PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL

FACULDADE DE BIOCIÊNCIAS

PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA CELULAR E MOLECULAR

MAILA PACHECO DIAS

CARACTERIZAÇÃO DE ISOLADOS DE *Streptomyces* spp. COMO RIZOBACTÉRIAS PROMOTORAS DE CRESCIMENTO E DE RESISTÊNCIA À *Pectobacterium carotovorum* subsp. *brasiliensis* EM PLANTAS DE *Solanum lycopersicum* (L.)

Prof.ª Dra. ELIANE ROMANATO SANTARÉM

Orientadora

Porto Alegre

2016

MAILA PACHECO DIAS

CARACTERIZAÇÃO DE ISOLADOS DE *Streptomyces* spp. COMO RIZOBACTÉRIAS PROMOTORAS DE CRESCIMENTO E DE RESISTÊNCIA À *Pectobacterium carotovorum* subsp. *brasiliensis* EM PLANTAS DE *Solanum lycopersicum* (L.)

Dissertação apresentada ao Programa de Pós-Graduação em Biologia Celular e Molecular da Faculdade de Biociências da Pontifícia Universidade Católica do Rio Grande do Sul como requisito para obtenção do título de mestre.

Orientadora: Prof.ª Dra. Eliane Romanato Santarém

Porto Alegre

2016

AGRADECIMENTOS

Em primeiro lugar, antes de agradecer, eu gostaria de pedir perdão, sim perdão. Foram dois anos de mestrado, de peleia, de correrias e durante este tempo eu provavelmente negligenciei muita coisa e muitas pessoas. O mestrado começou como uma ideia que virou um sonho e aos poucos foi virando realidade e eu detestaria chegar ao fim disto tudo sabendo que durante o processo as minhas decisões/atitudes fizeram de alguma forma alguém sofrer. Por isso peço perdão, pois eu sei que não deve ter sido fácil me "aturar" muitas vezes em meio a experimentos, no vai e vem diário, chegando cansada em casa. À minha família, orientadores e colegas um sincero "Sorry".

Bem, se eu tenho o que pedir perdão, imagina então agradecer!

Não poderia começar os meus agradecimentos de forma diferente, o meu maior OBRIGADA vai para Deus, meu melhor amigo, que me sustenta, que nada deixa me faltar, sem Ele eu nem estaria aqui. Por isso meu Deus, muito obrigada! Muito obrigada por renovar minhas forças a cada dia e me dar coragem para seguir em frente lutando, por estar "abrindo meus olhos" a cada dia e me mostrando como posso alcançar aquilo que o Senhor sonhou para mim e é claro, por me enviar todas as pessoas que cruzaram o meu caminho enquanto este sonho estava sendo realizado.

À minha família, muito obrigada! Cada um, a sua maneira, foi peça fundamental para esse resultado. Sempre acreditando em mim; me incentivando; lidando com as minhas dúvidas, meus medos; pela compreensão quando "mestrado" era meu único assunto; pela ajuda financeira; caronas de madrugada; lanchinhos; por todo carinho, apoio; por reconhecer a importância que essa experiência tem para mim; por me ouvir e principalmente pelo amor e por serem meu exemplo de perseverança, integridade, honestidade e humildade. AMO vocês!

Aos familiares e amigos, muitos que durante este tempo foram privados da minha companhia, eu tenho um carinho muito grande por todos e agradeço as mensagens de incentivo, as orações, caronas, preocupação e faço aqui um voto com vocês de que daqui para frente estarei mais presente em suas vidas. Um grande abraço!

Aos meus colegas de laboratório, devo muitos "muito obrigada" a vocês. Pela alegria e momentos de descontração; pela "mãozinha" nos experimentos e planejamento, acho que todos entraram "na dança"; pelas discussões relevantes sobre diversos temas da ciência e

da vida como um todo; pela companhia; pelo silêncio; pelo barulho; por me permitirem ensinar e por me ensinarem tantas coisas, provavelmente o resultado não seria tão legal sem vocês técnicos, iniciação científica, mestrandos, bolsistas, "novos" e "antigos", enfim, "família Biotec". Obrigada!

Um abraço especial à Janaína Belquis, que prestes a sair de férias me deu "aquela ajuda" para a finalização dos meus experimentos.

Ao querido professor Leandro Vieira Astarita, muito obrigada pela atenção e carinho com que sempre me tratou, sempre disponível para tentar sanar minhas dúvidas, me ajudando a pensar e a buscar a história do problema. Ah e quem melhor do que ele para ouvir as minhas reclamações sobre sideróforos? Professor, foi um prazer inenarrável ter a sua companhia em todas essas discussões.

À minha querida orientadora, a professora Doutora Eliane Romanato Santarém, MUITO obrigada! Por acreditar em mim; por sempre me tratar com carinho, sem me julgar pelas minhas limitações; por me ensinar, do simples ao complexo, sempre com a mesma paixão; pelas discussões entusiasmadas, aprendi muito; por incentivar o pensamento científico entre os "Biotecs"; por ser mais que uma orientadora formalmente, mas por ser um ser-humano preocupada, amiga, solidária, que como uma mãe educa os filhos para a vida, deixando-os livres para alçar seus próprios "voos". Ah, e obrigada por ouvir reclamações e perguntas como: "as plantas não crescem nesse clima, vai dar tudo errado." "As bactérias estão crescendo devagar e agora?" "O meio de cultura não ficou azul, não aguento mais!" "Eu tive uma ideia, queria fazer mais alguns experimentos."

À professora Doutora Renata Medina, muito obrigada pelas considerações na fase de projeto, todas as ideias foram muito bem recebidas e me deram uma nova visão do que poderia ser feito.

À comissão examinadora Dra. Andréia Mara Rotta de Oliveira (FEPAGRO), Dra. Luciane Maria Pereira Passaglia (UFRGS) e Dra. Sílvia Dias de Oliveira (PUCRS) muito obrigada pela disponibilidade e comprometimento. Tenho certeza que as considerações e sugestões de cada um serão muito importantes para o aprimoramento deste trabalho.

A PUCRS – PROBOLSAS pela bolsa de estudos concedida que possibilitou a realização deste trabalho.

RESUMO

O tomateiro (*Solanum lycopersicum* L.) é uma planta herbácea pertencente à família Solanaceae. Seus frutos são consumidos mundialmente, chegando à produção mundial de 160 milhões de toneladas por ano. No Brasil, é a segunda hortaliça em importância econômica. Contudo, o tomateiro é alvo de inúmeras doenças que levam à perda de produção e/ou má qualidade dos frutos, como por exemplo, a doença Talo oco causada por *Pectobacterium* spp. Devido ao elevado número de doenças causadas por fitopatógenos, o tomateiro é uma cultura onde se utiliza uma quantidade expressiva de agroquímicos, estando entre as hortaliças que apresentam maior quantidade de agrotóxicos residuais. Por isso, torna-se imprescindível o desenvolvimento de técnicas sustentáveis de defesa para o vegetal, a fim de reduzir o uso destes compostos. Para este fim, é fundamental compreender as alterações no metabolismo vegetal relacionado à defesa, para que novas estratégias e novos produtos agrícolas possam ser desenvolvidos. O controle de doenças utilizando microrganismos de solo tem sido considerado uma alternativa, uma vez que as rizobactérias, além de promoverem o crescimento vegetal, podem induzir à resistência como consequência da ativação da defesa vegetal. Estas, chamadas rizobactérias promotoras de crescimento vegetal (PGPR), vêm sendo exploradas quanto à capacidade biofertilizante, fito-estimuladora e biopesticida. Os objetivos deste estudo foram caracterizar seis isolados de *Streptomyces* spp. como PGPR, determinar o antagonismo contra *Pectobacterium carotovorum* subsp. *brasiliensis* (Pcb), determinar a capacidade de isolados de *Streptomyces* spp. na promoção do crescimento de plantas de tomate e avaliar a modulação do metabolismo relacionado à defesa das plantas de tomate quando tratadas com *Streptomyces* spp. A possível influência de *Streptomyces* spp. na redução da doença Talo oco em plantas de tomate também foi avaliada. A caracterização bioquímica de isolados de *Streptomyces* spp. foi realizada por meio da capacidade de produzir sideróforos, solubilizar fosfato, e da atividade de amilase e lipase, bem como a produção de compostos orgânicos voláteis. O antagonismo de *Streptomyces* spp. contra Pcb foi determinado pelo método de dupla cultura e placa com barreira para análise do efeito de compostos orgânicos voláteis (VOC). A promoção do crescimento das plantas foi avaliada por meio de emissão de VOC e pela interação direta com os isolados de *Streptomyces* spp. (PM1, PM3, PM4, PM5, PM6 e PM9). Enzimas relacionadas à resposta de defesa foram analisadas colorimetricamente em plantas tratadas com isolados de *Streptomyces* spp. A avaliação da doença Talo oco foi realizada

em plantas tratadas com *Streptomyces* spp. e desafiadas com Pcb através da área sob a curva de progresso da doença e da mortalidade das plantas em 24 dias. Os isolados de *Streptomyces* spp. mostraram características de PGPR e 32 compostos voláteis foram identificados como produtos dos diferentes isolados. PM3 foi o isolado mais eficiente quanto ao antagonismo contra Pcb. A maioria dos isolados promoveu o aumento do comprimento de raiz e da parte aérea do tomateiro por VOC, embora PM5 tenha sido também eficiente na promoção do crescimento através da interação direta com *Streptomyces* spp. O tratamento com *Streptomyces* spp. modulou a atividade de enzimas relacionadas à defesa e diminuiu a incidência da doença Talo oco.

Palavras-chave: Actinomycetes, Defesa vegetal, *Pectobacterium* spp., PGPR, tomateiro.

ABSTRACT

Solanum lycopersicum L., tomato, is an herbaceous plant belonging to the Solanaceae family. Its fruits are consumed worldwide, reaching the world production of 160 million tons per year. In Brazil, it is the second vegetable in economic importance. However, the tomato is attacked by numerous diseases that lead to loss of production and /or poor quality of the fruit, such as the hollow stem, the disease caused by *Pectobacterium* spp. Due to the large number of diseases caused by plant pathogens, the tomato is a culture in which a significant amount of agrochemicals is used. Therefore, this species is among the vegetables with the greatest amount of residual pesticides. Under these circumstances, it is essential to develop sustainable plant defense techniques in order to reduce the use of agrochemicals. Then, changes in plant metabolism related to defense must be understood so that new strategies and new products can be developed. Disease control using soil microorganisms has been considered as an alternative, since the rhizobacteria, in addition to promoting plant growth, may induce resistance as the result of activation of the natural plant defenses. These, calls plant growth promoting rhizobacteria (PGPR), has been explored for their biofertilizers, biopesticides and phyto-stimulating abilities. The aims of this study were to characterize biochemically the *Streptomyces* spp. isolates, to determine the antagonism against *Pectobacterium carotovorum* subsp. *brasiliensis* (Pcb), to determine the ability of *Streptomyces* spp. on promoting growth of tomato plants and to evaluate the modulation of the defense-related metabolism of tomato plants when treated with *Streptomyces* spp. The possible influence of *Streptomyces* spp. on reducing soft rot disease in tomato plants was also evaluated. Biochemical characterization was evaluated through the ability of *Streptomyces* spp. on producing siderophores, solubilizing phosphate, and activity of amylase and lipase, as well as volatile organic compounds (VOC) production. Antagonism of *Streptomyces* spp. against Pcb was determined by dual-culture method and I-plate for VOC effect analysis. Plant growth promotion was evaluated through VOC emission and by direct interaction with *Streptomyces* spp. isolates (PM1, PM3, PM4, PM5, PM6 e PM9). Enzymes related to plant defense were colorimetric analyzed in plants treated with isolates of *Streptomyces* spp. Evaluation of soft rot disease was performed on plants treated with *Streptomyces* spp. and challenged with Pcb through the area under the disease progression curve (AUDPC) and plant mortality. Isolates of *Streptomyces* spp. displayed characteristics of PGPR and 32 volatile compounds were identified from the different isolates. PM3 was

the isolate showing efficient antagonism against Pcb. Most of the isolates promoted increase of root and shoot length of tomato plants by VOC although PM5 was efficient on promoting growth by direct interaction with *Streptomyces* spp. Treatment with *Streptomyces* spp. modulated the activity of defense-related enzymes and decrease incidence of soft rot disease.

Key words: Actinomycetes, Plant defense, *Pectobacterium* spp., PGPR, tomato.

LISTA DE ABREVIATURAS E SIGLAS

ACC – Enzima 1-aminociclopropano-1-carboxilato desaminase;

AIA – Ácido 3- indolacético;

AS – Ácido Salicílico;

FAOSTAT – Base de dados sobre Alimentação, Agricultura e Fome da FAO (do inglês, *Food and Agricultural Organization Statistical*);

ISR – Indução de Resistência Sistêmica (do inglês, *Induced Systemic Resistance*);

PAL - Fenilalanina Amônia Liase;

PGPF – Fungos Promotores de Crescimento Vegetal (do inglês, *Plant Growth Promoting Fungi*);

PGPR – Rizobactérias Promotoras de Crescimento Vegetal (do inglês, *Plant Growth Promoting Rhizobacteria*);

POX – Peroxidase;

PPO - Polifenol Oxidase;

QS - *Quorum sensing*;

RH – Resposta de Hipersensibilidade;

SAR – Resistência Sistêmica Adquirida (do inglês, *Systemic Acquired Resistance*);

VOC – Compostos Orgânicos Voláteis (do inglês, *Volatile Organic Compounds*).

SUMÁRIO

Capítulo I

Introdução e Objetivos

1. INTRODUÇÃO

1.1 Rizobactérias Promotoras de Crescimento Vegetal

Muitos gêneros de microrganismos têm sido descritos como componentes vitais para o solo e saúde das plantas por auxiliar em processos como extração de nutrientes do solo, produção de reguladores de crescimento e mesmo proteção contra fitopatógenos pelo controle ou inibição destes. Dentre eles, destacam-se as rizobactérias, assim chamadas por viverem na rizosfera e em associação com raízes (Bakker et al. 2013). Tais rizobactérias apresentam efeito benéfico no desenvolvimento vegetal e, por isso, têm sido denominadas rizobactérias promotoras de crescimento vegetal ou PGPR (Kloepper et al. 1980; Ahemad & Kibret 2014), as quais são caracterizadas por, pelo menos, dois entre os três critérios a seguir: capacidade de colonizar a superfície de raízes das plantas, promover o crescimento e reduzir a incidência de doenças (Haas et al. 2005; Reddy 2013).

As PGPR alteram a comunidade microbiana da rizosfera e são bem sucedidas em competir com outros microrganismos por nutrientes ou nicho nas raízes (Reddy 2013; Ahemad & Kibret 2014). Os efeitos promotores de crescimento de PGPR, bem como o uso para o controle de doenças, têm sido amplamente discutidos, como por exemplo em *Pseudomonas* (Ahn et al. 2011; Ramos-Solano et al. 2014) e *Bacillus* (Chowdappa et al. 2013; Santiago et al. 2015; Niu et al. 2011). Assim, o uso de PGPR com a finalidade de aumentar a produção agrícola se tornou hoje uma alternativa promissora e PGPR tem sido alvo de interesse em pesquisa para obtenção de formulações para a fabricação de produtos comerciais (Reddy 2013; Walia et al. 2013).

Baseado no mecanismo de ação de PGPR, estas podem ser categorizadas em três formas gerais: biofertilizantes, fito-estimuladoras e biopesticidas (Bhattacharyya & Jha 2012). Investigações recentes mostraram que a promoção de crescimento pode ocorrer de maneira direta, facilitando a absorção de nutrientes pelas plantas (Ahemad & Kibret 2014; Reddy 2013), através de mecanismos como (i) fixação de nitrogênio, nutriente vital para o crescimento de plantas e que se encontra na atmosfera, em sua maioria, sob forma N_2, a qual é indisponível para os vegetais (Ahn et al. 2011; Nihorimbere et al. 2011); (ii) solubilização de fosfato, o qual encontra-se sob forma insolúvel no solo, não podendo ser absorvido pelas plantas (Jog et al. 2014; Mehta et al. 2014; Ramírez et al. 2015); (iii) produção de hormônios como o ácido indolacético (AIA), auxina envolvida no

alongamento celular (Bhattacharyya & Jha 2012; Gusain et al. 2015); (iv) habilidade de produzir sideróforos, quelantes de ferro, tornando este elemento indisponível para outros microrganismos da rizosfera (potenciais patógenos) e disponível para as plantas; os sideróforos podem ainda formar complexos com outros metais do solo e aliviar o estresse imposto à planta por altos níveis de metais pesados (Lee et al. 2012; Lakshmanan et al. 2015); (v) habilidade em reduzir os níveis endógenos de etileno produzido pela planta sob estresse biótico ou abiótico, através da enzima 1-aminociclopropano-1-carboxilato (ACC) desaminase, conferindo resistência a inúmeros estresses (Glick 2005; Glick 2014; Matsuoka et al. 2015).

A promoção de crescimento pode ainda ocorrer por mecanismos indiretos (Ahemad & Kibret 2014; Reddy 2013), como (i) habilidade de PGPR em sintetizar metabólitos com propriedades antibióticas ou antifúngicas, além de enzimas degradadoras de parede celular de fungos (Bhattacharyya & Jha 2012; Lin et al. 2014); (ii) interferência na sinalização de *quorum sensing* (QS), inibindo a formação de biofilme de bactérias fitopatogênicas presentes na rizosfera (Reddy 2014; Bhattacharyya & Jha 2012) e (iii) produção de compostos orgânicos voláteis (VOC), compostos de baixo peso molecular contendo carbono que evaporam facilmente à temperatura e pressão normais e podem se difundir através da atmosfera e do solo, sendo, capazes de induzir resistência contra patógenos biotróficos e necrotróficos e de promover simbiose entre a planta e outros microrganismos benéficos (Bitas et al. 2013; Farag et al. 2013; Wang et al. 2013a).

1.2 *Streptomyces* spp.

Streptomyces (actinobactérias) constituem um grupo de bactérias Gram-positivas, geralmente encontradas no solo, totalizando aproximadamente 10% do total da microbiota do solo (Tarkka et al. 2008; Schrey & Tarkka 2008). Alguns membros desse gênero são considerados exemplos de PGPR, pois apresentam, dentre outras características, produção de AIA (Salla et al. 2014), de proteases extracelulares (Palaniyandi et al. 2013b), de antibióticos (Palaniyandi et al. 2013a), VOC (Li et al. 2012; Wang et al. 2013b), de sideróforos e capacidade de solubilização de fosfato (Oliveira et al. 2010).

A promoção do crescimento por *Streptomyces* spp. tem sido reportada em algumas espécies como tomate e trigo (El-Tarabily 2008; Sadeghi et al. 2012). Além disso, Dalmas e cols. (2011) avaliaram o efeito de três isolados de *Streptomyces* spp. (PM1, PM4 e PM9) como promotores de crescimento e moduladores do metabolismo secundário de plântulas de *Araucaria angustifolia* (pinheiro do Paraná). Neste estudo, foi observado que todos os isolados foram capazes de produzir auxina e demonstraram competência em crescer e se desenvolver na superfície das raízes (Dalmas et al. 2011). Baseado neste estudo, Salla e cols. (2014) utilizaram isolados de *Streptomyces* spp. como indutores de crescimento e moduladores do metabolismo secundário de plantas de *Eucalyptus* spp. *in vitro*. Os resultados indicaram haver uma resposta sistêmica na modulação das enzimas relacionadas à defesa vegetal, sugerindo a utilização destes microrganismos como agentes de biocontrole (Salla et al. 2014). Posteriormente, plantas de *Eucalyptus* spp. desafiadas com o fungo patogênico *Botrytis cinerea*, após pré-tratamento com o isolado PM9 de *Streptomyces* spp., apresentaram menor incidência de doença em relação às plantas não tratadas, assim como maior atividade de enzimas relacionadas à defesa vegetal (Salla et al. 2016). A capacidade reportada de espécies de *Streptomyces* em promover o crescimento e modular o metabolismo de plantas sugere que mecanismos semelhantes possam ser detectados em *Solanum lycopersicum*, bem como a possibilidade de utilização de um destes isolados no controle biológico do patógeno *Pectobacterium carotovorum* subsp. *brasiliensis.*

1.3 *Solanum lycopersicum* (L.)

