from wastewater treatment sludge oxidizes ammonia to N_2 with a previously unknown pathway. Environmental Microbiology 23, 6965–6980. https://doi.org/10.1111/1462-2920.15751

Wu, Z.-F., Liu, G.-L., Zhou, Z., Wang, G.-X., Xia, L., Liu, J.-L., 2012. Induction of Immune-related Gene Expression in *Ctenopharyngodon idella* Kidney Cells by Secondary Metabolites from Immunostimulatory *Alcaligenes faecalis* FY-3. Scandinavian Journal of Immunology 76, 131–140. https://doi.org/10.1111/j.1365-3083.2012.02722.x

SUPPLEMENTARY MATERIAL

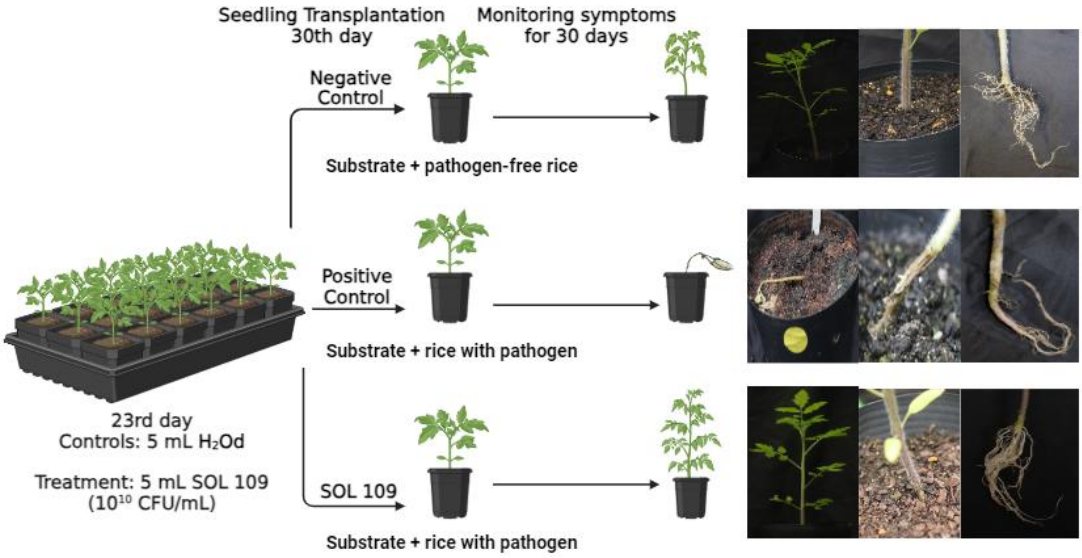

Figure S1. Conducting the *Rhizoctonia solani* biocontrol experiment with the *Alcaligenes nematophilus* SOL 109 strain and monitoring symptoms (thinning and darkening of the stem, superficial necrosis in the neck and seedling tipping over) in the control and treatment groups. Image created with BioRender.com.

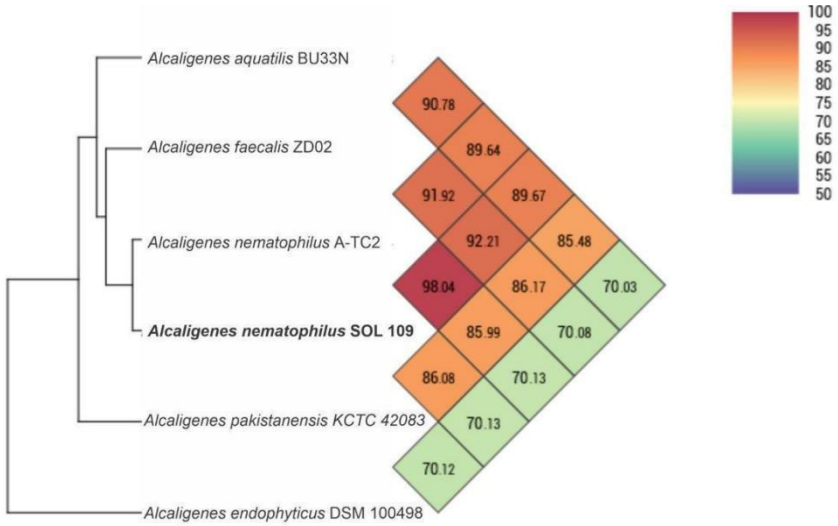

Figure S2. Heatmap and phylogram based on calculated ANI values using OAT software with the genome of isolate SOL 109 and reference species. Values greater than 95% indicate that the strains belong to the same species.