O tomateiro é uma planta dicotiledônea, pertencente à família Solanaceae, que varia de 1,2 a 2,5 m de comprimento, podendo chegar a 10 m em um ano e cujos frutos, dependendo da cultivar, podem atingir a massa média de 750 g. Originário da região Andina, incluindo Peru, Equador, Colômbia, Bolívia até o norte do Chile, o tomate foi domesticado e cultivado no México, sendo incorporado à cultura Asteca e de onde foi levado para a Europa pelos colonizadores espanhóis e portugueses (Alvarenga 2013; Graça 2013). No século XVIII, o tomate já era largamente consumido em vários países europeus e hoje, com a sua distribuição e consumo global, é considerado uma das mais importantes culturas hortícolas, com uma produção mundial de mais de 160 milhões de toneladas, a segunda em importância econômica no Brasil, cuja produção está entre os

dez países maiores produtores (Herman & Williams 2012; Vos et al. 2014; FAOSTAT 2013; FAOSTAT 2012).

No entanto, o sistema de produção do tomateiro tem apresentado limitações decorrentes da utilização de cultivares suscetíveis a doenças e pragas, responsáveis pelo excessivo uso de agrotóxicos, os quais geram danos ao meio ambiente e ao homem (Oliveira et al. 2010). Levantamentos efetuados pela ANVISA nesta cultura mostravam em 2002, que frutos de tomate apresentavam alto nível de resíduos, com valores acima dos permitidos pela legislação (MAPA 2008). Atualmente, o tomate ainda se encontra entre as hortaliças com o maior teor residual de agrotóxicos (ANVISA 2014).

O desenvolvimento de cultivares que atendam às diversas demandas de mercado tem crescido, assim como o uso de agroquímicos em lavouras. Porém, a consolidação de novas tecnologias como a utilização de microrganismos benéficos no controle biológico de doenças poderá contribuir para uma mudança neste cenário.

1.4 *Pectobacterium* spp. e a doença Talo Oco

A bactéria patogênica *P. carotovorum* apresenta-se como bacilos Gram-negativos, anaeróbias facultativas, com movimentação por meio de flagelos peritríqueos, formando colônias amarelas e mostra grande variação na patogenicidade. Temperaturas entre 25 e 30 ºC e umidade relativa acima de 90% são condições favoráveis ao seu desenvolvimento (Alvarenga 2013; Silva & Giordano 2000).

O gênero *Pectobacterium* consiste em linhagens de bactérias necrotróficas, que atacam os tecidos da planta através da secreção de fatores de virulência conhecidos como efetores (proteínas que induzem morte celular) e enzimas que degradam a parede das células vegetais (Lee et al. 2014; Hogan et al. 2013; Cui et al. 2015).

A subespécie *P. carotovorum brasiliensis* foi proposta por Duarte e cols. (2004), após análise de isolados de bactérias que causavam a doença "canela preta" em batata (*S. tuberosum* L.), no estado do Rio Grande do Sul, Brasil. Foi observado que estes isolados apresentavam características moleculares que os diferenciava de outras subespécies de *P. carotovorum* e que os mesmos apresentavam maior virulência que as subespécies até então conhecidas (Duarte et al. 2004). Desde então, algumas pesquisas vêm mencionando

P. carotovorum subsp. *brasiliensis* como um dos principais patógenos causadores de podridões no Brasil (Nabhan et al. 2012) e em países como África do Sul (van der Merwe et al. 2010), Canadá (De Boer et al. 2012), Coréia (Lee et al. 2014), Nova Zelândia (Panda et al. 2012), Quênia (Onkendi & Moleleki 2014) e Holanda (Leite et al. 2014).

A cultura de *S. licopersycum* é afetada por diversas doenças, o que leva a perdas substanciais de produção. Dentre as mais recorrentes destaca-se a podridão mole, responsável pelo apodrecimento dos órgãos suculentos da planta a partir de ferimentos no vegetal e provocada por espécies de bactérias pectolíticas dos gêneros *Pectobacterium* e *Dickeya* (Carvalho et al. 2014). A infecção no caule (Talo Oco) resulta em aparência externamente encharcada e enegrecida (Figura 1A), enquanto que os frutos geralmente morrem precocemente no início da colheita e ainda podem ficar presos à planta, como se fossem bolsas d'água (Podridão Mole - Figura 1B). A doença acentua-se a partir da frutificação (Alvarenga 2013; Carvalho et al. 2014).

Figura 1 –Sintoma na haste decorrente da destruição da medula da planta – Talo Oco (A). Apodrecimento de frutos – Podridão Mole (B). Adaptado de Carvalho et al. 2014.

1.5 Mecanismos de defesa vegetal

Por causa da natureza séssil das plantas, um sistema imune sensível a sinais de estresse e a transdução destes sinais em uma resposta de defesa apropriada é crucial para a adaptação e sobrevivência (Buscaill & Rivas 2014). Plantas não possuem células móveis e um sistema imune adaptativo, mas contam com a imunidade inata de cada célula e sinais sistêmicos que se deslocam a partir dos locais de infecção (Spoel & Dong 2012; Jones & Dangl 2006).

As plantas estão em constante contato com microrganismos potencialmente patogênicos. Globalmente, 10 a 30% do potencial de colheitas são perdidos como consequência do ataque de fitopatógenos e essas perdas podem chegar a um número maior em casos de surtos de doenças. No entanto, a doença é exceção e não a regra, pois comumente as plantas são resistentes à maioria das infecções dos patógenos (Herman & Williams 2012). Elas utilizam estratégias de defesa que podem ser constitutivas ou induzidas. A resistência constitutiva envolve barreiras estruturais, metabólitos secundários pré-formados e enzimas líticas, enquanto que a resistência induzida é desencadeada pelo contato com o patógeno (Dangl et al. 2013; Smith et al. 2014). De forma geral, quando a barreira física não é suficiente para impedir um patógeno, a planta desencadeia processos que iniciam pelo reconhecimento do microrganismo, emissão de um sinal primário ou mensageiro que irá desencadear uma série de outros sinais e por fim, ativam genes ligados à defesa ou ao aumento de atividade de enzimas importantes para reações de defesa (Buscaill & Rivas 2014).

Quando a planta é atacada por um patógeno, processos metabólicos são desencadeados, proporcionando a defesa. O aumento da resistência basal potencializa as respostas inatas contra vários patógenos, herbívoros e estresses abióticos (Lucas et al. 2014; Burketová et al. 2015). O aparecimento de respostas locais, como resposta de hipersensibilidade, pode induzir a resistência sistêmica adquirida (SAR) e esta, através da geração de sinais móveis, induz o acúmulo do hormônio de defesa ácido salicílico (AS) e secreção de proteínas PR, conferindo resistência de forma sistêmica a uma ampla gama de patógenos. Esse fenômeno é observado quando a mesma planta é atacada uma segunda vez (Fu & Dong 2013). Esta resposta foi descrita na década de 1960, quando folhas não infectadas de uma planta infectada por vírus apresentaram resistência a subsequentes infecções virais (Herman & Williams 2012). Por outro lado, a indução de resistência

sistêmica (ISR), diferentemente de SAR, não apresenta respostas locais como RH e parece não estar relacionada ao AS ou envolver proteínas PR, sugerindo haver outra rota de sinalização mais associada ao jasmonato e ao etileno. Pode ser induzida por indutores abióticos, porém é mais conhecida por envolver a participação de microrganismos não patogênicos (Kloepper et al. 1992).

Van Loon e cols. (1998) definiram a ISR como um estado de maior capacidade de defesa desenvolvida pela planta quando adequadamente estimulada, através da ativação de mecanismos de resistência latentes induzidos por diversos agentes, incluindo rizobabactérias (van Loon et al. 1998). O início de ISR requer microrganismos benéficos que eficientemente colonizem o sistema radicular das plantas hospedeiras. Para o estabelecimento de uma associação mutualista de sucesso, plantas e microrganismos precisam responder a sinais reciprocamente e, assim, priorizar suas respostas, de modo a desenvolver um estilo de vida que proporcione benefícios mútuos. Microrganismos como PGPR e fungos promotores de crescimento vegetal (PGPF) atuam na ISR, e acredita-se que um diálogo molecular também é essencial para estas interações (Pieterse et al. 2014; Burketová et al. 2015).

Em plantas de tomate, muitos PGPR e PGPF têm sido explorados quanto à capacidade de induzir resistência sistêmica, *Bacillus pumilis* (Kurabachew & Wydra 2014; Kurabachew et al. 2013), *B. subtilis* e *Trichoderma harzianum* (Chowdappa et al. 2013; Chen et al. 2013), *T. asperellum* (Fernández et al. 2014), *T. virens* e *T. atroviride* (Salas-Marina et al. 2015), *B. thuringiensis* (Hyakumachi et al. 2013), *Streptomyces griseus* subsp. *griseus* (Sousa et al. 2006), *Pseudomonas putita*, *Serratia marcescens* e *B. cereus* são alguns exemplos (Kurabachew & Wydra 2013).

A indução de respostas de defesa, inclui a ativação de genes promove a síntese e acúmulo de produtos do metabolismo secundário, como compostos fenólicos, macromoléculas estruturais como calose e lignina, proteínas relacionadas à patogênese, inibidoras de enzimas e enzimas hidrolíticas (Boller & Meins 2012). Isto resulta em mudanças na atividade de enzimas chaves do metabolismo, como a fenilalanina amônia-liase (PAL), polifenoloxidase (PPO) e peroxidases (POX). O aumento na atividade destas enzimas tem sido relatado como um fator importante para a indução de resistência contra *Ralstonia solanacearum*, *Alternaria solani* e *Fusarium oxysporum* em tomate (Kurabachew & Wydra 2014; Mandal et al. 2009; Mandal et al. 2013; Song et al. 2011).

A atividade da enzima PAL, dentre as diversas vias metabólicas envolvidas nas respostas de defesa vegetal, está intimamente relacionada à síntese de compostos de defesa. Ela é responsável por catalisar a conversão de fenilalanina em ácido cinâmico, além de proporcionar a formação da maior parte dos compostos fenólicos vegetais. O produto da PAL, ácido cinâmico, está diretamente ligado a processos de lignificação e os mais altos níveis de atividade da PAL geralmente ocorrem cerca de 24 horas após a infecção inicial, sendo importante também na biossíntese de AS. Sua atividade é altamente induzida durante a interação planta-patógeno e a inibição desta enzima resulta no desenvolvimento de doença (Mauch-Mani & Slusarenko 1996; Montesinos 2000; Silva et al. 2004).

As PPOs catalisam duas reações distintas, mediadas pelas enzimas monofenolase que catalisa a hidroxilação de monofenóis para difenóis e pela difenolase, responsável pela oxidação de difenóis a quinonas (Núñez-Delicado et al. 2005; Webb et al. 2013). A oxidação de compostos fenólicos a quinonas geralmente proporciona resistência contra doenças, pelo fato de quinonas serem frequentemente mais tóxicas aos microrganismos que os compostos fenólicos originais (Mayer & Staples 2002). Portanto, o aumento na atividade dessas enzimas resulta em altas concentrações de compostos antimicrobianos, contribuindo para a resistência a infecções (Quiroga et al. 2000).

Outro grupo de enzimas são as peroxidases (POX), que catalisam a reação de oxidação de compostos fenólicos através da utilização de peróxido de hidrogênio (H_2O_2) como doador de elétrons; estão relacionadas ao processo de proteção oxidativa, como a oxidação do ácido indol-3- acético (AIA), a biossíntese de etileno, a cicatrização de ferimentos e a regulação do alongamento das células (crescimento e senescência), além de também promover a síntese de lignina. Quando as células são atacadas por microrganismos patogênicos, as POX promovem o aumento da síntese de lignina, que fortalece a parede celular contra enzimas líticas, levando ao aumento da resistência da planta (Kvaratskhelia et al. 1997; Zámocký et al. 2001).

2. JUSTIFICATIVA

O controle de doenças de plantas tem dependido de grandes quantidades de agroquímicos e os métodos tradicionais de produção têm causado grandes problemas

ambientais e de saúde. Um grande desafio do século XXI é a produção de alimentos de forma sustentável e ecologicamente dirigida, visto que o aumento da produção de alimentos é necessário para suprir a demanda de uma população em crescimento (Berg 2009).

Métodos alternativos de controle de doenças são desejáveis devido ao aumento da demanda de produtos seguros para agricultura, ao surgimento de resistência à fungicida e aos problemas ambientais decorrentes da aplicação de agrotóxicos. Na última década, o uso de PGPR para o biocontrole de patógenos em plantas cultivadas tem sido amplamente estudado, por ser uma estratégia para auxiliar a superação da maioria dos problemas associados aos métodos de controle químico (An et al. 2010; Marcuzzo 2010).

A associação das rizobactérias com as plantas e seu antagonismo contra microrganismos patogênicos sugerem sua aplicação na redução das quantidades de agrotóxicos utilizados na agricultura e abre novas perspectivas para práticas alternativas de manejo de lavouras, menos agressivas e danosas ao meio ambiente e aos seres humanos (Palaniyandi et al. 2013a; Whipps 2001).

Na comparação com os pesticidas e fertilizantes químicos/sintetizados, os inoculantes microbianos apresentam inúmeras vantagens, tais como; (i) maior segurança, pois apresentam danos ambientais reduzidos e riscos potencialmente menores para a saúde humana, (ii) apresentam a atividade muito mais específica, (iii) são eficazes em pequenas quantidades, (iv) multiplicam-se, mas são controlados pela planta, bem como pelas populações microbianas nativas, (v) se decompõem mais rapidamente do que pesticidas químicos convencionais, (vi) o desenvolvimento de resistência é reduzido devido a vários mecanismos e (vii) podem ser também usados nos sistemas de manejo convencional ou integrado de pragas (Berg 2009).

Contudo, os mecanismos envolvidos nas respostas celulares de interação de plantas com microrganismos, patogênicos ou não, ainda precisam ser estudados. Desta forma, um estudo que associe o conhecimento de possíveis indutores de crescimento e de respostas celulares resultantes da interação com PGPR representa um avanço para o desenvolvimento de formulações comerciais de baixa toxidade para uso agronômico.

3. HIPÓTESES

1 Os isolados PM1, PM3, PM4, PM5, PM6 e PM9 de *Streptomyces* spp. apresentam características de PGPR.

2 Os isolados de *Streptomyces* spp. promovem o crescimento e modulam o metabolismo secundário de plantas de *S. lycopersicum.*

3 O pré-tratamento com isolados de *Streptomyces* spp. induz resistência em tomateiros.

4. OBJETIVOS

4.1 Objetivo geral

Avaliar isolados de rizobactérias *Streptomyces* spp. como promotores de crescimento e moduladores de mecanismos envolvidos no metabolismo de resistência à bactéria necrotrófica *Pectobacterium carotovorum* subsp. *brasiliensis* em *Solanum lycopersicum*, assim como determinar a atividade antimicrobiana destes isolados sobre o fitopatógeno.

4.2 Objetivos específicos

1. Caracterizar bioquimicamente os isolados de *Streptomyces* spp., PM1, PM3, PM4, PM5, PM6 e PM9.
2. Determinar o potencial de antagonismo dos isolados de *Streptomyces* spp. contra *P. carotovorum* subsp. *brasiliensis*, através de contato direto ou por possíveis compostos voláteis.
3. Determinar a promoção do crescimento de plantas de tomateiro pelos isolados de *Streptomyces* spp., selecionando três isolados (promotor, intermediário e não promotor do crescimento vegetal).
4. Avaliar o metabolismo bioquímico de plantas tratadas com três isolados selecionados de *Streptomyces* spp., através da análise das enzimas de defesa, bem como o de acúmulo de compostos fenólicos.
5. Avaliar a possível resistência de plantas de tomateiro pré-tratadas com *Streptomyces* spp. e desafiadas com a bactéria necrotrófica *P. carotovorum* subsp. *brasiliensis*.
6. Identificar isolados de *Streptomyces* spp. com potencial uso em formulações como promotoras de crescimento/indutores de defesa vegetal.

Capítulo II

Manuscrito a ser submetido:

Plant growth and resistance promoted by *Streptomyces* spp. in tomato

M. P. Dias, M. B. Scherer, V. B. Xavier, E. Cassel, L. V. Astarita, E. R. Santarém

Manuscrito a ser submetido
para a revista Plant
Physiology and Biochemistry
(Fator de impacto 2,928).

Guia para autores:
https://www.elsevier.com/wps/find/journaldescription.cws_home/600784?generatepdf=true

Nota: Os autores optaram por incluir Tabelas e Figuras ao longo do texto para facilitar a leitura e interpretação pelos avaliadores da dissertação. No entanto, no momento da submissão do artigo, as normas serão seguidas e as tabelas e figuras serão apresentadas separadamente.

Plant growth and resistance promoted by *Streptomyces* spp. in tomato

Maila P. Dias[1], Matheus S. Bastos[1], Vanessa B. Xavier[2], Eduardo Cassel[2], Leandro V. Astarita[1], Eliane R. Santarém[1]

[1]Laboratório de Biotecnologia Vegetal, Faculdade de Biociências; Pontifícia Universidade Católica do Rio Grande do Sul, Av. Ipiranga 6681, Porto Alegre, RS 90619-900, Brazil

[2]Laboratório de Operações Unitárias, Faculdade de Engenharia, Pontifícia Universidade Católica do Rio Grande do Sul, Av. Ipiranga 6681, Porto Alegre, RS 90619-900, Brazil

∗Corresponding author

E.R. Santarém

Tel.: +55 51 3353 4148; fax: +55 51 3320 3568. E-mail address: esantarem@pucrs.br

Abstract

Streptomyces spp. have been recognized as Plant Growth Promoting Rhizobacteria (PGPR) and as agents of biocontrol. This study characterized the *Streptomyces* isolates as PGPR, determined the antagonism against *Pectobacterium carotovorum* subsp. *brasiliensis* (Pcb), and evaluated the ability of *Streptomyces* on promoting growth and modulating the defense-related metabolism of tomato plants. Antagonism of *Streptomyces* spp. against Pcb was determined by dual-culture method and partitioned plate (I-plate) for VOC effect analysis. Plant growth promotion was assessed through VOC emission and by direct interaction with *Streptomyces* spp. isolates. Enzymes related to plant defense were analyzed in plants treated with isolates of *Streptomyces* spp. Evaluation of soft rot disease was performed on plants treated with *Streptomyces* spp. and challenged with Pcb through the area under the disease progression curve and plant mortality. *Streptomyces* spp. isolates displayed characteristics of PGPR. PM3 was the isolate showing efficient antagonism against Pcb by dual-culture. Most of the isolates promoted increase of root and shoot length of tomato plants by VOC, although PM5 also promoted growth by direct interaction with *Streptomyces* spp. Treatment with *Streptomyces* spp. modulated the activity of defense-related enzymes and decrease incidence of soft rot disease. PM5 was able to promote growth of tomato plants, although decreased incidence and mortality of plants were obtained with PM1, indicating that both strains could be used as PGPR.

Keywords: Actinomycetes**,** Antagonism, PGPR, Phenolics compounds, *Solanum lycopersicum*, VOC

1. Introduction

Rhizobacteria, so called due to their association with roots in the rhizosphere, play a very important role on plant growth promotion and protection by controlling or inhibiting pathogens (Bakker et al., 2013). Due to the beneficial effect that many rhizobacteria have on plant development, they have been termed plant growth-promoting rhizobacteria or PGPR (Ahemad and Kibret, 2014), which are characterized by at least two of the three following criteria: competitively root colonization, stimulation of growth and reduction of disease incidence (Haas et al., 2005; Reddy, 2013). PGPR effects in promoting plant growth and disease biocontrol have been widely reported for bacteria, such as *Pseudomonas* and *Bacillus* (Chowdappa et al., 2013; Ramos-Solano et al., 2014; Walia et al., 2014). In recent years, the use of PGPR has become an attractive tool for crop disease management, being considered as a component for sustainable agriculture (Gopalakrishnan et al., 2015).