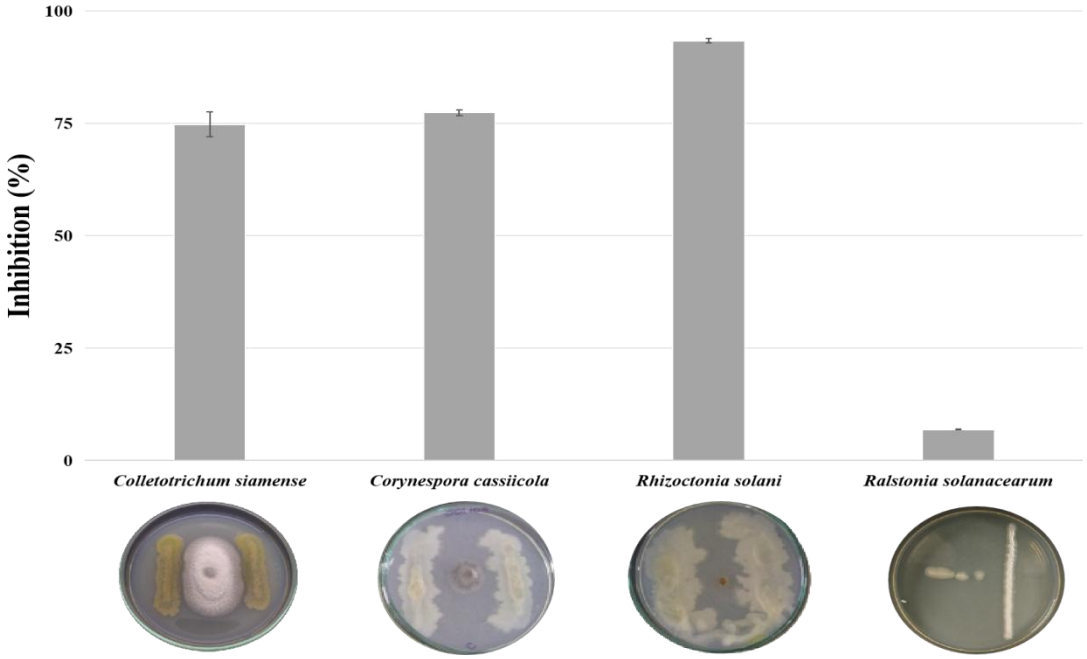

Figure S5. *In vitro* inhibition dual culture of *Alcaligenes nematophilus* SOL 109 against *Colletotrichum siamense, Corynespora cassiicola, Rhizoctonia solani* and *Ralstonia solanacearum*, phytopathogens of agricultural relevance in Amazonas.

Table S1. Analysis of dDDH0, dDDH4 and dDDH6 of the *Alcaligenes nematophilus* SOL 109 lineage with the close lineages identified by TYGS.

Query strain	Subject strain	dDDH0 (%)	dDDH4 (%)	dDDH6 (%)	G+C (%)
A. nematophilus SOL109	*Alcaligenes nematophilus* A-TC2	88,4	83	90,2	0,08
A. nematophilus SOL109	*A. faecalis* subsp. *phenolicus* DSM 16503	77,3	66,5	77,9	0,08
A. nematophilus SOL109	*Alcaligenes faecalis* NBRC 13111	83,4	47,6	77,5	0,32
A. nematophilus SOL109	*A. faecalis* subsp. *parafaecalis* DSM 13975	75,9	35,8	66	0,31
A. nematophilus SOL109	*Alcaligenes ammonioxydans* HO-1	68,4	31,6	58	0,84
A. nematophilus SOL109	*Alcaligenes pakistanensis* KCTC 42083	71,9	30,5	59,8	0,93
A. nematophilus SOL109	*Bordetella avium* NCTC 12033	13,1	19,3	13,5	5,26
A. nematophilus SOL109	*Kerstersia gyiorum* DSM 16618	13,1	19,2	13,5	6,02
A. nematophilus SOL109	*Eoetvoesiella caeni* DSM 25520	13,1	19,1	13,4	2,58
A. nematophilus SOL109	*Pusillimonas caeni* KCTC 42353	13,2	18,7	13,5	6,79
A. nematophilus SOL109	*Paenalcaligenes hominis* DSM 26613	12,9	18,3	13,3	8,06
A. nematophilus SOL109	*Paenalcaligenes hominis* CCM 7698	12,9	18,2	13,3	8,06
A. nematophilus SOL109	*Alcaligenes endophyticus* DSM 100498	13,7	17,8	13,9	6,28
A. nematophilus SOL109	*Acidovorax facilis* DSM 649	12,6	17,8	13	7,75

Table S2. Analysis of dDDH0, dDDH4 and dDDH6 of the *Alcaligenes endophyticus* lineage with the close lineages identified by TYGS.