Studies on PGPR revealed that growth promotion might occur directly, by facilitating the absorption of nutrients by plants such as nitrogen fixation, phosphate solubilization, and siderophore production (Reddy, 2013; Ahemad and Kibret, 2014). Moreover, production of hormones such as IAA (indoleacetic acid) and the ability to reduce endogenous levels of ethylene produced by plant, through of the enzyme ACC-deaminase (1-aminocyclopropane-1-carboxylate deaminase), are also attributes related to promotion of plant growth (Glick, 2014; Mehta et al., 2015). On the other hand, indirect mechanisms of stimulating growth mediated by PGPR are based on the reduction or complete elimination of the harmful effect of pathogenic organisms. Usually, PGPR modify the rhizospheric environment by producing antagonistic molecules with antibiotic or antifungal properties, or by synthesizing cell walls-degrading enzymes and volatile organic compounds (VOC), which act against pathogens, disrupting bacterial cell–cell communication (*quorum sensing*) (Grobelak et al., 2015; Kanchiswamy et al., 2015). PGPR may also be capable of inducing systemic resistance (ISR) against biotrophic and necrotrophic pathogens. This mechanism is dependent on jasmonic acid and ethylene signaling in the plant (Pieterse et al., 2014) and result in faster and/or stronger activation of defense responses when plant is subsequently challenged by microbes, insects, or abiotic stress. This response is frequently associated to development of local and systemic immunity (Lucas et al., 2014).

When attacked by a pathogen, plants respond with complex changes in the metabolism. Besides the synthesis of pathogenesis-related proteins, defense enzymes and inhibitors of hydrolytic enzymes (Coll et al., 2011), the activation of synthesis, accumulation or oxidation of secondary metabolic products such as phenolic compounds has been reported (Lavania et al., 2006; Salla et al., 2016). PGPRs are also known to induce host resistance through activation of the phenylpropanoid pathway. Alterations on the activity of key metabolic enzymes, such as phenylalanine ammonia lyase (PAL), polyphenoloxidase (PPO) and peroxidases (POX) have been reported as an important factor in the induction of resistance against *Ralstonia solanacearum, Alternaria solani* and *Fusarium oxysporum* (Song et al., 2011; Mandal et al., 2013; Kurabachew and Wydra, 2014). Although induction of defense enzymes in pathogen–host interactions has been studied, the ability of PGPR on stimulating the activity of defense enzymes and host defense–related compounds is little explored.

Streptomyces (actinomycetes) are a group of Gram-positive bacteria, usually found in soil, comprising approximately 10% of the total soil microbes (Tarkka et al., 2008). Some members of this genus

are considered PGPR since they are capable of producing IAA (Salla et al., 2014), extracellular proteases (Palaniyandi et al., 2013a), antibiotics (Palaniyandi et al., 2013b), VOC (Li et al., 2012; Wang et al., 2013), siderophores as well as solubilizing phosphate (Jog et al., 2014; Lakshmanan et al., 2015). Plant growth promotion by *Streptomyces* spp. has been reported in some species such as tomato, wheat, rice, chickpea and eucalypt (El-Tarabily, 2008; Sadeghi et al., 2012; Gopalakrishnan et al., 2014; Salla et al., 2014; Gopalakrishnan et al., 2015).

Tomato (*Solanum lycopersicum* L.) belongs to the *Solanaceae* family and it has been considered one of the most important horticultural crop worldwide (Vos et al., 2014). However, tomato production has shown limitations arising from the use of cultivars susceptible to diseases and pests causing substantial production losses. The high incidence of disease in this species is responsible for excessive use of pesticides, which generate damage to the environment and man (De Oliveira et al., 2010). Soft rot of tomato caused by species of pectolytic bacteria from *Pectobacterium* and *Dickeya* genera is a serious disease. Pectolytic enzymes produced by these bacteria destroy the middle lamella in the plant infected site (Carvalho et al., 2014).

Overall, 10 to 30% of the potential crop is lost as a consequence of the attack of plant pathogens and these losses can reach a greater number of cases of disease outbreaks and are mostly controlled by the use of agrochemicals (Herman and Williams, 2012). As an alternative for an environment-friendly approach, the use of PGPRs became a tool for disease management. In addition, *Streptomyces* spp. and other actinobacteria are surprisingly under explored for plant-growth promotion, as compared to *Pseudomonas* spp. or *Bacillus* spp. (Doumbou et al., 2001). Thus, the aims of the present study were (i) to characterize the *Streptomyces* spp. isolates as PGPR, (ii) to determine the antagonism against *Pectobacterium carotovorum* subsp. *brasiliensis*, (iii) to determine the ability of *Streptomyces* spp. on promoting growth of tomato (*S. lycopersicum*) plants and (iv) to evaluate the modulation of the defense-related metabolism of tomato plants when treated with *Streptomyces* spp. The potential effect of *Streptomyces* spp. on reducing soft rot disease in tomato plants was also evaluated.

2. Material and Methods

2.1. Culture of microorganisms

Six isolates of rhizobacteria *Streptomyces* spp. (PM1, PM3, PM4, PM5, PM6 and PM9) were previously isolated from soil of Araucaria Forest at São Francisco de Paula, Rio Grande do Sul, Brazil (29°29'18.4''S, 50°12'23.5''W), and taxonomically identified by amplification and partial sequencing of theirs 16S rDNA genes (Dalmas et al., 2011; Salla et al., 2014). Isolates, stored at -80 °C with 20% glycerol, were grown in ISP_4 liquid medium (Shirling and Gottlieb, 1966), under agitation at 100 rpm for 7 days at 26 ± 2 °C (stationary phase). Each suspension was centrifuged (2,500 *g*, 10 min, room temperature), resuspended in sterile distilled water, and adjusted to a final concentration of 10^7-10^8 CFU mL^{-1} (OD_{600nm}= 1) for use as an inoculum for the assays. When blocks of medium containing rhizobacteria were required, *Streptomyces* spp. isolates were cultured on ISP_4 semi-solid medium for 7 to 10 days at 28 ± 2 °C.

Pectobacterium carotovorum subsp. *brasiliensis* (therein named Pcb) was grown in liquid Luria-Bertani medium at 26 ± 2 °C for 24 h at 100 rpm. Cultures were centrifuged at 2,500 *g* for 10 min at room

temperature. The supernatant was discarded and the pellet was washed three times in sterile distilled water. Final bacterial concentration was adjusted to 10^8-10^9 CFU mL^{-1} (OD_{600nm}= 0.5 and 1, respectively).

2.2. Biochemical characterization of *Streptomyces* isolates

Biochemical characteristics of the isolates such as siderophore production (Lakshmanan et al. 2015), phosphate (P) solubilization (Lucas et al., 2014), as well as amylase and lipase activities (Hankin and Anagnostakis, 1975; Jesus et al., 2013) were determined following the standard procedures with some modifications. Briefly, for the assessment of siderophore production, each isolate was previously grown in Fe-deficient medium and then cultivated according the cup plate method (5 mm-well; Dingle et al., 1953) using CAS (Chrome Azurol S) agar-LB plates. The change of the medium color from bluish to yellowish-orange after incubation indicates the presence of siderophores. Sterile distilled water was used as negative control; as positive control, a 1M pyrocatechol solution was used. Measurements were taken considering the width of the halo border (cm) after 6 days of culture. The assay for P-solubilization was performed by inoculating *Streptomyces* spp. on potato dextrose agar (PDA) supplemented with yeast extract (PDYA, pH 7.0), containing calcium chloride (50 ml K_2HPO_4 10% and 100 ml $CaCl_2$ 10% added in 1 L of sterile PDYA). Each isolate was inoculated onto the PDYA plate, using a disc of sterile filter paper (1 cm) previously scraped on a 7-day grown semi-solid culture. The appearance of clearing zones surrounding the discs indicates tricalcium phosphate solubilization by the bacterial isolates. The capacity of P-solubilization was scored by the width of the halo border (cm) after 14 days of cultivation. The ability to hydrolyze starch was determined by the appearance of a clear halo around the colony on nutrient agar plates containing soluble 1% starch. After the addition of lugol 4% on the plate, the presence of starch was indicated by the development of a blue color and therefore, a clear halo indicates starch hydrolysis. Detection of lipase activity was carried out in minimal medium supplemented with 1% Tween 20 (v/v). The cup plate method was used. After 4 days of cultivation, plates were kept at 4 ˚C for 48 h in order to detect the degradation halo. All biochemical analyses were carried out at 28 ± 2 °C, in at least three replicates, and results were considered positives when the halo border around either well or colony was larger than 2 mm. Data were expressed as mean of the halo zone ± standard error.

2.3. Collection and analysis of VOC by GC/MS

The VOC produced by *Streptomyces* spp. isolates were collected by the headspace solid-phase microextraction (HS-SPME) technique (Gu et al., 2007) from 7-day old cultures of *Streptomyces* spp. A SPME fiber coated with divinylbenzene-carboxene-PDMS (DCP, 50/30 mm) was used for extracting the VOC. The gas chromatograph (Agilent 7890A) was equipped with mass spectrometer (GC/ME). A HP-5MS fused silica capillary column (30 m x 0.25 mm i.d., 0.25 µm-thick) was used to separate the volatiles. Helium was used as the carrier gas at 0.8 mL min^{-1}. The temperature for the volatile-separation column was programmed as follows: set at 40 °C for 3 min at the beginning, increased to 150 °C at 4 °C min^{-1} held at 150 °C for 1 min, and further increased to 250 °C at 8 °C min^{-1} held at 250 °C for 2 min. The injector temperature was maintained at 250 °C (Wang et al., 2013). Compounds were identified by comparing their

retention indices (RI) determined relative to a homologous series of pure n-alkane standards or values reported in the literature (Jennings and Shibamoto, 1980). Fragmentation patterns in the mass spectra were also compared with those available in the Library of the National Institute of Standards and Technology (NIST05).

2.4. Antagonism between *Streptomyces* spp. and *P. carotovorum brasiliensis*

Rhizobacterial isolates were tested for *in vitro* direct antagonistic activity against Pcb. Three agar blocks containing the *Streptomyces* spp. isolate were placed separately on a LB plate previously spread with Pcb (OD_{600nm}= 1). Two control treatments were used: (i) plates with agar blocks without *Streptomyces* spp. and (ii) LB plates with Pcb, as viability control. The dual-cultures were incubated at 28 ± 2 °C for 7 days, when evaluation of antibiosis was carried out. Halos of inhibition were measured and results were expressed as mean ± standard error.

The antagonism between *Streptomyces* spp. and Pcb via VOC was tested on two-section plastic Petri dishes, containing a center partition (I-plates, Fisher Scientific). *Streptomyces* spp. isolates were grown on ISP_4 medium in one half of the I-plate. After 7 days of cultivation, a suspension of Pcb (20 µL; 10^8 CFU mL^{-1}) was dropped onto a sterile paper disc, which was then rapidly placed on the other half of the I-plate and then removed. For the control, sterile distilled water was used. Plates were completely sealed and incubated at 28 ± 2 °C for 7 days. The growth of Pcb was assessed using the mean of horizontal and vertical axes of the bacterial growth (mm). The percentage of relative growth inhibition (GI) was calculated as follows (Wang et al. 2011).

$$GI\ (\%) = \frac{(\text{Diameter of negative control} - \text{Diameter of sample})}{\text{Diameter of negative control}} \times 100$$

and data were expressed as mean ± standard error.

2.5. Promotion of plant growth by VOC from *Streptomyces* spp.

Seeds of tomato (*S. lycopersicum* cv. Santa Cruz Kada; Isla Sementes, Brazil) were surface-disinfected and sown in Magenta™ boxes (Life Technologies) containing 50 mL of ½ MS medium (Murashige and Skoog, 1962). Fifteen days after sprouting, a block (1.5 cm) of agar containing each *Streptomyces* spp. isolate was placed in a dish (3 cm diameter), which was placed on medium suface on the Magenta™ box. The contact of *Streptomyces* either with the medium or with the plant was avoided. The control consisted of an agar block without rhizobacteria. After 15 days, growth was assessed using length, fresh and dry weight of shoots and roots and root volume of tomato plants. Roots and shoots were dried at 65 °C to constant weight to evaluate their dry matter. The contents of chlorophylls *a, b* and *total* were also analyzed at 645, 652 and 663 nm (Ni et al., 2009). Results were expressed as the ratio *chl a*/*chl b* and in mg of chlorophyll g^{-1} of fresh weight (FW) for *chl total*. Fourteen plants were used per treatment (PM1, PM3, PM4, PM5, PM6, PM9, and control).

2.6. Promotion of plant growth by direct interaction with *Streptomyces* spp. isolates

Tomato seeds were sown in a mixture of organic soil and sand (70:30 (v/v), in 13x20 cm pots and were kept in culture chamber at 26 ± 2 °C and photoperiod of 14 h until the development of the first leaf pair. Nutrient solution (10 mL of ¼ MS salts) was supplied to the plants every 15 days. Plants were inoculated with 5 mL aliquots of rhizobacteria suspension (PM1, PM3, PM4, PM5, PM6 or PM9; 10^7-10^8 CFU mL^{-1}) by wetting the substrate directly in contact with the roots. A second addition of rhizobacterial suspension (10 mL) was done after 21 days. The control plants were inoculated with water. Plants were irrigated every two days. Tomato plants were harvested at 30 and 45 days after the first *Steptomyces* spp. inoculation, and then assayed to determine length, fresh and dry weight of shoots and roots, leaf number and root volume, as well as chlorophyll contents. Each treatment consisted of 15 plants.

2.7. Modulation of secondary metabolism of tomato plants by *Streptomyces* spp.

Three *Streptomyces* spp. isolates were selected based on their characteristics of PGPR. The criteria of selection was the isolate that most promoted growth (PM5), one with intermediate effect (PM3) and one that had no effect on growth (PM1). The choice of isolates with different effects on growth was based on the hypothesis that rhizobacteria that does not promote growth might be effective on inducing metabolic changes that could be useful on defense against pathogens.

In order to evaluate the changes on the secondary metabolism mediated by *Streptomyces* spp., tomato plants (two true leaves) were inoculated with 10 mL of each selected isolates (10^7-10^8 CFU mL^{-1}) and grown as described above. The control treatment received only water. Specific activities of PAL (EC 4.3.1.24), PPO (EC 1.14.18.1) and POX (EC 1.11.17), as well as the levels of induced phenolic compounds were determined in shoots and roots separately. Before analyses, plants were washed under running tap water, blotted dry and frozen in liquid nitrogen. Colorimetric assays for enzymatic activities were done by spectrophotometer. Total protein concentration was determined according to Bradford's method (Bradford 1976), using bovine serum albumin as standard. Activity of enzymes was measured at 0, 1, 3 e 9 days post inoculation (dpi) of *Streptomyces* spp. isolates. Forty plants were used per time point of analysis per treatment (PM isolates or control).

For PAL activity, samples of plant material (250 mg) were ground in ice-cold Tris- HCL 50 mM buffer pH 8.8, supplemented with 1% (p/v) of polyvinylpyrrolidone (PVP), 1 mM EDTA pH 8.8 and 0.2% (v/v) of Triton X-100. The tissues extracts were centrifuged at 3,200 *g* for 20 min at 4 °C, and the supernatant was collected for the enzyme assays and for determination of the protein content. PAL activity was determined at 290 nm, in a reaction solution of 62.5 mM sodium borate buffer with addition of 20 mM L-phenylalanine. Absorbancies were read before and after incubation at 37 °C for 1 h in darkness. Calibration curve was established using *trans*-cinnamic acid and the enzyme activity was calculated as the amount of enzyme converting 1 nmol *trans*-cinnamic acid h^{-1}. The activities of the enzymes PPO and POX were determined according to Salla et al. (2016). Briefly, PPO activity was determined at 400 nm, using chlorogenic acid (1 mM) as substrate. Specific enzyme activity was defined as the change in absorbance min^{-1} mg^{-1} protein. For peroxidases, oxidation of guaiacol was measured by the increase in absorbance at

420 nm for 30 s at intervals of 5 s. Specific enzyme activity was calculated as µkatal mg^{-1} protein. For quantification of the total phenolic compounds, samples of shoots and roots (0.125 g of FW) of tomato plants were taken from each treatment and ground in 2.5 mL of 80% (v/v) methanol at room temperature. Extracts were centrifuged at 1,250 *g* for 15 min. Total phenolic compounds were analyzed in the supernatant by the colorimetric Folin-Ciocalteu method as described previously (Salla et al. 2016). Gallic acid was used as the standard. The contents of total phenolic compounds were calculated as mg g^{-1} of FW. Results were expressed as Relative activity of enzymes (%), calculated as [(Activity on the treatment – activity on the control)/ activity on the control] x 100. Same calculation was used to express the levels of phenolic compounds.

2.8. Evaluation of soft rot disease

Plants of tomato were cultivated in an *in vitro* system according to Salla et al. (2014), with modifications. Briefly, 25 mL of ½ MS medium was poured into a Petri dish (9 cm in diameter), and after the agar solidification, a semicircle of medium was discarded and one plant (approximately, 5 cm) was placed on the remaining medium semicircle. Plates were maintained at 26 ± 2 ˚C. Treatments consisted of (i) plants inoculated with sterile distilled water (absolute control); (ii) plants inoculated with Pcb on the stem (OD_{600nm}= 0.5); (iii) plants treated with *Streptomyces* spp. isolates (OD_{600nm}=1) and; (iv) plants treated with *Streptomyces* spp. isolates and challenged with Pcb. Inoculation of rhizobacterium was performed by adding 200 µL of each isolate of *Streptomyces* spp. suspension along the root surface. A swab wetted in Pcb suspension was used for challenging on the stem of the plant 4 days after the treatment with *Streptomyces* spp. Experiment was evaluated using 10 plants per treatment.

Disease incidence and development of soft rot symptoms were evaluated in the shoots. A scale of 0%, 50% and 100% was used, representing: 0% - no symptoms; 50% - plant with symptoms of hollow stem (thinning and browning of the stem), and 100%- wilting and yellowing of leaves. Data were collected from observations made every two-three days from the beginning of the experiment, until at least 80% of control plants showed 100% of wilting, i.e, 24 dpi of Pcb. Values of the area under the disease progress curve (AUDPC) were normalized and corrected (AUDPC-nc) by dividing the values by the number of days until the final incidence reading for each treatment, and multiplying the resulting values by the number of days until the final incidence evaluation (Graichen et al., 2010; Zambonato et al., 2012), as shown below:

$$\text{AUDPC-nc} = \{\{\Sigma[(y_{i+1} + y_i) \times 0.5]*[t_{i+1} - t_i]\}/n\}*c,$$

where yi = percentage of shoot affect by soft rot (severity at the i^{th} observation); ti = time (in days) after inoculation of Pcb at the i^{th} observation; n = number of days between the disease onset and the last disease assessment; c = longest period of epidemic duration among the plants evaluated. Disease severity was evaluated and expressed as percentage of plant mortality.

2.9. Statistical analysis

Experiments were performed in a fully randomized design, tested for variance homogeneity by Levene's test ($\alpha \leq 0.05$). Data from experiments of characterization of *Streptomyces* spp. as PGPR, antagonism against Pcb and disease progression, were subjected to one-way ANOVA and mean differences were determined by Duncan Test at a significance level of $\alpha \leq 0.05$. Results obtained from plant growth promoting experiments and modulation of secondary metabolism of tomato plants were analyzed by Student's *t*-Test ($\alpha \leq 0.05$). All statistical analyses were performed using the software SPSS v. 17.5. Data from the experiments were expressed as mean ± standard error.

3. Results

3.1. Biochemical characterization of *Streptomyces* spp. isolates

The potential attributes for plant growth promotion mediated by *Streptomyces* spp. isolates were evaluated *in vitro* based on the siderophore production, P-solubilization on agar plates, amylase and lipase activities and production of volatile organic compounds. All isolates were able to colonize the iron-deficient medium, as well as to removing iron from Fe-CAS complex, and therefore, were considered positive for siderophore production (Table 1). Color of halos varied from yellow to brownish (data not shown), suggesting differences on the type of the siderophore produced by the isolates. Production of siderophore was significantly higher for *Streptomyces* PM5 (Table 1; Fig. 1a). Moreover, four out of the six *Streptomyces* spp. isolates showed P-solubilizing ability on PDYA medium (Table 1). PM3, PM4, PM5 and PM9 isolates showed significant activity after 14 days (Table 1), and PM9 resulted in the highest P-solubilization (Fig. 1b). Solubilization activity was not detected on PM1 and PM6. In addition, all isolates showed amylolytic activity, and the largest halos were observed in PM6 and PM9 (Table 1; Fig. 1c). Similarly, PM9 presented the highest ability to degrade lipids, although PM4 and PM6 also showed clear halos (Table 1; Fig. 1d).

Table 1 Siderophore production, phosphate solubilization, enzymatic activity of amylase and lipase by different *Streptomyces* spp. isolates

	Biochemical characteristics			
Isolates	Siderophore production	Phosphate solubilization	Activity of Amylase	Activity of Lipase
PM1	0.44±0.01 e*	NA	0.98±0.02 b	NA
PM3	1.15±0.09 b	0.22±0.06 b	0.58±0.03 c	NA
PM4	0.86±0.07 c	0.31±0.04 b	0.98±0.02 b	0.54±0.13 b
PM5	1.80±0.08 a	0.31±0.04 b	1.00±0.06 b	NA
PM6	0.63±0.04 d	NA	1.23±0.12 a	0.59±0.01 b
PM9	0.68±0.11 d	0.48±0.05 a	1.33±0.05 a	1.52±0.12 a

*Values are the average width of the halo zone (cm) of at least three replicates. Data are presented as mean ±SE. Means followed by the different letters in the columns indicate significant difference at $p \leq 0.05$ according to Duncan Test

NA: no activity detected

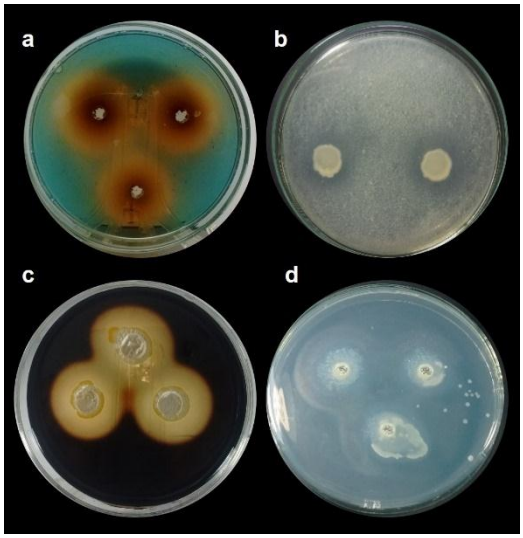

Fig. 1 Effect of *Streptomyces* spp. on (a) siderophore production (PM5); (b) P-solubilization (PM9); (c) amylase activity (PM9); (d) lipase activity (PM9).

Through solid-phase microextraction (HS-SPME) technique (SPME) and gas chromatography-mass spectrometer (GC/MS) the volatile organic compounds produced by the isolates of *Streptomyces* spp. were determined. Thirty-two compounds were identified using mass spectra and retention index (Table 2). All isolates analyzed showed production of VOC, although there was large variation among the rhizobacteria (Table 2). Twenty-four out of 32 identified compounds were unique to one isolate. This was specifically true for the profile of PM5 isolate, which was the isolate producing more VOC (17 compounds), with 2-methyl-isoborneol as the major compound followed by PM9 with the same major compound. PM3 produced seven compounds, being anisole the major compound, whereas PM1, PM4 and PM6 showed 4 or 6 identified compounds, as majority compounds being 1,1-diethoxy-ethane, 2-methyl-isoborneol and α-muurolol (=torreyol), respectively. Geosmin was the only volatile compound common to all the isolates analyzed (Table 2).

Table 2 Composition of volatile organic compounds (VOC) of the six isolates of *Streptomyces* spp. extracted by SPME (solid-phase microextraction)

RI[a]	Compound[d]	Relative Peak Area (%)[e]					
		Isolates					
		PM1	PM3	PM4	PM5	PM6	PM9
-[b]	Isobutanol	2.33	6.28				2.00
-	1-butanol	4.93	13.82	2.04			3.18
-	1,1-diethoxy-ethane	79.28		15.32	5.85		2.73
-	2-methyl-butanoic acid, methyl ester			16.91			2.78
-	3-methyl-butanoic acid, methyl ester			13.25			1.98
846[c]	3-methyl-1-butanol						2.02
849	2-methyl-1-butanol						3.08
860	1,3-dimethyl-benzene				2.01		
876	Isopentyl acetate	3.2					
919	Anisole		34.8				
937	γ-valerolactone		11.9				
961	Benzaldehyde				1.74		
983	2-pentyl-furan						2.45
1032	2-ethyl-1-hexanol		12.09				
1101	Linalool				2.21		
1182	2-methyl-isoborneol	0.61		26.1	26.56		47.27
1193	α-terpineol				1.69		
1226	1H-Indene,1-ethylideneoctahydro-7a-methyl-,(1Z,3a,alpha,7a,beta)				1.65		
1235	1H-Indene,1-ethylideneoctahydro-7a-methyl-,cis-				1.45		
1257	p-anisaldehyde				2.5		
1348	α-cubebene				1.34		
1385	β-elemene				1.81		
1406	Geosmin	2.57	7.68	12.16	22.03	37.72	21.03
1440	Coumarin				2.99		
1449	Seychellene				1.24		
1475	n-dodecanol				2.65		
1482	γ-muurolene				3.37		
1517	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-				4.28		
1530	Cis-calamenene					1.35	
1578	Caryolan-8-ol					6.4	
1636	α-muurolol (=torreyol)					44.06	
1875	Hexadecanoic acid, methyl ester		6.11				
	Total identified in each isolated:	**92.92%**	**92.68%**	**85.78%**	**85.10%**	**89.53%**	**88.52%**

[a] Retention time. The components were reported according to their order of elution on the HP-5 MS.

[b] Identity assigned by comparing mass spectra with those obtained from NIST.

[c] Identity assigned by comparing retention time with a homologous series of pure n-alkane standards and by comparing mass spectra with those obtained from NIST.

[d] The identification of peaks is based on a comparison of their linear retention indices with pure standards and a comparison of their mass spectra (MS) with the literature (NIST05).

[e] Relative proportions of the isolated constituents were expressed as percentages obtained by peak-area normalization, all relative response factors being taken as one.

3.2. Antagonism between *Streptomyces* spp. and Pcb

The effect of *Streptomyces* spp. on Pcb growth was evaluated by either direct (dual-culture plate assay) or VOC antagonism. On the direct approach, results were observed 24 h after the contact between microorganisms had been established. Inhibition of growth was seen on PM1, PM3, PM5 and PM6 and significant differences were observed among the isolates. PM3 was the most effective isolate (Table 3). PM4 and PM9 showed no antagonism against Pcb (Table 3). Using the partitioned petri dishes method (I-plate), the effects of VOC from *Streptomyces* spp. on growth of Pcb could be seen. All isolates, previously tested for the production of VOC, exhibit inhibition of Pcb verified by growing inhibition (GI) ranging from 4.2 to 25.2% at 7 days post culture (Table 3). Isolates PM1 and PM3 showed the highest GI, while PM6 was the isolate with the lowest GI (Table 3). The effect of these isolates on inhibiting Pcb growth can be observed in Figure 2.

Table 3 Growth inhibition of *P. carotovorum brasiliensis* (Pcb) on agar plates by bacterial isolates in dual-culture plate assay and *Streptomyces* spp. volatiles in I-plate assay

Isolates	**Antagonism against *Pcb* dual-culture**	**Antagonism against *Pcb* via VOC**
	Halo (cm)	**GI (%)**
PM1	0.7±0.08 b	21.9±5.54 a
PM3	1.1±0.20 a	25.2±2.79 a
PM4	NA	19.0±5.52 ab
PM5	0.3±0.08 c	11.2±4.47 abc
PM6	0.7±0.14 b	4.2±1.55 bc
PM9	NA	16.1±7.15 ab

*Values are the average of the width of the halo zone (cm) or the percentage of relative growth inhibition (GI), at least three replicates. Data are presented as mean ±SE. Means followed by the different letters in the columns indicate significant difference at $p \leq 0.05$ according to Duncan Test

NA: No antagonism detected.

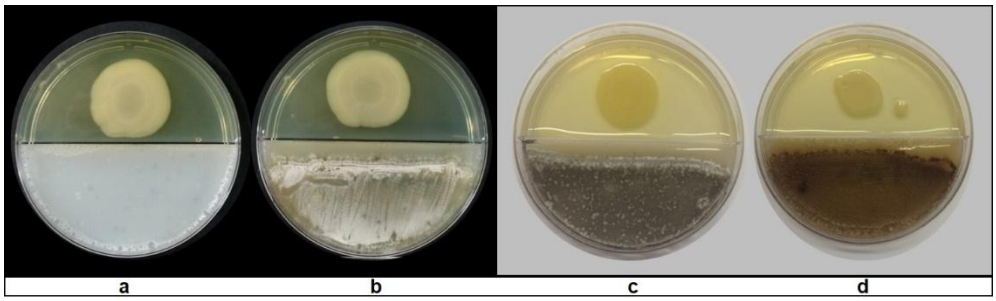

Fig. 2 Effect of VOC produced by *Streptomyces* spp. on growth of *P. carotovorum brasiliensis* at 7 days post culture. (a) Pcb alone (control), (b) Pcb in the presence of PM6, (c) Pcb in presence of PM1, (d) Pcb in presence of PM3. *Note*: Microorganisms are physically separated in a partitioned I-plate. Pcb was cultured on the upper half of the I-plate

3.3. Promotion of plant growth by VOC from *Streptomyces* spp.

Volatiles from *Streptomyces* spp. promoted root length of plants cultivated in presence of all isolates, with exception of PM1 when compared to the control treatment (Table 4). Similar response was observed for shoot length, although PM1 and PM9 effect did not differ from the control (Table 4). The chlorophyll levels varied in relation to the control and increased *chla/chlb* ratio and *chl total* was observed when plants were exposed to PM6 volatiles. On the contrary, a significant reduction of the *chl total* was noted in plants from PM9 treatment (Table 4). The roots and shoots DW were not affected, regardless the isolate used (Table 5). However, shoot fresh weight was promoted by *Streptomyces* spp. and plants exposed to PM3 showed the highest value (518.9 mg). The volume of the roots was only increased by volatiles produced by PM3.

Table 4 Growth and chlorophyll contents of tomato plants under the effect of VOC from *Streptomyces* spp.

Treatments	Root length (cm)	Shoot length (cm)	*Chl a/ Chl b*	*Chl total*
Control	5.9±0.9 b	11.6±0.6 b	0.125 ab	1.96±0.1 b
PM1	4.6±0.9 b	11.8±0.6 b	0.114 b	1.97±0.2 b
PM3	10.4±0.7 a	15.3±0.6 a	0.117 b	1.92±0.1 b
PM4	9.2±0.9 a	14.3±0.8 a	0.119 ab	1.31±0.1 c
PM5	8.8±0.6 a	14.4±0.7 a	0.149 a	1.99±0.1 b
PM6	11.1±0.6 a	14.6±0.4 a	0.133 a	2.76±0.2 a
PM9	11.0±0.7 a	13.6±0.9 b	0.112 b	1.87±0.1 b

Values are the average of 14 plants. Data are presented as mean ±SE. Means followed by different letters within the columns indicate significant difference at $p \leq 0.05$ according to Duncan test

Table 5 Biomass of tomato plants under the effect of VOC from *Streptomyces* spp.

Treatments	Root FW (mg)	Root DW (mg)	Root volume (mL)	Shoot FW (mg)	Shoot DW (mg)
Control	111.5±10.3 a	4.1±0.4 a	0.3±0.6 b	255.0±20.9 c	15.5±1.6 a
PM1	113.7±21.0 a	3.6±0.5 a	0.3±0.4 ab	285.5±22.8 bc	16.2±1.6 a
PM3	138.2±16.1 a	4.8±0.5 a	0.4±0.6 a	518.9±42.1 a	19.5±1.6 a
PM4	113.8±19.0 a	3.2±0.5 a	0.3±0.7 ab	370.6±41.8 b	16.0±1.7 a
PM5	115.9±13.6 a	4.2±0.4 a	0.3±0.8 ab	378.9±41.0 b	16.2±1.3 a
PM6	113.3±13.3 a	4.2±0.5 a	0.4±1.2 ab	386.4±35.0 b	16.4±1.6 a
PM9	125.9±14.9 a	3.6±0.5 a	0.3±0.8 ab	380.6±27.0 b	15.3±1.1 a

Values are the average of 14 plants. Data are presented as mean ±SE. Means followed by different letters within the columns indicate significant difference at $p \leq 0.05$ according to Duncan test

3.4. Promotion of plant growth by direct interaction with *Streptomyces* spp. isolates

Growth of tomato plants was affected by inoculation with rhizobacteria *Streptomyces* spp. At 30 dpi, inoculation with PM5 showed significant positive results, increasing root FW and DW and shoot FW (Fig. 3a, b). Shoot FW was also increased by treatment with PM3, PM6 and PM9 (Fig. 3b). Plants showed highest number of leaves (8.13) when treated with PM3 (Table 6). At the same time point, contents of chlorophyll were also altered by root interaction with *Streptomyces* spp. The highest ratio of *Chl a/ Chl b* was recorded on PM5 treatment, *chl total* significantly increased with PM1, PM3, PM4 and PM5 (Table 6).

Marked effect on plant growth could be seen at 45 dpi. At this time point, growth parameters showed positive responses when compared to the control plants (Fig. 3; Table 6). Root length was significant increased on PM4 and PM5, reaching 31.0 and 29.2 cm, respectively, when compared to 24.7 cm from the control plants (Fig. 3c). Shoot growth, measured by fresh and dry weight, as well as root FW, were promoted by plant-inoculation with PM5 (Fig. 3a, b). Shoot FW was also increased when plants were treated with PM3 and PM6 (Fig. 3b). Contrary to what was observed at 30 dpi, the number of leaves showed no difference from the control treatment at 45 dpi. However, root volume was increased from 0.93 mL at 30 dpi to 2.35 mL at 45 dpi when plants were inoculated with PM5 (Table 6). Chlorophyll levels also showed alterations at 45 dpi. Level of *chl a*/*chl b* and *chl total* was increased in PM9-plants, although PM3, PM5 and PM6-inoculation also resulted in higher levels of *chl total* when compared to the non-inoculated plants (Table 6). Interestingly, no *Streptomyces* spp. isolates decreased plant growth, although PM1 showed no effect on any of the analyzed parameters (Fig. 3; Table 6).

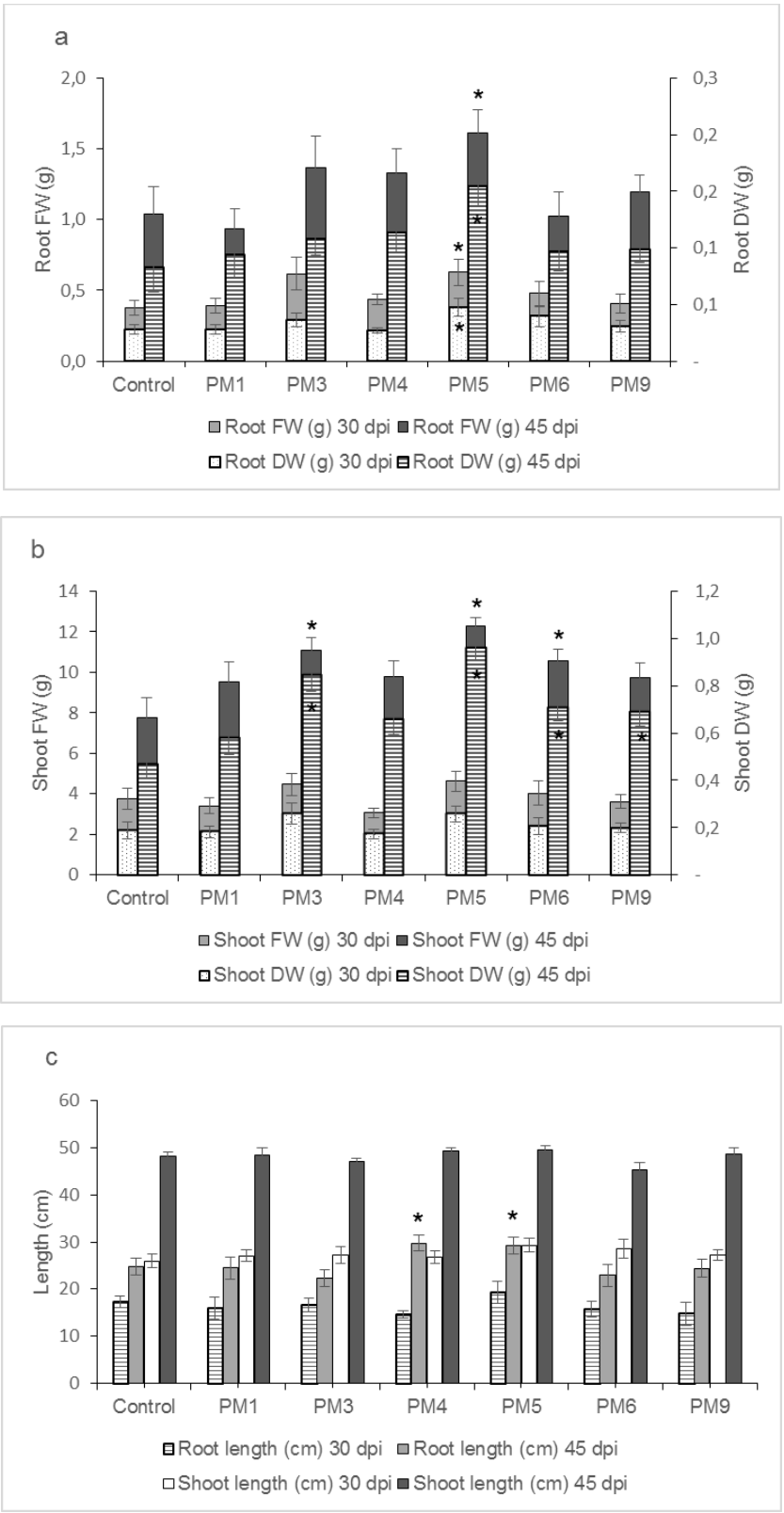

Fig. 3 Growth promotion of tomato plants induced by *Streptomyces* spp. isolates evaluated at 30 and 45 dpi. (a) Fresh and dry weight of roots, (b) Fresh and dry weight of shoots, (c) Length of roots and shoots. Values are the average of 15 plants. Data are presented as mean ±SE. Bars with different letters within the parameter indicate significant difference at $p \leq 0.05$ according to Duncan test

Table 6 Number of leaves, root volume and chlorophyll contents in tomato plants inoculated with different isolates of *Streptomyces* spp. evaluated at 30 and 45 dpi.