Query strain	Subject strain	dDDH0 (%)	dDDH4 (%)	dDDH6 (%)	G+C (%)
A.endophyticus	*Eoetvoesiella caeni* DSM 25520	12,8	21,7	13,2	8,87
A. endophyticus	*Pusillimonas minor* YC-7-48T	12,8	21,3	13,2	7,32
A. endophyticus	*Pusillimonas thiosulfatoxidans* ye3	12,8	20	13,2	9,29
A. endophyticus	*Corticimicrobacter populi* 3d-2-2T	12,7	19,9	13,1	10,98
A.endophyticus	*Paenalcaligenes niemegkensis* NGK35	13,2	19,6	13,5	2,07
A.endophyticus	*Pusillimonas harenae* JCM 16917	12,8	18,8	13,2	6,21
A.endophyticus	*Alcaligenes ammonioxydans* HO-1	13,6	18,3	13,9	7,12
A.endophyticus	*Alcaligenes pakistanensis* KCTC 42083	13,5	18,2	13,8	5,35
A. endophyticus	*Alcaligenes nematophilus* A-TC2	13,6	18,2	13,9	6,36
A. endophyticus	*A. faecalis* subsp. *parafaecalis* DSM 1397	13,6	18,1	13,8	5,97
A. endophyticus	*A. faecalis* subsp. *phenolicus* DSM 16503	13,7	18	13,9	6,36
A. endophyticus	*Alcaligenes faecalis* NBRC 13111	13,7	18	13,9	6,6
A. endophyticus	*Paenalcaligenes hominis* DSM 26613	12,9	17,9	13,3	1,78

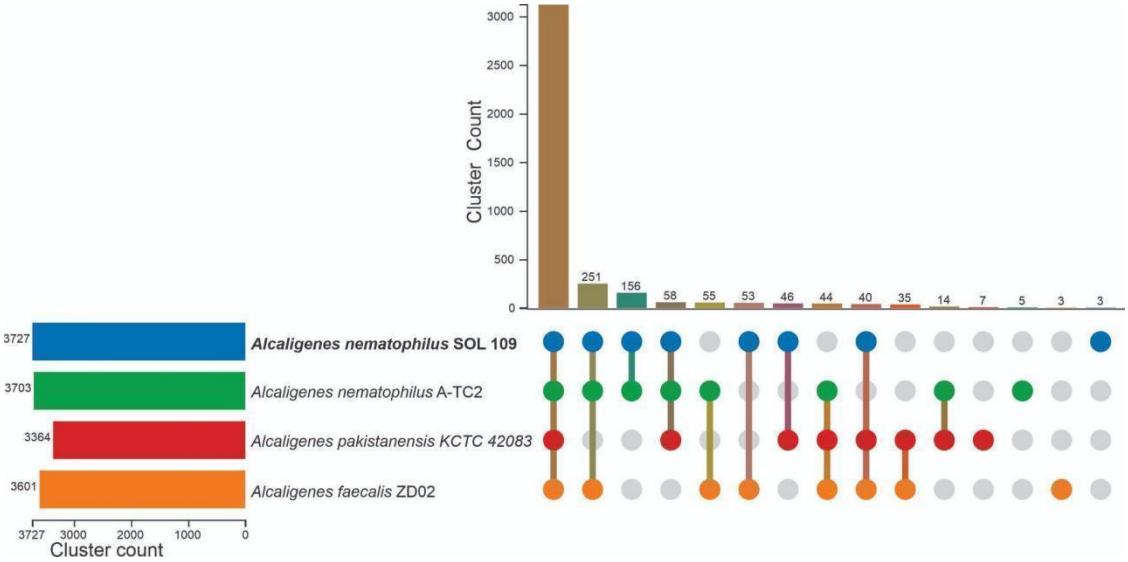

Figure S3. Venn diagram demonstrating the number of proteins shared between *Alcaligenes nematophilus* SOL 109, *Alcaligenes nematophilus* A-TC2, *Alcaligenes pakistanensis* KCT 42083 and *Alcaligenes faecalis* ZD02.