	Number of leaves		Root volume mL		*Chl a/ Chl b*		*Chl total*	
Treatments	30 dpi	45 dpi	30 dpi	45 dpi	30 dpi	45 dpi	30 dpi	45 dpi
Control	7.29±0.29 b	8.06±0.31 a	0.79±0.07 a	1.51±0.26 b	0.11 b	0.14 a	2.31±0.09 c	1.36±0.09 b
PM1	7.29±0.19 b	7.73±0.28 a	0.8±0.11 a	1.41±0.21 b	0.11 b	0.13 b	2.70±0.05 ab	1.53±0.05 ab
PM3	8.13±0.42 a	8.08±0.55 a	0.77±0.12 a	1.88±0.22 ab	0.12 ab	0.13 b	2.66±0.11 b	1.62±0.04 a
PM4	6.87±0.22 b	7.71±0.29 a	0.7±0.07 a	1.82±0.24 ab	0.12 ab	0.13 b	2.98±0.10 a	1.52±0.06 ab
PM5	7.17±0.21 b	8.06±0.27 a	0.93±0.13 a	2.35±0.26 a	0.14 a	0.13 b	2.62±0.09 b	1.66±0.03 a
PM6	7.43±0.27 ab	8.06±0.31 a	0.82±0.14 a	1.43±0.24 b	0.12 ab	0.13 b	2.32±0.10 c	1.61±0.05 a
PM9	7.27±0.21 b	7.75±0.13 a	0.87±0.17 a	1.62±0.15 b	0.13 ab	0.13 b	2.58±0.14 bc	1.61±0.06 a

Values are the average of 15 plants. Data are presented as mean ±SE. Means followed by different letters within the columns indicate significant difference at $p \leq 0.05$ according to Duncan test

3.5. Modulation of secondary metabolism of tomato plants by *Streptomyces* spp.

Responses of the defense-related enzymes PAL, PPO and POX were much more intense in the roots than in the shoots (Table 7). The maximum of activity of PAL and PPO was approximately 6.9-fold higher in the roots than in the shoots. The difference regarding activity of POX was 3.2-fold higher. On the other hand, higher concentration of phenolic compounds was observed in the shoots (13.3-fold) than in the roots (Table 7).

In the Figure 4, all results of enzyme activities were expressed as relative percentage of the activity observed in the control treatment. In the roots of plants treated with *Streptomyces* spp. changes on the activity of the enzymes analyzed occurred depending on the isolated tested (Fig. 4a, c, e). Inoculation of tomato plants with PM1 resulted in decline of PAL activity from 0 dpi (0.355 nmol mL^{-1} min^{-1} mg^{-1} protein) to 3 dpi (0.184 nmol mL^{-1} min^{-1} mg^{-1} protein). At 9 dpi, PAL activity was recorded at 0.061 nmol mL^{-1} min^{-1} mg^{-1} protein), 145% higher than the control treatment (Fig. 4a). Similar trend was observed for POX activity, showing the maximum decrease at 1 dpi and an increase of activity at 9 dpi (0.095 µk mg^{-1} protein). On the other hand, difference on PPO activity was only seen at 9 dpi (Fig. 4a). Levels of phenolic compounds showed no difference in comparison with control treatment (Fig. 4a). When plants were inoculated with PM3 and analyzed at 1 dpi, roots showed a significant reduction of PAL activity followed by a decrease of phenolic compounds, which reached the minimal concentration at 3 dpi (0.163 mg g^{-1} FW; Fig. 4c). A slight increment of POX activity at 9 dpi was accompanied by a notable rising on phenolic levels (Fig. 4c). On PM5-treated roots, activity of enzymes was lower than the control in all time points assessed, with exception of PAL at 1 dpi and POX at 9 dpi (Fig 4e). Phenolic compounds exhibited the same trend of PM3-plants, decreasing up to 3 dpi (Fig. 4e).

Shoots also showed alterations in the metabolism of defense-related enzymes when plants were inoculated with *Streptomyces* spp. showing a possible systemic response to the rhizobacteria interaction (Fig. 4b, d, f). In the shoots of PM1-inoculated plants, high PPO and POX activities (0.906 ΔAbs min^{-1} mg $proteína^{-1}$ and 0.233 µk mg^{-1} protein s^{-1}, respectively) were observed at 0, and such levels were kept similar to the control treatment thereafter (Fig. 4b). A significant decline in PAL activity was detected at 1 and 3 dpi (0.023 nmol mL^{-1} min^{-1} mg^{-1} protein; Fig. 4b). The activity of the oxidation enzymes associated with the diminishing of PAL activity is coincident with the continuous decreasing of phenolic compounds from 0 to 9 dpi (2.52 mg g^{-1} FW; Fig. 4b). Similar to PM1, shoots of plants inoculated with PM3 also showed markedly decrease of phenolics from 0 to 9 dpi, whereas activity of PAL (0.099 nmol mL^{-1} min^{-1} mg^{-1} protein) and POX (0.184 µk mg^{-1} protein s^{-1}) were higher than control plants at 0 dpi (Fig. 4d). An increase of PPO activity was recorded at 9 dpi in PM3-shoots.

PM5-shoots responded to the interaction with *Streptomyces* spp. with a fast increase of PAL activity at 0 dpi (0.069 nmol mL^{-1} min^{-1} mg^{-1} protein), although activity on the following time points was lower than the non-treated plants, reaching 0.030 nmol mL^{-1} min^{-1} mg^{-1} protein at 9 dpi (Fig. 4f). In addition, at 9 dpi PPO activity (0.067 ΔAbs min^{-1} mg $proteín^{-1}$) decreased when compared to the control (0.093 ΔAbs min^{-1} mg $proteín^{-1}$) contrasting with a significant increment of POX (0.18 µk mg^{-1} $proteins^{-1}$; Fig. 4f). The level of phenolic compounds reduced from 2.32 (0 dpi) to 1.94 mg g^{-1} FW (9 dpi) (Fig. 4f).

Table 7 Variation range, in absolute values, of enzyme activity (PAL, PPO and POX) and phenolic compounds in roots and shoots of tomato plants, regardless the isolates of *Streptomyces* spp. (PM1, PM3 and PM9) and time of analysis

Secondary metabolism	**Roots**		**Shoots**	
	Minimal	Maximum	Minimal	Maximum
PAL (nmol mL^{-1} min^{-1} mg^{-1} protein)	0.051	0.680	0.023	0.099
PPO (ΔAbs min^{-1} mg $protein^{-1}$)	0.193	4.167	0.060	0.906
POX (µk mg^{-1} protein s^{-1})	0.064	0.756	0.056	0.233
Phenolic compounds (mg g^{-1} FW)	0.0211	0.223	1.456	2.966

PAL, phenylalanine ammonia lyase; PPO, polyphenoloxidase; POX, peroxidases

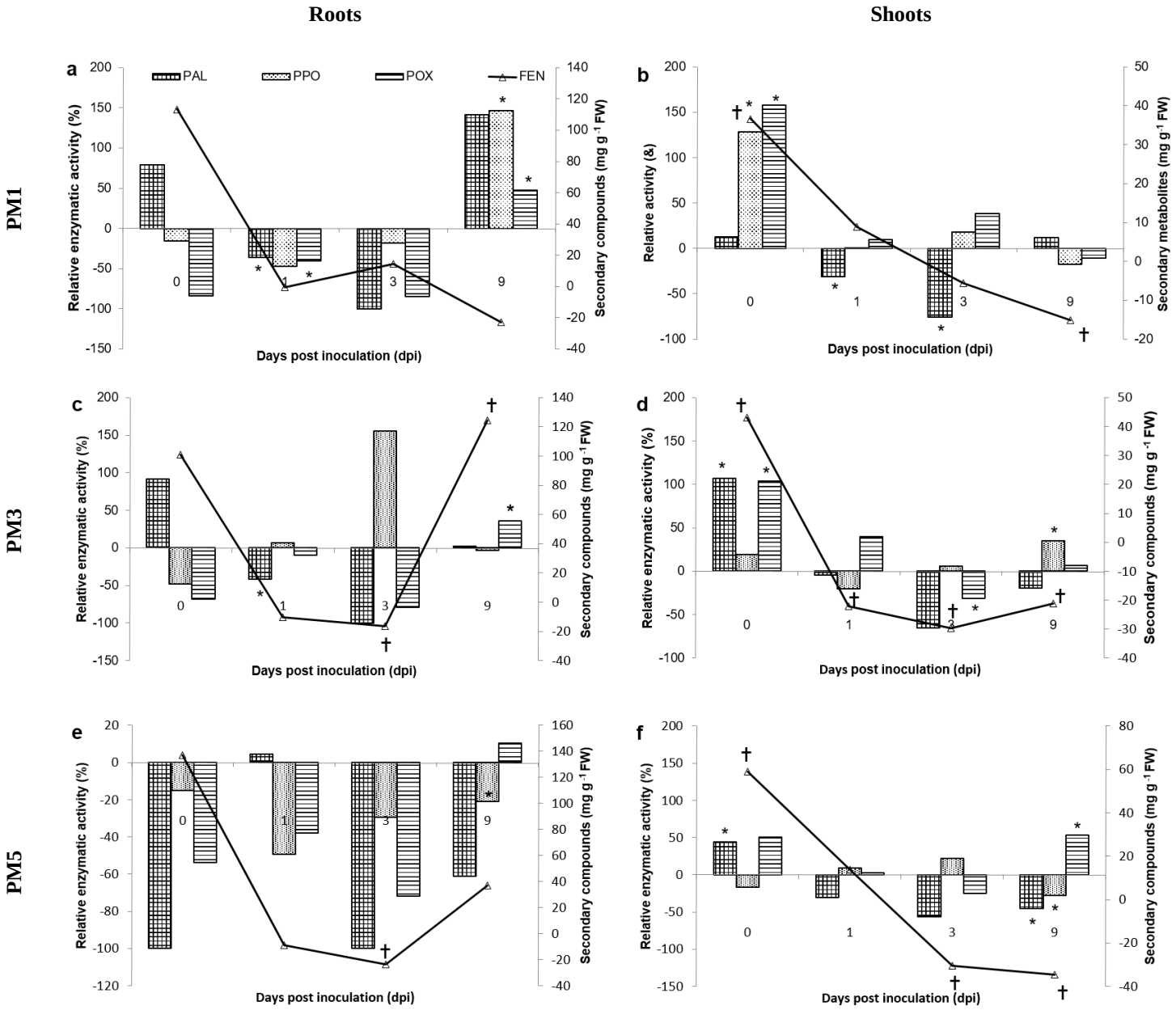

Fig. 4 Relative activity of the enzymes PAL, PPO, and POX, and phenolic compounds on tomato plants treated with three isolates of *Streptomyces* spp. (PM1, PM3 and PM5). PAL, phenylalanine ammonia lyase; PPO, polyphenoloxidase; POX, peroxidases. Asterisk on the bar and a cross on the line indicates significant difference within the time point when compared to the control treatment (Student's *t*-Test, at $p \leq 0.05$)

3.6. Evaluation of soft rot disease

Evaluation of the area under disease progress curve (AUDPC-nc) in tomato plants showed differences among the treatments. Plants from the control treatment showed initially browning and thinning of the stem. As disease developed, wilting and yellowing of leaves could be seen. Nonetheless, no disease symptoms were observed in plants from either the absolute control (H_2O) or rhizobacteria treatments (Table 8). On the other hand, all isolates were effective in promoting plant resistance against Pcb and reduced disease compared to the positive control (Pcb control) (Table 8; Fig. 5). Plants treated with rhizobacteria and challenged with Pcb showed significant smaller AUDPC-nc value when compared to the Pcb control and PM1 pretreatment resulted in the lowest AUDPC-nc during the period of analysis (Table 8). Interestingly, PM1 was also the isolate that induced the highest number of adventitious roots, in both the unchallenged and Pcb-challenged plants (Table 8). The response observed with AUDPC-nc was ratified when disease incidence was recorded during cultivation period. All isolates were efficient in reducing mortality when compared to Pcb-infected plants (Fig. 5). Although, none of the isolates delayed the onset of disease, PM1 was the *Streptomyces* isolate that showed the lowest percentage of diseased plants from the onset on (Fig. 5).

Table 8 Mean AUDPC-nc values and adventitious roots in tomato plants elicited with different isolates of *Streptomyces* spp. and challenged with *P. carotovorum brasiliensis*, cultivated for 24 days post-inoculation with the pathogen

Treatments	AUDPC-nc	Number of adventitious roots
Control	0 ± 0.0 d	1.7
PM1	0 ± 0.0 d	10.5
PM3	0 ± 0.0 d	3.8
PM4	0 ± 0.0 d	3.7
PM5	0 ± 0.0 d	4.3
PM6	0 ± 0.0 d	5.7
PM9	0 ± 0.0 d	2.8
Pcb	25,836.92 ± 415.69 a	1.7
PM1+Pcb	13,188.46 ± 0.00 c	10.1
PM3+Pcb	17,662.50 ± 1,748.45 bc	5.8
PM4+Pcb	16,148.08 ± 1,681.59 bc	6.5
PM5+Pcb	16,587.06 ± 1,900.07 bc	4.5
PM6+Pcb	18,411.06 ± 1,923.57 b	3.9
PM9+Pcb	17,606.64 ± 1,879.81 bc	2.7

Values are the average of 15 plants. Data are presented as mean ±SE. Means followed by the different letters in the columns indicate significant difference at $p \leq 0.05$ according to Duncan Test

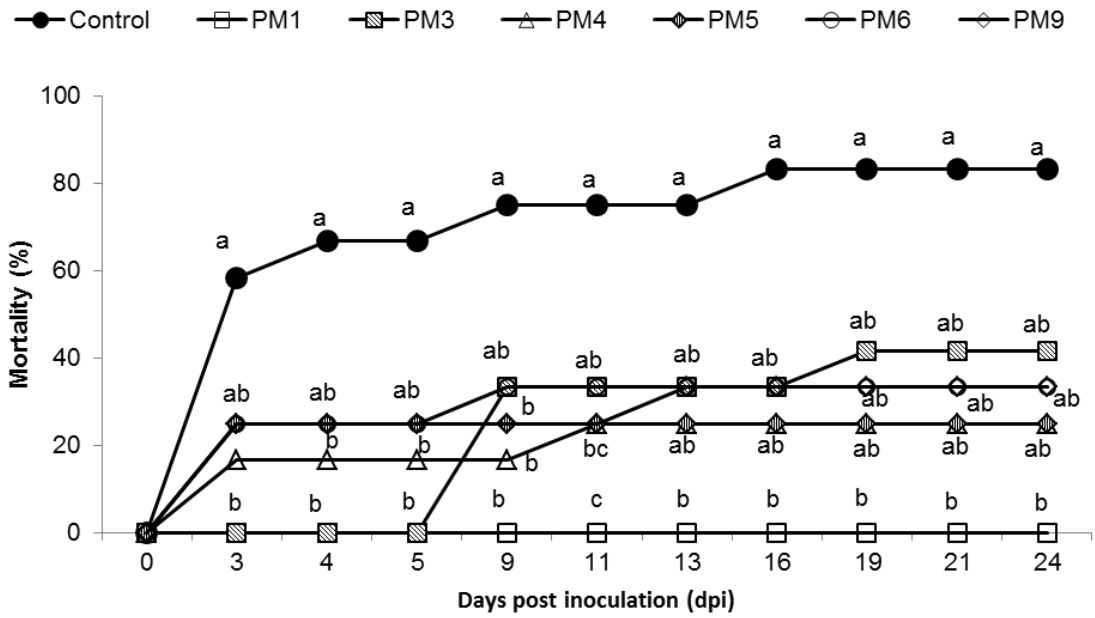

Fig. 5 Plant mortality after inoculation with *P. carotovorum brasiliensis* evaluated during 24 days. Treatments consisted of control (Pcb alone), plants pretreated with *Streptomyces* spp. isolates and challenged with Pcb (PM1+Pcb; PM3+Pcb; PM4+Pcb; PM5+Pcb; PM6+Pcb and PM9+Pcb). Different letters mean significant difference at each time point according Duncan Test at $p \leq 0.05$

4. Discussion

Many studies have reported that some rhizobacteria have the ability to solubilize phosphates, produce siderophores, fix N_2 and secrete IAA, which are some important characteristics of beneficial microorganisms. PGPR inoculation enhanced the fertilizer P efficiency and biomass in *Fraxinus americana* (Liu et al., 2013), *Oriza sativa* (Gusain et al., 2015) and *Zea mays* (Adesemoye et al., 2008), although screening of bacteria for this trait has shown that P-solubilization is not the most common ability among these microorganisms (Beneduzi et al., 2008; Ambrosini et al., 2012). Siderophore production is also a typical feature of PGPR and plants are capable of using this siderophore-Fe complex of microorganisms as a source of obtaining iron. Rhizobacteria from the genus *Streptomyces* have played a crucial role in increasing the availability of nutrients in the soil and thus promoting an increase in plant growth (Ahemad and Kibret, 2014). *Streptomyces* sp. are known to promote plant growth either by producing siderophores and IAA (Dalmas et al., 2011; Gopalakrishnan et al., 2015). All the tested *Streptomyces* isolates were previously confirmed for IAA production and significant differences were detected. The isolate PM9 showed the highest production of IAA (0.991 mg g^{-1} FM), followed by isolate PM5 (0.724 mg g^{-1} FM) (Salla et al., 2014).

Production of hydrolytic enzymes by *Streptomyces* spp. such as amylase and lipases may confer capability for competing in the rhizosphere environment (Bach et al., 2016) as well as for interacting with

cell wall of pathogens, making these organisms promising for biocontrol uses. Production of lipases by *Pseudomonas* spp. and *Bacillus* spp. was suggested to be related to inhibition of *Fusarium* spp. growth (Muleta et al., 2007). In the present study, the six *Streptomyces* spp. isolates showed capability of producing siderophores and hydrolyzing starch. Only four out of six isolates were able to solubilize phosphate. PM4 and PM9 presented all the evaluated characteristics.

Another interesting feature of rhizobacteria is the production of volatile organic compounds (VOC) that may be involved with plant growth and defense (Kanchiswamy et al., 2015). VOC have low molecular masses, are mostly lipophilic in nature, and may act on modulating the physiology of plants and microorganisms. *Streptomyces* species are well known for their ability to produce antibiotics and VOC (Chater et al., 2010; Wu et al., 2015). Variation on the profile of VOC was detected in *Streptomyces* spp. isolates. In PM5, 17 compounds were identified, followed by 10 compounds in PM9. Some of the volatiles were previously detected in *S. alboflavus* TD-1, such as 2-methyl isoborneol , α-cubebene, 1H-Indene, 1-ethylideneoctahydro-7a-methyl-,(1Z,3a,alpha,7a,beta), 1H-Indene,1-ethylideneoctahydro-7a-methyl-, cis-, and geosmin (Wang et al., 2013). Geosmin is a common molecule in *Streptomyces* species and it is responsible for the musty aroma (Cordovez et al., 2015). 1-butanol and benzoaldehyde showed antimicrobial activities (Bitas et al., 2013), and β-elemene, found in *Thricoderma virens*, was related to growth promotion and induction of defense responses of *Arabidopsis thaliana* against *Botrytis cinerea* (Kanchiswamy et al., 2015). Anisole and 2-pentyl-furan, which were also found in *S. albulus* NJZJSA2 (Wu et al. 2015), were exclusively detected in PM3. VOC from *Pseudomonas fluorescens* and *B. subtilis* have been reported to increase growth, as well as essential oil production in peppermint plants (Santoro et al., 2011). Moreover, volatiles produced by *S. platensis* demonstrated antifungal activity against *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, and *Botrytis cinerea* (Wan et al., 2008). Thus, the isolates of *Streptomyces* spp. in this study present, in their majority, plant-beneficial properties, which indicated that they might have a role as plant growth promoter.

The antagonism of *Streptomyces* spp. against Pcb was evaluated by two different methods. The dual-culture plate assay, PM3 was the most efficient on inhibiting growth of Pcb. Similarly, on I-plate method, where VOC effect on antagonism was evaluated, PM3 was also effective (25.2% of inhibition), although PM1 showed similar capacity of inhibiting Pcb growth. Interestingly, PM3 isolate produced 13.82% 1-butanol and 34.8% anisole, compounds with antimicrobial proprieties (Wu et al., 2015). 1-butanol was also detected in PM1. VOC generated by *S. albulus* NJZJSA2 inhibited mycelial growth of *S. sclerotiorum* and *Fusarium oxysporum* by 100 and 56.3%, respectively, likely because the production of 4-methoxystyrene, 2-pentylfuran, and anisole, which were proved to have antifungal activity (Wu et al., 2015). The basis of antibiosis as a biocontrol mechanism of plant growth promoting bacteria has become increasingly better understood and a variety of antibiotics have been identified in *Pseudomonas* and *Bacillus* (Compant et al., 2005). Olygomycin A, in *S. libani*, and streptochlorin, nigericin and piericidin A1 in *S. anulatus* S37, were identified and characterized as antifungal compounds (Kim et al. 1999; Couillerot et al., 2014).

In order to evaluate the potential of *Streptomyces* spp. to promote tomato growth, the six isolates were tested. On evaluating growth promotion mediated by VOC, significant increases of shoot and root length and shoot FW were obtained with all isolates, with exception of PM1. Considering that in this method

no physical contact between roots and bacteria was established, the effect of growth could be attributed to the production of VOC from *Streptomyces* spp. When suspension of each isolate of *Streptomyces* spp. was added to the soil around the roots of tomato plants, the results were also evident and differences were seen between 30 and 45 dpi. PM5 was effective on promoting growth at both time points assessed, mainly in biomass accumulation. However, at 45 dpi PM3, PM6 and PM9 were also efficient on increasing shoot FW. The promotion of growth of *Streptomyces* sp. had previously been reported in various agriculturally important crops, such as tomato, rice and sunflower (El-Tarabily, 2008; Ambrosini et al., 2012; Gopalakrishnan et al., 2012). In chickpea, biomass and yield were improved by treatment with several strains of *Streptomyces* spp. and quantitative real-time PCR analysis of selected plant growth promoting genes revealed the selective up-regulation of IAA-related and siderophore-related genes (Gopalakrishnan et al., 2015). Additionally, the observed enhancement of chlorophyll content observed with PM3 and PM5 at 30 and 45 dpi might lead to an enhanced photosynthesis in PGPR-treated tomato plants, and could be related to the promotion of growth. Alteration in chlorophyll contents and its relation to tomato growth and productivity mediated by rhizobacteria *B. subtilis* and *Azotobacter chroococcum* was previously reported (Babu et al., 2015) and by *Brevundimonas diminuta* (NBRI012) in rice plants (Singh et al., 2016).

In addition to the beneficial effect on growth of tomato plants, *Streptomyces* spp. treatment was able to modulate the secondary metabolism of roots and shoots during 9 days of culture. Levels of the defense-related enzymes PAL, POX and PPO were altered in *Streptomyces*-inoculated plants. In general, roots responded to the *Streptomyces* spp. inoculation in a higher intensity than aerial parts, which was expected since roots are the organ of naturally contact with the rhizobacteria. Treatment with *Streptomyces* PM1 and PM3 reduced the levels of enzymes in the roots at 1 and 3 dpi when compared to the control plants. Likewise, the contents of phenolic compounds were reduced within the same period. In roots of plants treated with PM5, however, the decrease on the activity of enzymes was evident at all the time points assessed. However, in spite of the reduction observed at 1 and 3 dpi, roots treated with PM1 and PM3 showed a significant stimulation of POX activity at 9 dpi. Different responses were observed in the shoots, where a fast increase of PPO and POX activities could be detected at 0 dpi on PM1-plants and PAL and POX in PM3-plants. Thereafter the levels of enzymes were maintained mostly similar to the control plants. On the other hand, PM5 inoculation resulted in few alterations in the shoots regarding the enzymes.

Increased activity of PAL, POX and PPO have been correlated with defense against pathogens in betelvine and pigeon pea (Chen et al., 2000; Lavania et al., 2006; Dutta et al., 2008). However, they also have their levels altered in benefic interactions with microorganisms, namely *Streptomyces* spp. (Dalmas et al., 2011; Salla et al., 2014). Non-pathogenic rhizobacteria such as PGPR interact with roots without activating the defense responses in the host plants, and only cause the accumulation of transcription factors related to defense genes that reduce the response time to pathogen attack (Van der Ent et al., 2009). It noteworthy that enzymes decreased their activity at 1 and 3 dpi in the tomato roots, likelihood in an attempt to permit the plant-rhizobacteria interaction. Likewise, the lowest levels of phenolic compounds observed in tomato roots at 1 and 3 dpi were also reported for *Eucalyptus* plants treated with *Streptomyces* PM9 (Salla et al., 2014). Nevertheless, different results have been reported in the literature. When cucumber roots were treated with *P. corrugata* 13 or *P. aureofaciens* 63-28, PAL activity was stimulated in root tissues in 2 days whereas POX and PPO activities were increased 2-5 days after bacterization with *P.*

corrugate (Chen et al., 2000). Increase of phenolic compounds is often reported as a defense response against pathogen, mainly the synthesis of flavonoids, molecules which display antimicrobial activity (Chen et al., 2000; Lavania et al., 2006). In our study, the decrease of phenolic compounds could be a result of the low PAL activity and basal activity of POX and PPO, since pathogen was not involved in the interaction.

An interesting effect of inoculation of *Streptomyces* spp. in the roots was the induction of adventitious roots. PM1 inoculation resulted the highest number of roots, even when challenge with Pcb was performed. *Streptomyces* spp. isolates used in the current study also proved to be efficient on decreasing the incidence of soft rot disease. All isolates inoculated in the roots of tomato plants reduced the AUDPC-nc in comparison to control plants. In addition, treating with *Streptomyces* spp. lead to a reduction on plant mortality caused by Pcb. Rhizobacteria have been reported as agents of biocontrol. The participation of PGPR in the induction of systemic resistance is related to the activation of the defense responses of plant cells (Tarkka, 2008). These responses include production of phytoalexins and re-enforcement of plant physical defense barriers by increased deposition of lignin and tannins (Małolepsza, 2006) which together produce non-favorable conditions for pathogen infection and invasion. The combination of the ability of producing siderophores and VOC with antimicrobial properties, with the alteration of POX activity later in the culture (9 dpi) by the PM1 and PM3 isolates may have prepared the plants to respond to the infection by Pcb, resulting in diminished plant mortality.

5. Conclusions

The present study demonstrates that the *Streptomyces* spp. isolates were able to produce siderophores, phosphate solubilizing and produce volatile organic compounds, which are desirable characteristics for their use as PGPR. *Streptomyces* PM5 promoted growth of tomato plants by direct contact between rhizobacteria and roots. Antagonism by dual-culture against *P. carotovorum* subsp. *brasiliensis* was obtained with PM3. The potential *in vivo* antagonism of PM1, along with reducing the incidence of the soft rot disease indicates that this *Streptomyces* isolate could be used as an inducer of resistance against Pcb. The worldwide efforts in the search of natural products for the crop protection market have progressed significantly and actinomycetes, especially those belonging to the genus *Streptomyces*, appear to be good candidates to find new approaches to manage plant diseases. This study established an efficient interaction of at least one isolated from *Streptomyces* spp. with tomato plants, promoting the growth and/or induction of plant defense against *P. carotovorum* subsp. *brasiliensis.*

Acknowledgments

Authors thank Andréia Oliveira from Fundação Estadual de Agropecuária (FEPAGRO), for providing the *Pectobacterium carotovorum brasiliensis* and to Janaina Belquis da S. P. Langois for technical assistance. This work was supported by PROBOLSAS-PUCRS (fellowship of first author) and by the National Council for Scientific and Technological Development (CNPq/Brazil; 403843/2013-8).

Conflict of interest

The authors declare that there is no conflict of interest.

Author contribution statement

M.P.Dias and E.R. Santarém designed the experiments. M.P.Dias performed the experiments. M.S.Bastos helped on conducting the experiments on disease. V.B.Xavier and E.Cassel performed the VOC analysis. L.V.Astarita contributed to data analyses and discussion of the results. M.P.Dias and E.R.Santarém wrote the manuscript. All authors read and approved the manuscript.

References

Adesemoye, A.O., Torbert, H.A., Kloepper, J.W., 2008. Enhanced plant nutrient use efficiency with PGPR and AMF in an integrated nutrient management system. Can. J. Microbiol. 54, 876–86. doi: 10.1139/w08-081

Ahemad, M., Kibret, M., 2014. Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. J. King Saud Univ. Sci. 26, 1–20. doi:10.1016/j.jksus.2013.05.001

Ambrosini, A., Beneduzi, A., Stefanski, T., Pinheiro, F.G., Vargas, L.K., Passaglia, L.M.P., 2012. Screening of plant growth promoting rhizobacteria isolated from sunflower (*Helianthus annuus* L.). Plant Soil 356, 245–264. doi:10.1007/s11104-011-1079-1

Babu, A.N., Jogaiah, S., Ito, S-I., Nagaraj, A.K., Tran, L-S. P., 2015. Improvement of growth, fruit weight and early blight disease protection of tomato plants by rhizosphere bacteria is correlated with their beneficial traits and induced biosynthesis of antioxidant peroxidase and polyphenol oxidase. Plant Sci. 231, 62–73. doi: 10.1016/j.plantsci.2014.11.006

Bach, E., Seger, G.D.S., Fernandes, G.C., Lisboa, B.B., Passaglia, L.M.P., 2016. Evaluation of biological control and rhizosphere competence of plant growth promoting bacteria. App. Soil Ecol. 99, 141–149. doi: 10.1016/j.apsoil.2015.11.002

Bakker, P.A.H.M., Berendsen, R.L., Doornbos, R.F., Wintermans, P.C.A., Pieterse, C.M.J., 2013. The rhizosphere revisited: root microbiomics. Front. Plant Sci. 4, 165. doi: 10.3389/fpls.2013.00165

Beneduzi, A., Peres, D., Vargas, L.K., Bodanese-Zanettini, M.H., Passaglia, L.M.P., 2008. Evaluation of genetic diversity and plant growth promoting activities of nitrogen-fixing bacilli isolated from rice fields in South Brazil. Appl. Soil Ecol. 39, 311–320. doi: 10.1016/j.apsoil.2008.01.006

Bitas, V., Kim, H-S., Bennett, J.W., Kang, S., 2013. Sniffing on microbes: diverse roles of microbial volatile organic compounds in plant health. Mol. Plant Microbe Interact. 26(8), 835–43. doi: 10.1094/MPMI-10-12-0249-CR

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254. doi: 10.1016/0003-2697(76)90527-3

Carvalho, R.D.C.P., Resende, R.O., Duval, A.Q., Costa, H., Lopes, C.A., Boiteux, L.S., Lima, M.F., Pinheiro, J.B., Souza, Z.A., 2014. Doenças do tomate, SBF. 16p.

Chater, K.F., Biró, S., Lee, K.J., Palmer, T., Schrempf, H., 2010. The complex extracellular biology of *Streptomyces*: Review Article. FEMS Microbiol. Rev. 34(2), 171–198. doi: 10.1111/j.1574-6976.2009.00206.x

Chen, C., Bélanger, R.R., Benhamou, N., Paulitz, T.C., 2000. Defense enzymes induced in cucumber roots by treatment with plant growth-promoting rhizobacteria (PGPR) and *Pythium aphanidermatum*. Physiol. Mol. Plant Pathol. 56, 13–23. doi:10.1006/pmpp.1999.0243

Chowdappa, P., Mohan Kumar, S.P., Jyothi Lakshmi, M., Upreti, K.K., 2013. Growth stimulation and induction of systemic resistance in tomato against early and late blight by *Bacillus subtilis* OTPB1 or *Trichoderma harzianum* OTPB3. Biol. Control 65(1), 109–117. doi:10.1016/j.biocontrol.2012.11.009.

Coll, N.S., Epple, P., Dangl, J.L., 2011. Programmed cell death in the plant immune system. Cell Death Differ. 18(8), 1247–56. doi: 10.1038/cdd.2011.37

Compant, S., Duffy, B., Nowak, J., Cle, C., Barka, E.A., 2005. Use of plant growth-promoting bacteria for biocontrol of plant diseases : Principles, mechanisms of action and future prospects. Appl. Environ. Microbiol. 71(9), 4951–4959. doi: 10.1128/AEM.71.9.4951

Cordovez, V., Carrion, V.J., Etalo, D.W., Mumm, R., Zhu, H., van Wezel, G.P., Raaijmakers, J.M., 2015. Diversity and functions of volatile organic compounds produced by *Streptomyces* from a disease-suppressive soil. Front. Microbiol. 6, 1–13. doi: 10.3389/fmicb.2015.01081

Couillerot, O., Loqman, S., Toribio, A., Hubert, J., Gandner, L., Nuzillard, J-M., Ouhdouch, Y., Clément, C., Barka, E.A., Renault, J-H., 2014. Purification of antibiotics from the biocontrol agent *Streptomyces anulatus* S37 by centrifugal partition chromatography. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 944, 30–4. doi: 10.1016/j.jchromb.2013.11.008

Dalmas, F.R., Pereira, T.C.B., Bogo, M.R., Astarita, L.V., 2011. Autochthonous *Streptomyces* regulate the metabolism of seedlings of *Araucaria angustifolia* (Coniferales) during root colonisation. Aust. J. Bot. 59(2), 118–125. doi: 10.1071/BT10175

Dingle, J., Reid, W.W., Solomons, G.L., 1953. The enzymic degradation of pectin and other polysaccharides. II—Application of the "Cup-plate" assay to the estimation of enzymes. J. Sci. Food Agr. 4(3), 149–155. doi: 10.1002/jsfa.2740040305

Doumbou, C.L., Hamby Salove, M.K., Crawford, D.L., Beaulieu, C., 2001. Actinomycetes, promising tools to control plant diseases and to promote plant growth. Phytoprotection 82(3), 85–102. doi: 10.7202/706219ar

Dutta, S., Mishra, A.K., Dileep Kumar, B.S., 2008. Induction of systemic resistance against fusarial wilt in pigeon pea through interaction of plant growth promoting rhizobacteria and rhizobia. Soil Biol. Biochem. 40(2), 452–461. doi: 10.1016/j.soilbio.2007.09.009

El-Tarabily, K.A., 2008. Promotion of tomato (*Lycopersicon esculentum* Mill.) plant growth by rhizosphere competent 1-aminocyclopropane-1-carboxylic acid deaminase-producing streptomycete actinomycetes. Plant Soil 308(1-2), 161–174. doi: 10.1007/s11104-008-9616-2

Van der Ent, S., Van Wees, S.C.M., Pieterse, C.M.J., 2009. Jasmonate signaling in plant interactions with resistance-inducing beneficial microbes. Phytochemistry 70 (13-14), 1581–1588. doi: 10.1016/j.phytochem.2009.06.009

Glick, B.R., 2014. Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiol. Res. 169(1), 30–9. doi: 10.1016/j.micres.2013.09.009

Gopalakrishnan, S., Srinivas, V., Alekhya, G., Prakash, B., Kudapa, H., Rathore, A., Varshney, R.K., 2015. The extent of grain yield and plant growth enhancement by plant growth-promoting broad-spectrum *Streptomyces* sp. in chickpea. SpringerPlus 4(1), 31. doi: 10.1186/s40064-015-0811-3

Gopalakrishnan, S., Upadhyaya, Hd., Vadlamudi, S., Humayun, P., Vidya, M.S., Alekhya, G., Singh, A., Vijayabharathi, R., Bhimineni, R.K., Seema, M., Rathore, A., Rupela, O., 2012. Plant growth-promoting traits of biocontrol potential bacteria isolated from rice rhizosphere. SpringerPlus 1(1), 71. doi: 10.1186/2193-1801-1-71

Gopalakrishnan, S., Vadlamudi, S., Bandikinda, P., Sathya, A., Vijayabharathi, R., Rupela, O., Kudapa, H., Katta, K., Varshney, R.K., 2014. Evaluation of *Streptomyces* strains isolated from herbal vermicompost for their plant growth-promotion traits in rice. Microbiol. Res. 169(1), 40–48. doi: 10.1016/j.micres.2013.09.008

Graichen, F.A.S., Martinelli, J.A., Federizzi, L.C., Pacheco, M.T., Chaves, M.S., Wesp, C.L., 2010. Inheritance of resistance to oat crown rust in recombinant inbred lines. Sci. Agric. 67(4), 435–440. doi: 10.1590/S0103-90162010000400010

Grobelak, A., Napora, A., Kacprzak, M., 2015. Using plant growth-promoting rhizobacteria (PGPR) to improve plant growth. Ecol. Eng. 84, 22–28. doi: 10.1016/j.ecoleng.2015.07.019

Gu, Y.Q., Mo, M.H., Zhou, J.P., Zou, C.S., Zhang, K.Q., 2007. Evaluation and identification of potential organic nematicidal volatiles from soil bacteria. Soil Biol. Biochem. 39(10), 2567–2575. doi: 10.1016/j.soilbio.2007.05.011

Gusain, Y.S., Kamal, R., Mehta, C.M., Singh, U.S., Sharma, A.K., 2015. Phosphate solubilizing and indole-3-acetic acid producing bacteria from the soil of Garhwal Himalaya aimed to improve the growth of rice. J. Environ. Biol. 36(1), 301–307.

Haas, D., Defago, G., Défago, G., 2005. Biological control of soil-borne pathogens by fluorescent pseudomonads. Nat. Rev. Microbiol. 3, 307-319. doi:10.1038/nrmicro1129

Hankin, L., Anagnostakis, S.L., 1975. The use of solid media for detection of enzyme production by fungi. Mycological Soc. Amer. 67(3), 597–607. doi: 10.2307/3758395

Herman, M., Williams, M., 2012. Fighting for their lives: Plants and pathogens. Plant Cell 24(6), 1-15. doi: 10.1105/tpc.112.tt0612

Jennings, W., Shibamoto, T., 1980. Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography. Elsevier 472p.