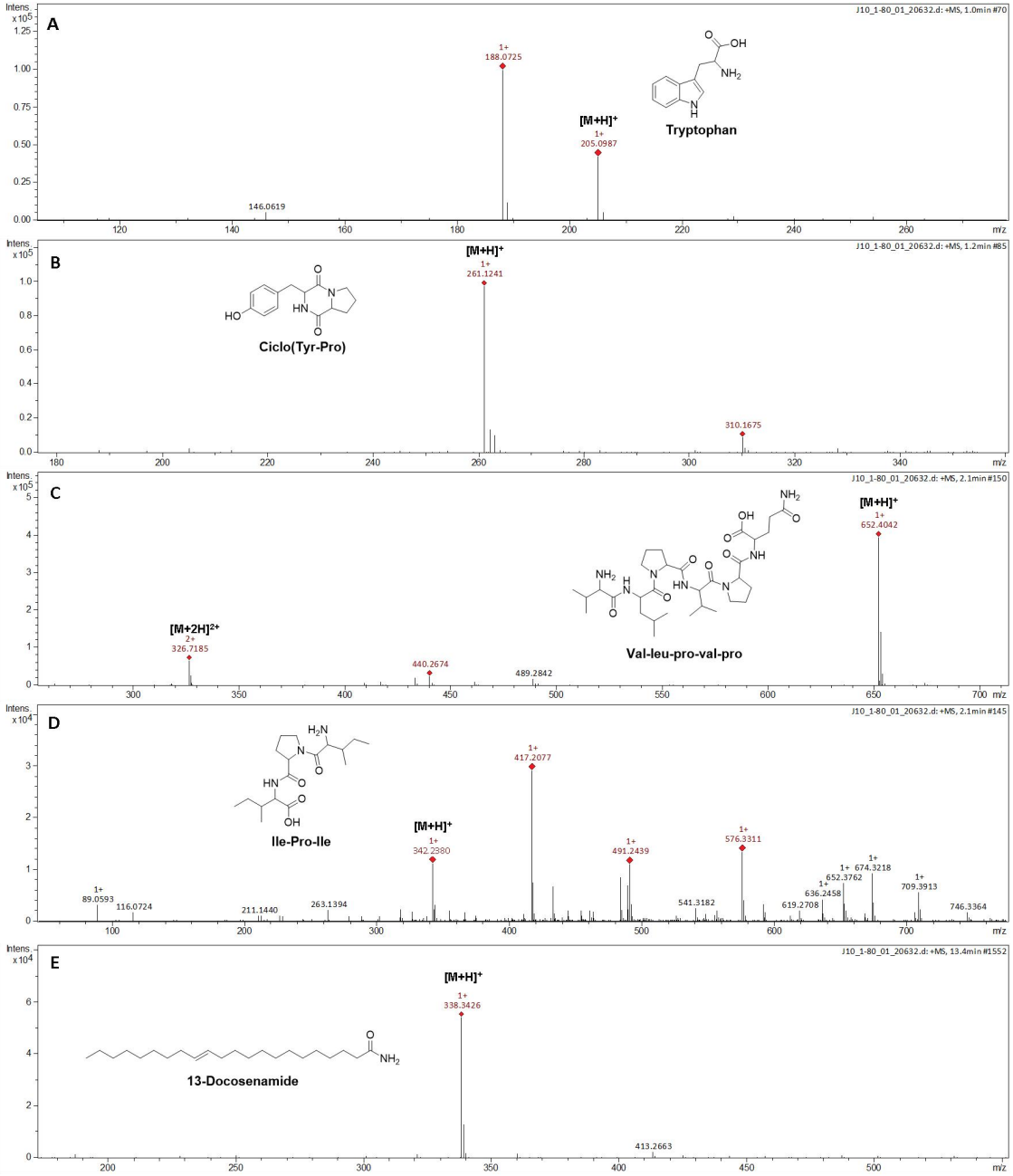

Figura S4. MS1 spectra of the compounds (A) Tryptophan, (B) Cyclo(Tyr-Pro), (C) Val-Leu-Pro-Val-Pro Ile-Pro-Ile, (C), (D) 13-docosenamide and (E) noted in extract of *Alcaligenes nematophilus* SOL 109 grown in LB medium.