Jesus, J.G.R., Lessa, G.S., Rodrigues, T.B., Ferrão-Gonzales, A.D., Freire, E., Hanna, A.S., Moreau, V.H., 2013. Seleção e identificação de micro-organismos produtores de amilases isolados da microbiota associada a resíduos agrícolas de cacau e dendê. Diálogos & Ciência 11(33), 7–12. doi: 10.7447/dc.2013.002

Jog, R., Pandya, M., Nareshkumar, G., Rajkumar, S., 2014. Mechanism of phosphate solubilization and antifungal activity of *Streptomyces* spp. isolated from wheat roots and rhizosphere and their application in improving plant growth. Microbiology+ 160, 778–788. doi: 10.1099/mic.0.074146-0

Kanchiswamy, C.N., Malnoy, M., Maffei, M.E., 2015. Bioprospecting bacterial and fungal volatiles for sustainable agriculture. Trends Plant Sci. 20(4), 206–211. doi: 10.1016/j.tplants.2015.01.004

Kim, B.S., Moon, S.S., Hwang, B.K., 1999. Isolation, identification, and antifungal activity of a macrolide antibiotic, oligomycin A, produced by *Streptomyces libani*. Can. J. Bot. 77(6), 850–858. doi: 10.1139/b99-044

Kurabachew, H., Wydra, K., 2014. Induction of systemic resistance and defense-related enzymes after elicitation of resistance by rhizobacteria and silicon application against *Ralstonia solanacearum* in tomato (*Solanum lycopersicum*). Crop Prot. 57, 1–7. doi: 10.1016/j.cropro.2013.10.021

Lakshmanan, V., Shantharaj, D., Li, G., Seyfferth, A.L., Janine Sherrier, D., Bais, H.P., 2015. A natural rice rhizospheric bacterium abates arsenic accumulation in rice (*Oryza sativa* L.). Planta 242(4), 1037–1050. doi: 10.1007/s00425-015-2340-2

Lavania, M., Chauhan, P.S., Chauhan, S.V.S., Singh, H.B., Nautiyal, C.S., 2006. Induction of plant defense enzymes and phenolics by treatment with plant growth-promoting rhizobacteria *Serratia marcescens* NBRI1213. Curr. Microbiol. 52(5), 363–368. doi: 10.1007/s00284-005-5578-2

Li, Q., Ning, P., Zheng, L., Huang, J., Li, G., Hsiang, T., 2012. Effects of volatile substances of *Streptomyces globisporus* JK-1 on control of *Botrytis cinerea* on tomato fruit. Biol. Control 61(2), 113–120. doi: 10.1016/j.biocontrol.2011.10.014

Liu, F., Xing, S., Ma, H., Du, Z., Ma, B., 2013. Plant growth-promoting rhizobacteria affect the growth and nutrient uptake of *Fraxinus americana* container seedlings. Appl. Microbiol. Biotechnol. 97(10), 4617–4625. doi: 10.1007/s00253-012-4255-1

Lucas, J.A., García-Cristobal, J., Bonilla, A., Ramos, B., Gutierrez-Mañero, J., 2014. Beneficial rhizobacteria from rice rhizosphere confers high protection against biotic and abiotic stress inducing systemic resistance in rice seedlings. Plant Physiol. Bioch. 82, 44–53. doi: 10.1016/j.plaphy.2014.05.007

Małolepsza, U., 2006. Induction of disease resistance by acibenzolar-S-methyl and o-hydroxyethylorutin against *Botrytis cinerea* in tomato plants. Crop Prot. 25(9), 956–962. doi: 10.1016/j.cropro.2005.12.009

Mandal, S., Kar, I., Mukherjee, A.K., Acharya, P., 2013. Elicitor-induced defense responses in *Solanum lycopersicum* against *Ralstonia solanacearum*. Sci. World J. doi: 10.1155/2013/561056

Mehta, P., Walia, A., Kulshrestha, S., Chauhan, A., Shirkot, C.K., 2015. Efficiency of plant growth-promoting P-solubilizing *Bacillus circulans* CB7 for enhancement of tomato growth under net house conditions. J. Basic Microbiol. 55(1), 33–44. doi: 10.1002/jobm.201300562

Muleta, D., Assefa, F., Granhall, U., 2007. In vitro antagonism of rhizobacteria isolated from *Coffea arabica* L. against emerging fungal coffee pathogens. Eng. Life Sci. 7(6), 577–586. doi: 10.1002/elsc.200700004

Murashige, T., Skoog, F., 1962. A Revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15(3), 473–497. doi: 10.1111/j.1399-3054.1962.tb08052.x

Ni, Z., Kim, E-D., Chen, Z.J., 2009. Chlorophyll and starch assays. doi: 10.1038/nprot.2009.12

De Oliveira, M.F., da Silva, M.G., Van Der Sand, S.T., 2010. Anti-phytopathogen potential of endophytic actinobacteria isolated from tomato plants (*Lycopersicon esculentum*) in southern Brazil, and characterization of *Streptomyces* sp. R18(6), a potential biocontrol agent. Res. Microbiol. 161(7), 565–572. doi: 10.1016/j.resmic.2010.05.008

Palaniyandi, S.A., Yang, S.H., Suh, J.W., 2013a. Extracellular proteases from *Streptomyces phaeopurpureus* ExPro138 inhibit spore adhesion, germination and appressorium formation in *Colletotrichum coccodes*. J. Appl. Microbiol. 115(1), 207–217. doi: 10.1111/jam.12212

Palaniyandi, S.A., Yang, S.H., Zhang, L., Suh, J-W., 2013b. Effects of actinobacteria on plant disease suppression and growth promotion. Appl. Microbiol. Biotechnol. 97(22), 9621–9636. doi: 10.1007/s00253-013-5206-1

Pieterse, C.M.J., Zamioudis, C., Berendsen, R.L., Weller, D.M., Van Wess S.C.M., Bakker, P.A.H.M., 2014. Induced systemic resistance by beneficial microbes. Annu. Rev. of Phytopathol. 52, 347–375. doi: 10.1146/annurev-phyto-082712-102340

Ramos-Solano, B., Garcia-Villaraco, A., Gutierrez-Mañero, F.J., Lucas, J.A., Bonilla, A., Garcia-Seco, D., 2014. Annual changes in bioactive contents and production in field-grown blackberry after inoculation with *Pseudomonas fluorescens*. Plant Physiol. Bioch. 74, 1–8. doi: 10.1016/j.plaphy.2013.10.029

Reddy, P.P., 2013. Recent advances in crop protection. Springer, India. doi: 10.1007/978-81-322-0723-8

Sadeghi, A., Karimi, E., Dahaji, P.A., Javid, M.G., Dalvand, Y., Askari, H., 2012. Plant growth promoting activity of an auxin and siderophore producing isolate of *Streptomyces* under saline soil conditions. World J. Microb. Biotechnol. 28(4), 1503–1509. doi: 10.1007/s11274-011-0952-7

Salla, T.D., da Silva T.R., Astarita, L.V., Santarém, E.R., 2014. *Streptomyces* rhizobacteria modulate the secondary metabolism of *Eucalyptus* plants. Plant Physiol. Bioch. 85, 14–20. doi: 10.1016/j.plaphy.2014.10.008

Salla, T.D., Astarita, L.V., Santarém, E.R., 2016. Defense responses in plants of *Eucalyptus* elicited by *Streptomyces* and challenged with *Botrytis cinerea*. Planta 243. doi: 10.1007/s00425-015-2460-8

Santoro, M.V., Zygadlo, J., Giordano, W., Banchio, E., 2011. Volatile organic compounds from rhizobacteria increase biosynthesis of essential oils and growth parameters in peppermint (*Mentha piperita*). Plant Physiol. Bioch. 49(10), 1177–1182. doi: 10.1016/j.plaphy.2011.07.016

Shirling, E.B., Gottlieb, D., 1966. Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16(3), 313–340. doi: 10.1099/00207713-16-3-313

Singh, N., Marwa, N., Mishra, S., Mishra, J., Verma, P., Rathaur, S., Singh, N., 2016. *Brevundimonas diminuta* mediated alleviation of arsenic toxicity and plant growth promotion in *Oryza sativa* L. Ecotox. Environ. Safe. 125, 25–34. doi: 10.1016/j.ecoenv.2015.11.020

Song, W., Ma, X., Tan, H., Zhou, J., 2011. Abscisic acid enhances resistance to *Alternaria solani* in tomato seedlings. Plant Physiol. Bioch. 49(7), 693–700. doi: 10.1016/j.plaphy.2011.03.018

Tarkka, M.T., Lehr, M.A., Hampp, R., Schrey, S.D., 2008. Plant behavior upon contact with Streptomycetes. Plant Signal Behav. 3(11), 917–9. doi: 10.4161/psb.5996

Vos, C.M., Yang, Y., De Coninck, B., Cammue, B.P.A., 2014. Fungal (-like) biocontrol organisms in tomato disease control. Biol. Control 74, 65–81. doi: 10.1016/j.biocontrol.2014.04.004

Walia, A., Mehta, P., Chauhan, A., Shirkot, C.K., 2014. Effect of *Bacillus subtilis* strain CKT1 as inoculum on growth of tomato seedlings under net house conditions. Proc. Natl. Acad. Sci., India, Sect B Biol. Sci. 84(1), 145–155. doi: 10.1007/s40011-013-0189-3

Wan, M., Li, G., Zhang, J., Jiang, G., Huang, H-C., 2008. Effect of volatile substances of *Streptomyces platensis* F-1 on control of plant fungal diseases. Biol. Control 46(3), 552–559. doi: 10.1016/j.biocontrol.2008.05.015

Wang, Y., Luo, Q., Zhang, X., Wang, W., 2011 Isolation and purification of a modified phenazine, griseoluteic acid, produced by *Streptomyces griseoluteus* P510. Res. Microbiol. 162(3), 311–319. doi: 10.1016/j.resmic.2011.01.005

Wang, C., Wang, Z., Qiao, X., Li, Z., Li, F., Chen, M., Wang, Y., Huang, Y., Cui, H., 2013. Antifungal activity of volatile organic compounds from *Streptomyces alboflavus* TD-1. FEMS Microbiol. Lett. 341(1), 45–51. doi: 10.1111/1574-6968.12088

Wu, Y., Yuan, J.E.Y., Raza, W., Shen, Q., Huang, Q., 2015. Effects of volatile organic compounds from *Streptomyces albulus* NJZJSA2 on growth of two fungal pathogens. J. Basic Microbiol. 55(9), 1104–1117. doi: 10.1002/jobm.201400906

Zambonato, F., Federizzi, L.C., Pacheco, M.T., de Arruda, M.P., Martinelli, J.A., 2012. Phenotypic and genetic characterization of partial resistance to crown rust in *Avena sativa* L. Cbab 12(4), 261–268. doi: 10.1590/S1984-70332012000400005

Capítulo III

Considerações Finais:

5. CONSIDERAÇÕES FINAIS

Os resultados deste estudo permitem concluir que:

- Os isolados de *Streptomyces* spp. foram capazes de produzir sideróforos, solubilizar fosfato e produzir compostos orgânicos voláteis, características desejáveis para utilização de rizobactérias como PGPR.

- Todos os isolados foram capazes de hidrolisar amido e os isolados PM4, PM6 e PM9 apresentaram capacidade de hidrolisar lipídeos através da enzima lipase.

- A atividade promotora do crescimento de plantas de tomate, via VOC ou contato direto rizobacteria-raízes, permite sugerir que o isolado *Streptomyces* PM5 possa ser utilizado como promotor de crescimento vegetal.

- O potencial antagonismo do isolado PM1 contra *P. carotovorum* subsp. *brasiliensis*, juntamente com a diminuição da incidência da doença talo oco em plantas de tomate resultante da inoculação com PM1, permitem indicar este isolado como indutor de resistência do tomateiro contra Pcb.

Os resultados deste projeto de pesquisa permitiram estabelecer uma interação eficiente de, pelo menos, um isolado de *Streptomyces* spp. com plantas de tomate. Promovendo o crescimento ou a indução da defesa de plantas contra *P. carotovorum* subsp. *brasiliensis*.

6. REFERÊNCIAS BIBLIOGRÁFICAS

Adesemoye, A.O., Torbert, H.A. & Kloepper, J.W., 2008. Enhanced plant nutrient use efficiency with PGPR and AMF in an integrated nutrient management system. *Canadian journal of microbiology*, 54(10), pp.876–86. Available at: http://www.nrcresearchpress.com/doi/abs/10.1139/W08-081#.Vrt081grLIX

Ahemad, M. & Kibret, M., 2014. Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. *Journal of King Saud University - Science*, 26(1), pp.1–20.

Ahn, I.P. et al., 2011. Priming by rhizobacterium protects tomato plants from biotrophic and necrotrophic pathogen infections through multiple defense mechanisms. *Molecules and Cells*, 32(1), pp.7–14.

Alvarenga, M.A.R. ed., 2013. *Tomate: Produção em campo, casa de vegetação e hidroponia* 2ª ed., Lavras, MG: Editora Universitária de Lavras.

Ambrosini, A. et al., 2012. Screening of plant growth promoting Rhizobacteria isolated from sunflower (*Helianthus annuus* L.). *Plant and Soil*, 356(1-2), pp.245–264. Available at: http://link.springer.com/10.1007/s11104-011-1079-1

An, Y. et al., 2010. Enhanced defense responses of tomato plants against late blight pathogen *Phytophthora infestans* by pre-inoculation with rhizobacteria. *Crop Protection*, 29(12), pp.1406–1412.

ANVISA, 2014. *Programa de Análise de Resíduos de Agrotóxicos em Alimentos (PARA)*, Brasília. Available at: http://portal.anvisa.gov.br/wps/wcm/connect/d67107004634368583a5bfec1b28f937/Relatório+PARA+2012+2ª+Etapa+-+17_10_14-Final.pdf?MOD=AJPERES.

Babu, A.N. et al., 2015. Improvement of growth, fruit weight and early blight disease protection of tomato plants by rhizosphere bacteria is correlated with their beneficial traits and induced biosynthesis of antioxidant peroxidase and polyphenol oxidase. *Plant Science*, 231, pp.62–73.

Bach, E. et al., 2016. Evaluation of biological control and rhizosphere competence of plant growth promoting bacteria. *Applied Soil Ecology*, 99, pp.141–149.

Bakker, P. a H.M. et al., 2013. The rhizosphere revisited: root microbiomics. *Frontiers in plant science*, 4(May), p.165.

Bashan, Y. et al., 2014. Advances in plant growth-promoting bacterial inoculant technology: Formulations and practical perspectives (1998-2013). *Plant and Soil*, 378(1-2), pp.1–33.

Beneduzi, A. et al., 2008. Evaluation of genetic diversity and plant growth promoting activities of nitrogen-fixing bacilli isolated from rice fields in South Brazil. *Applied Soil Ecology*, 39(3), pp.311–320.

Berg, G., 2009. Plant-microbe interactions promoting plant growth and health: Perspectives for controlled use of microorganisms in agriculture. *Applied Microbiology and Biotechnology*, 84(1), pp.11–18.

Bhattacharyya, P.N. & Jha, D.K., 2012. Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World journal of microbiology & biotechnology*, 28(4), pp.1327–50.

Bitas, V. et al., 2013. Sniffing on microbes: diverse roles of microbial volatile organic compounds in plant health. *Molecular plant-microbe interactions : MPMI*, 26(8), pp.835–43.

Blancard, D., 2012. *A colour handbook : tomato diseases : identification, biology and control* / 2ª ed., Manson Publishing Ltd.

De Boer, S.H., Li, X. & Ward, L.J., 2012. *Pectobacterium* spp. Associated with Bacterial Stem Rot Syndrome of Potato in Canada. *Phytopathology*, 102(10), pp.937–947.

Boller, T. & Meins, F. eds., 2012. *Genes Involved in Plant Defense*, Springer Science & Business Media.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1-2), pp.248–254.

Burketová, L. et al., 2015. Bio-based resistance inducers for sustainable plant protection against pathogens. *Biotechnology Advances*, 33(6), pp.999–1004.

Buscaill, P. & Rivas, S., 2014. Transcriptional control of plant defence responses. *Current opinion in plant biology*, 20, pp.35–46.

Carvalho, R.D.C.P. et al., 2014. *Doenças do tomate*, Brasília. Available at: http://ptdocz.com/doc/108672/doenças-do-tomate---sociedade-brasileira-de-fitopatologia.

Chater, K.F. et al., 2010. The complex extracellular biology of *Streptomyces*: Review Article. *FEMS Microbiology Reviews*, 34(2), pp.171–198.

Chen, C. et al., 2000. Defense enzymes induced in cucumber roots by treatment with plant growth-promoting rhizobacteria (PGPR) and *Pythium aphanidermatum*. *Physiological and Molecular Plant Pathology*, 56, pp.13–23.

Chen, Y. et al., 2013. Biocontrol of tomato wilt disease by *Bacillus subtilis* isolates from natural environments depends on conserved genes mediating biofilm formation. *Environmental Microbiology*, 15(3), pp.848–864.

Chowdappa, P. et al., 2013. Growth stimulation and induction of systemic resistance in tomato against early and late blight by *Bacillus subtilis* OTPB1 or *Trichoderma harzianum* OTPB3. *Biological Control*, 65(1), pp.109–117.

Coll, N.S., Epple, P. & Dangl, J.L., 2011. Programmed cell death in the plant immune system. *Cell death and differentiation*, 18(8), pp.1247–56.

Compant, S. et al., 2005. Use of Plant Growth-Promoting Bacteria for Biocontrol of Plant Diseases : Principles , Mechanisms of Action , and Future Prospects MINIREVIEW Use of Plant Growth-Promoting Bacteria for Biocontrol of Plant Diseases : Principles , Mechanisms of Action , and. *Applied and environmental microbiology*, 71(9), pp.4951–4959.

Cordovez, V. et al., 2015. Diversity and functions of volatile organic compounds produced by *Streptomyces* from a disease-suppressive soil. *Frontiers in Microbiology*, 6(OCT), pp.1–13.

Couillerot, O. et al., 2014. Purification of antibiotics from the biocontrol agent *Streptomyces anulatus* S37 by centrifugal partition chromatography. *Journal of chromatography. B, Analytical technologies in the biomedical and life sciences*, 944, pp.30–4.

Cui, H., Tsuda, K. & Parker, J.E., 2015. Effector-triggered immunity: from pathogen perception to robust defense. *Annual review of plant biology*, 66(1), pp.487–511.

Dalmas, F.R. et al., 2011. Autochthonous *Streptomyces* regulate the metabolism of seedlings of *Araucaria angustifolia* (Coniferales) during root colonisation. *Australian Journal of Botany*, 59(2), pp.118–125.

Dangl, J.L., Horvath, D.M. & Staskawicz, B.J., 2013. Pivoting the plant immune system from dissection to deployment. *Science (New York, N.Y.)*, 341(6147), pp.746–51.

Dimkpa, C., Weinand, T. & Asch, F., 2009. Plant-rhizobacteria interactions alleviate abiotic stress conditions. *Plant, Cell and Environment*, 32(12), pp.1682–1694.

Dingle, J., Reid, W.W. & Solomons, G.L., 1953. The enzymic degradation of pectin and other polysaccharides. II—Application of the "Cup-plate" assay to the estimation of enzymes. *Journal of the Science of Food and Agriculture*, 4(3), pp.149–155.

Doumbou, C.L. et al., 2001. Actinomycetes, promising tools to control plant diseases and to promote plant growth. *Phytoprotection*, 82(3), pp.85–102.

Duarte, V. et al., 2004. Characterization of atypical *Erwinia carotovora* strains causing blackleg of potato in Brazil. *Journal of Applied Microbiology*, 96(3), pp.535–545.

Dutta, S., Mishra, A.K. & Dileep Kumar, B.S., 2008. Induction of systemic resistance against fusarial wilt in pigeon pea through interaction of plant growth promoting rhizobacteria and rhizobia. *Soil Biology and Biochemistry*, 40(2), pp.452–461.

El-Tarabily, K.A., 2008. Promotion of tomato (*Lycopersicon esculentum* Mill.) plant growth by rhizosphere competent 1-aminocyclopropane-1-carboxylic acid deaminase-producing streptomycete actinomycetes. *Plant and Soil*, 308(1-2), pp.161–174.

Van der Ent, S., Van Wees, S.C.M. & Pieterse, C.M.J., 2009. Jasmonate signaling in plant interactions with resistance-inducing beneficial microbes. *Phytochemistry*, 70(13-14), pp.1581–8.

FAOSTAT, 2013. Food and Agricultural Organization Statistical. *Annual Tomato Production*. Available at: http://faostat3.fao.org/browse/Q/QC/E [Accessed February 5, 2016].

FAOSTAT, 2012. Food and Agricultural Organization Statistical. *Top production - tomatoes*. Available at: http://faostat.fao.org/site/339/default.aspx [Accessed November 11, 2014].

Farag, M. a., Zhang, H. & Ryu, C.M., 2013. Dynamic Chemical Communication between Plants and Bacteria through Airborne Signals: Induced Resistance by Bacterial Volatiles. *Journal of Chemical Ecology*, 39(7), pp.1007–1018.

Fernández, E., Segarra, G. & Trillas, M.I., 2014. Physiological effects of the induction of resistance by compost or *Trichoderma asperellum* strain T34 against *Botrytis cinerea* in tomato. *Biological Control*, 78, pp.77–85.

Fu, Z.Q. & Dong, X., 2013. Systemic acquired resistance: turning local infection into global defense. *Annual review of plant biology*, 64, pp.839–63.

Glick, B.R., 2014. Bacteria with ACC deaminase can promote plant growth and help to feed the world. *Microbiological research*, 169(1), pp.30–9.

Glick, B.R., 2005. Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. *FEMS Microbiology Letters*, 251(1), pp.1–7.

Gopalakrishnan, S. et al., 2014. Evaluation of *Streptomyces* strains isolated from herbal vermicompost for their plant growth-promotion traits in rice. *Microbiological Research*, 169(1), pp.40–48.

Gopalakrishnan, S. et al., 2012. Plant growth-promoting traits of biocontrol potential bacteria isolated from rice rhizosphere. *SpringerPlus*, 1(1), p.71.

Gopalakrishnan, S. et al., 2015. The extent of grain yield and plant growth enhancement by plant growth-promoting broad-spectrum *Streptomyces* sp. in chickpea. *SpringerPlus*, 4(1), p.31.

Graça, A.J.P. da, 2013. *Heterose e Capacidade Combinatória de Linhagens de Tomateiro (Solanum lycopersicum L .) Pospectadas Para Dupla Finalidade*. Universidade Estadual do Norte Fluminense Darcy Ribeiro.

Graichen, F.A.S. et al., 2010. Inheritance of resistance to oat crown rust in recombinant inbred lines. *Scientia Agricola*, 67(4), pp.435–440.

Grobelak, A., Napora, A. & Kacprzak, M., 2015. Using plant growth-promoting rhizobacteria (PGPR) to improve plant growth. *Ecological Engineering*, 84, pp.22–28.

Gu, Y.Q. et al., 2007. Evaluation and identification of potential organic nematicidal volatiles from soil bacteria. *Soil Biology and Biochemistry*, 39(10), pp.2567–2575.

Gusain, Y.S. et al., 2015. Phosphate solubilizing and indole-3-acetic acid producing bacteria from the soil of Garhwal Himalaya aimed to improve the growth of rice. *Journal of Environmental Biology*, 36(1), pp.301–307.

Haas, D., Defago, G. & Défago, G., 2005. Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nature reviews. Microbiology*, 3(4), pp.307–19.

Hankin, L. & Anagnostakis, S.L., 1975. The Use of Solid Media for Detection of Enzyme Production by Fungi. *Mycological Society of America*, 67(3), pp.597–607.

Herman, M. & Williams, M., 2012. Fighting for their lives: Plants and pathogens. *The Plant Cell*, 24(6), p.tpc.112.tt0612–tpc.112.tt0612.

Hogan, C.S. et al., 2013. The type III secreted effector DspE is required early in solanum tuberosum leaf infection by *Pectobacterium carotovorum* to cause cell death, and requires Wx(3-6)D/E motifs. *PloS one*, 8(6), p.e65534.

Hyakumachi, M. et al., 2013. *Bacillus thuringiensis* suppresses bacterial wilt disease caused by *Ralstonia solanacearum* with systemic induction of defense-related gene expression in tomato. *Microbes and environments / JSME*, 28(1), pp.128–34.

JENNINGS, W. & SHIBAMOTO, T., 1980. *Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography*, Elsevier. Available at: http://www.sciencedirect.com/science/article/pii/B978012384250350009X

Jesus, J.G.R. et al., 2013. Seleção e identificação de micro-organismos produtores de amilases isolados da microbiota associada a resíduos agrícolas de cacau e dendê. *Diálogos & Ciência*, 11(33), pp.7–12.

Jog, R. et al., 2014. Mechanism of phosphate solubilization and antifungal activity of *Streptomyces* spp. isolated from wheat roots and rhizosphere and their application in improving plant growth. *Microbiology*, 160(2014), pp.778–788.

Jones, J.D.G. & Dangl, J.L., 2006. The plant immune system. *Nature*, 444(7117), pp.323–329.

Kanchiswamy, C.N., Malnoy, M. & Maffei, M.E., 2015. Bioprospecting bacterial and fungal volatiles for sustainable agriculture. *Trends in Plant Science*, 20(4), pp.206–211.

Kim, B.S., Moon, S.S. & Hwang, B.K., 1999. Isolation, identification, and antifungal activity of a macrolide antibiotic, oligomycin A, produced by *Streptomyces libani*. *Canadian Journal of Botany*, 77(6), pp.850–858.

Kloepper, J.W. et al., 1980. *Pseudomonas* siderophores: A mechanism explaining disease-suppressive soils. *Current Microbiology*, 4(5), pp.317–320.

Kloepper, J.W., Tuzun, S. & Kuć, J.A., 1992. Proposed definitions related to induced disease resistance. *Biocontrol Science and Technology*, 2(4), pp.349–351.

Kurabachew, H., Stahl, F. & Wydra, K., 2013. Global gene expression of rhizobacteria-silicon mediated induced systemic resistance in tomato (*Solanum lycopersicum*) against *Ralstonia solanacearum*. *Physiological and Molecular Plant Pathology*, 84(1), pp.44–52.

Kurabachew, H. & Wydra, K., 2013. Characterization of plant growth promoting rhizobacteria and their potential as bioprotectant against tomato bacterial wilt caused by *Ralstonia solanacearum*. *Biological Control*, 67(1), pp.75–83.

Kurabachew, H. & Wydra, K., 2014. Induction of systemic resistance and defense-related enzymes after elicitation of resistance by rhizobacteria and silicon application against *Ralstonia solanacearum* in tomato (*Solanum lycopersicum*). *Crop Protection*, 57, pp.1–7.

Kvaratskhelia, M., George, S.J. & Thorneley, R.N.F., 1997. Salicylic Acid Is a Reducing Substrate and Not an Effective Inhibitor of Ascorbate Peroxidase. *Journal of Biological Chemistry*, 272(34), pp.20998–21001.

Lakshmanan, V. et al., 2015. A natural rice rhizospheric bacterium abates arsenic accumulation in rice (*Oryza sativa* L.). *Planta*, 242(4), pp.1037–1050.

Lavania, M. et al., 2006. Induction of plant defense enzymes and phenolics by treatment with plant growth-promoting rhizobacteria Serratia marcescens NBRI1213. *Current Microbiology*, 52(5), pp.363–368.

Lee, D.H. et al., 2014. Genetic Diversity of *Pectobacterium carotovorum* subsp. brasiliensis Isolated in Korea. *The plant pathology journal*, 30(2), pp.117–24.

Lee, J. et al., 2012. Siderophore production by actinomycetes isolates from two soil sites in Western Australia. *Biometals : an international journal on the role of metal ions in biology, biochemistry, and medicine*, 25(2), pp.285–96.

Leite, L.N. et al., 2014. First report of potato blackleg caused by *Pectobacterium carotovorum* subsp. *brasiliensis* in the Netherlands. *New Disease Reports*, (29), p.24.

Li, Q. et al., 2012. Effects of volatile substances of *Streptomyces globisporus* JK-1 on control of *Botrytis cinerea* on tomato fruit. *Biological Control*, 61(2), pp.113–120.

Lin, Y. et al., 2014. Potential biocontrol *Bacillus* sp. strains isolated by an improved method from vinegar waste compost exhibit antibiosis against fungal pathogens and promote growth of cucumbers. *Biological Control*, 71, pp.7–15.

Liu, F. et al., 2013. Plant growth-promoting rhizobacteria affect the growth and nutrient uptake of *Fraxinus americana* container seedlings. *Applied Microbiology and Biotechnology*, 97(10), pp.4617–4625.

van Loon, L.C., Bakker, P. a & Pieterse, C.M., 1998. Systemic resistance induced by rhizosphere bacteria. *Annual review of phytopathology*, 36, pp.453–483.

Lucas, J.A. et al., 2014. Beneficial rhizobacteria from rice rhizosphere confers high protection against biotic and abiotic stress inducing systemic resistance in rice seedlings. *Plant Physiology and Biochemistry*, 82, pp.44–53.

Lugtenberg, B. & Kamilova, F., 2009. Plant-Growth-Promoting Rhizobacteria. *Annual Review of Microbiology*, 63(1), pp.541–556.

Małolepsza, U., 2006. Induction of disease resistance by acibenzolar-S-methyl and o-hydroxyethylorutin against Botrytis cinerea in tomato plants. *Crop Protection*, 25(9), pp.956–962.

Mandal, S. et al., 2013. Elicitor-induced defense responses in *Solanum lycopersicum* against *Ralstonia solanacearum*. *The Scientific World Journal*, 2013, pp.1–9.

Mandal, S., Mallick, N. & Mitra, A., 2009. Salicylic acid-induced resistance to *Fusarium oxysporum* f. sp. lycopersici in tomato. *Plant Physiology and Biochemistry*, 47(7), pp.642–649.

MAPA, 2008. *Produção integrada no Brasil: agropecuária sustentável - alimentos seguros* 1ª ed. Laércio Zambolim et al., eds., Brasília: Ministério da Agricultura, Pecuária E Abastecimento.

Marcuzzo, L.L., 2010. Efeito de Rizobactérias sobre o Biocontrole e Promoção de Crescimento de Plantas. *Ágora: Revista de Divulgação Científica*, 17(1), pp.1–11.

Matsuoka, H. et al., 2015. Changes in volatiles in carrots inoculated with ACC deaminase-producing bacteria isolated from organic crops. *Plant and Soil*, pp.1–14.

Mauch-Mani, B. & Slusarenko, A.J., 1996. Production of Salicylic Acid Precursors Is a Major Function of Phenylalanine Ammonia-Lyase in the Resistance of *Arabidopsis* to *Peronospora parasitica*. *The Plant cell*, 8(2), pp.203–212.

Mayer, A.M. & Staples, R.C., 2002. Laccase: New functions for an old enzyme. *Phytochemistry*, 60(6), pp.551–565.

Mehta, P. et al., 2014. Efficiency of plant growth-promoting P-solubilizing *Bacillus circulans* CB7 for enhancement of tomato growth under net house conditions. *Journal of Basic Microbiology*, (c), pp.1–12.

van der Merwe, J.J. et al., 2010. *Pectobacterium carotovorum* subsp. *brasiliensis* causing blackleg on potatoes in South Africa. *European Journal of Plant Pathology*, 126(2), pp.175–185.

Montesinos, E., 2000. Pathogenic plant-microbe interactions. What we know and how we benefit. *International microbiology : the official journal of the Spanish Society for Microbiology*, 3(2), pp.69–70.

Murashige, T. & Skoog, F., 1962. A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. *Physiologia Plantarum*, 15(3), pp.473–497.

Nabhan, S. et al., 2012. Taxonomic relatedness between *Pectobacterium carotovorum* subsp. *carotovorum*, *Pectobacterium carotovorum* subsp. *odoriferum* and *Pectobacterium carotovorum* subsp. *brasiliense* subsp. nov. *Journal of Applied Microbiology*, 113(4), pp.904–913.

Naue, C.R., Rocha, D.J.A. & Moura, A.B., 2014. Biological control of tomato bacterial spot by seed microbiolization. *Tropical Plant Pathology*, 39(5), pp.413–416.

Ni, Z., Kim, E.-D. & Chen, Z.J., 2009. Chlorophyll and starch assays. *Protocol Exchange*, (ml), pp.4–6.

Nihorimbere, V. et al., 2011. Beneficial effect of the rhizosphere microbial community for plant growth and health. *Biotechnologie Agronomie Societe Et Environnement*, 15(2), pp.327–337. Available at: <Go to ISI>://000293062900010.

Niu, D.-D. et al., 2011. The plant growth-promoting Rhizobacterium *Bacillus cereus* AR156 induces systemic resistance in arabidopsis thaliana by simultaneously activating salicylate- and jasmonate/ethylene-dependent signaling pathways. *Molecular plant-microbe interactions : MPMI*, 24(5), pp.533–542.

Núñez-Delicado, E. et al., 2005. Polyphenol oxidase from Dominga table grape. *Journal of agricultural and food chemistry*, 53(15), pp.6087–93.

Oliveira, M.F. de, Silva, M.G. da & Van Der Sand, S.T., 2010. Anti-phytopathogen potential of endophytic actinobacteria isolated from tomato plants (*Lycopersicon esculentum*) in southern Brazil, and characterization of *Streptomyces* sp. R18(6), a potential biocontrol agent. *Research in Microbiology*, 161(7), pp.565–572.

Onkendi, E.M. & Moleleki, L.N., 2014. Characterization of *Pectobacterium carotovorum* subsp. *carotovorum* and *brasiliense* from diseased potatoes in Kenya. *European Journal of Plant Pathology*, 139(3), pp.557–566.

Palaniyandi, S.A., Yang, S.H., et al., 2013. Effects of actinobacteria on plant disease suppression and growth promotion. *Applied Microbiology and Biotechnology*, 97(22), pp.9621–9636.

Palaniyandi, S.A., Yang, S.H. & Suh, J.W., 2013. Extracellular proteases from *Streptomyces phaeopurpureus* ExPro138 inhibit spore adhesion, germination and appressorium formation in *Colletotrichum coccodes*. *Journal of Applied Microbiology*, 115(1), pp.207–217.

Panda, P. et al., 2012. First report of blackleg and soft rot of potato caused by *Pectobacterium carotovorum* subsp . *brasiliensis* in New Zealand. *New Disease Reports*, (25), p.15.

Pieterse, C.M.J. et al., 2014. Induced Systemic Resistance by Beneficial Microbes. *Annual review of phytopathology*, 52, pp.347–375.

Quiroga, M. et al., 2000. A tomato peroxidase involved in the synthesis of lignin and suberin. *Plant physiology*, 122(4), pp.1119–1127.

Rajkumar, M. et al., 2010. Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. *Trends in biotechnology*, 28(3), pp.142–9.

Ramírez, M.L. et al., 2015. Molecular identification of phosphate-solubilizing native bacteria isolated from the rhizosphere of *Prosopis glandulosa* in Mexicali valley. *Genetics and Molecular Research*, 14(1), pp.2793–2798.

Ramos-Solano, B. et al., 2014. Annual changes in bioactive contents and production in field-grown blackberry after inoculation with *Pseudomonas fluorescens*. *Plant Physiology and Biochemistry*, 74, pp.1–8.

Reddy, P.P., 2014. *Plant Growth Promoting Rhizobacteria for Horticultural Crop Protection*, Available at: https://books.google.com/books?id=sWeSBAAAQBAJ&pgis=1.

Reddy, P.P., 2013. *Recent advances in crop protection*, Available at: http://www.springerlink.com/index/10.1007/978-81-322-0723-8.

Sadeghi, A. et al., 2012. Plant growth promoting activity of an auxin and siderophore producing isolate of *Streptomyces* under saline soil conditions. *World Journal of Microbiology and Biotechnology*, 28(4), pp.1503–1509.

Salas-Marina, M. a. et al., 2015. The Epl1 and Sm1 proteins from *Trichoderma atroviride* and *Trichoderma virens* differentially modulate systemic disease resistance against different life style pathogens in *Solanum lycopersicum*. *Frontiers in Plant Science*, 6(February), pp.1–13.

Salla, T.D. et al., 2014. *Streptomyces* rhizobacteria modulate the secondary metabolism of *Eucalyptus* plants. *Plant Physiology and Biochemistry*, 85, pp.14–20.

Salla, T.D., Astarita, L. V. & Santarém, E.R., 2016. Defense responses in plants of *Eucalyptus* elicited by *Streptomyces* and challenged with *Botrytis cinerea*. *Planta*. Available at: http://link.springer.com/10.1007/s00425-015-2460-8.

Sambrook, J., Fritsch, E.F. & Maniatis, T., 1989. *Molecular Cloning. A Laboratory Manual* 2ª ed., Cold Spring Harbor Laboratory Press.

Santiago, T.R. et al., 2015. Biological control of eucalyptus bacterial wilt with rhizobacteria. *Biological Control*, 80, pp.14–22.

Santoro, M.V. et al., 2011. Volatile organic compounds from rhizobacteria increase biosynthesis of essential oils and growth parameters in peppermint (*Mentha piperita*). *Plant Physiology and Biochemistry*, 49(10), pp.1177–1182.

Schöller, C.E.G. et al., 2002. Volatile metabolites from actinomycetes. *Journal of Agricultural and Food Chemistry*, 50(9), pp.2615–2621.

Schrey, S.D. & Tarkka, M.T., 2008. Friends and foes: Streptomycetes as modulators of plant disease and symbiosis. *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology*, 94(1), pp.11–19.

Sharma, A. et al., 2007. Molecular characterization of plant growth promoting rhizobacteria that enhance peroxidase and phenylalanine ammonia-lyase activities in chile (*Capsicum annuum* L.) and tomato (*Lycopersicon esculentum* Mill.). *Archives of microbiology*, 188, pp.483–494.

Shirling, E.B. & Gottlieb, D., 1966. Methods for characterization of *Streptomyces* species. *International Journal of Systematic Bacteriology*, 16(3), pp.313–340.

Silva, H.S.A. et al., 2004. Rhizobacterial induction of systemic resistance in tomato plants: non-specific protection and increase in enzyme activities. *Biological Control*, 29(2), pp.288–295.

Silva, J.B.C. da & Giordano, L. de B. eds., 2000. *Produção de Tomate para Processamento Industrial* 1ª ed., Brasília: Embrapa Hortaliças.

Singh, N. et al., 2016. *Brevundimonas diminuta* mediated alleviation of arsenic toxicity and plant growth promotion in *Oryza sativa* L. *Ecotoxicology and Environmental Safety*, 125, pp.25–34.

Smith, J.E. et al., 2014. Resistance to *Botrytis cinerea* in *Solanum lycopersicoides* involves widespread transcriptional reprogramming. *BMC genomics*, 15(1), p.334.

Song, W. et al., 2011. Abscisic acid enhances resistance to *Alternaria solani* in tomato seedlings. *Plant physiology and biochemistry : PPB / Société française de physiologie végétale*, 49(7), pp.693–700.

Sousa, C.D.S. et al., 2006. Estreptomicetos no controle da meloidoginose em mudas de tomateiro. *Pesquisa Agropecuaria Brasileira*, 41(12), pp.1759–1766.

Spoel, S.H. & Dong, X., 2012. How do plants achieve immunity? Defence without specialized immune cells. *Nature Reviews Immunology*, 12(2), pp.89–100.

Tarkka, M.T. et al., 2008. Plant behavior upon contact with Streptomycetes. *Plant signaling & behavior*, 3(11), pp.917–9.

Vos, C.M. et al., 2014. Fungal (-like) biocontrol organisms in tomato disease control. *Biological Control*, 74, pp.65–81.

Walia, A. et al., 2013. Effect of *Bacillus subtilis* Strain CKT1 as Inoculum on Growth of Tomato Seedlings Under Net House Conditions. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, 84(1), pp.145–155.

Wan, M. et al., 2008. Effect of volatile substances of *Streptomyces platensis* F-1 on control of plant fungal diseases. *Biological Control*, 46(3), pp.552–559.

Wang, C. et al., 2013. Antifungal activity of volatile organic compounds from *Streptomyces alboflavus* TD-1. *FEMS Microbiology Letters*, 341(1), pp.45–51.

Wang, Y. et al., 2011. Isolation and purification of a modified phenazine, griseoluteic acid, produced by Streptomyces griseoluteus P510. *Research in Microbiology*, 162(3), pp.311–319.

Wang, Z. et al., 2013. Fumigant activity of volatiles from *Streptomyces alboflavus* TD-1 against *Fusarium moniliforme* Sheldon. *Journal of Microbiology*, 51(4), pp.477–483.

Webb, K.J. et al., 2013. Gene expression patterns, localization, and substrates of polyphenol oxidase in red clover (*Trifolium pratense* L.). *Journal of agricultural and food chemistry*, 61(31), pp.7421–30.

Whipps, J.M., 2001. Microbial interactions and biocontrol in the rhizosphere. *Journal of Experimental Botany*, 52(suppl 1), pp.487–511.

Wu, Y. et al., 2015. Effects of volatile organic compounds from *Streptomyces albulus* NJZJSA2 on growth of two fungal pathogens. *Journal of Basic Microbiology*, 55(9), pp.1104–1117.

Zambonato, F. et al., 2012. Phenotypic and genetic characterization of partial resistance to crown rust in *Avena sativa* L. *Crop Breeding and Applied Biotechnology*, 12(4), pp.261–268.

Zámocký, M. et al., 2001. The molecular peculiarities of catalase-peroxidases. *FEBS Letters*, 492(3), pp.177–182.