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# *STREPTOMYCES* SPP. PROMOVE O CRESCIMENTO VEGETATIVO DE PLANTAS DE MILHO (*ZEA MAYS* L.) SOB ESTRESSE SALINO

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# *Streptomyces* spp. promove o crescimento vegetativo de plantas de milho (*Zea mays* L.) sob estresse salino

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Orientadora: Profa. Dra. Eliane Romanato Santarém

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**RESUMO**

As rizobactérias promotoras do crescimento vegetal (PGPR) são microrganismos com reconhecida ação sobre as plantas, afetando diretamente o metabolismo da planta, como produção de hormônios, da enzima ACC desaminase, de sideróforos, solubilização de fosfato e fixação de nitrogênio. Indiretamente, atuam no antagonismo a microrganismos fitopatogênicos (produzindo antibióticos e fenazinas), na promoção da tolerância a estresses bióticos e abióticos e na indução de resistência sistêmica. O estresse salino é um dos estresses abióticos que mais compromete o rendimento das culturas, como o milho. O excesso de sais no solo resulta em estresse osmótico e iônico, o que gera danos nutricionais e metabólicos à planta. As PGPR podem diminuir o estresse salino da planta por mecanismos de tolerância sistêmica induzida, que implica em alterações hormonais, em estímulo da defesa antioxidante, em ajuste osmótico e na expressão de genes de resposta ao estresse. O objetivo deste trabalho foi caracterizar dez isolados de rizobactérias *Streptomyces* spp. como PGPR e avaliar a ação dessas bactérias no crescimento e na atenuação dos efeitos do estresse salino em milho. A caracterização dos isolados foi realizada pela quantificação de compostos indólicos, de sideróforos e de fenazinas, por meio da tolerância à salinidade e da promoção do crescimento de plantas de milho. Para os experimentos de crescimento, as sementes de milho (*Zea mays* L.) foram microbiolizadas com os isolados de *Streptomyces* spp. e cultivadas em casa de vegetação, sendo o crescimento de parte aérea e de raiz (comprimento e matéria seca) avaliado 45 dias após plantio (estágio V5). A tolerância das plantas de milho à salinidade foi avaliada pelo cultivo de plantas submetidas às concentrações de 0, 50, 100, 150, 200 e 300 mM de NaCl, através de medidas de crescimento vegetativo. Concentrações subletais de NaCl foram determinadas e utilizadas nos experimentos seguintes. A tolerância dos isolados de *Streptomyces* spp. à salinidade foi avaliada pelo crescimento das suspensões bacterianas em presença de diferentes concentrações de NaCl. Quatro isolados de *Streptomyces* spp. caracterizados como PGPR e tolerantes à salinidade foram selecionados para testar sua ação na atenuação dos efeitos do estresse salino em plantas de milho. Plantas provenientes de sementes microbiolizadas com isolados de *Streptomyces* spp. foram submetidas às concentrações 100 e 300 mM de NaCl. Os parâmetros de crescimento vegetativo foram avaliados 20 dias após o tratamento salino. A atividade das enzimas antioxidantes ascorbato peroxidase (APX) e catalase (CAT) foi avaliada em 0, 6, 12 e 24 horas após o tratamento com NaCl. Os resultados demostraram que os isolados de *Streptomyces* spp. selecionados apresentam características de PGPR como produção de compostos indólicos e de sideróforos, sendo o CLV178 o que mais produziu esses dois compostos. Fenazinas foram encontradas somente em CLV186 e CLV194. Os isolados foram tolerantes à salinidade, crescendo em concentrações de até 300 mM. O crescimento de plantas de milho foi promovido pela interação com rizobactérias *Streptomyces*. CLV95 promoveu crescimento de raiz e de parte aérea, enquanto que o tratamento das sementes com CLV179 resultou em plantas com mais matéria seca de raiz (40%) e de parte aérea (120%) quando comparado com plantas não bacterizadas. O tratamento de sementes de milho

com CLV179 resultou na promoção significativa do crescimento sob estresse salino, independente da concentração de NaCl testada. Foi registrado aumento de 61,3% e 73% no crescimento da parte aérea a 100 e 300 mM de NaCl, respectivamente. De forma geral, a atividade de APX foi mais intensa em raízes do que em partes aéreas de plantas de milho. Resultado contrário foi observado com CAT. Especificamente, a atividade de CAT foi modulada nas raízes por CLV97, CLV178 e CLV179 em 100 mM de NaCl, enquanto que o efeito sobre a atividade de APX foi mais expressivo nas partes aéreas na mesma concentração.

**Palavras-chaves**: Actinomycetos, Estresse salino, PGPR, Rizobactérias, Sistema antioxidante.

**ABSTRACT**

Plant growth promoting rhizobacteria (PGPR) are microorganisms recognized by their positive effect on plants, directly affecting plant metabolism by production of hormones, ACC deaminase enzyme, and siderophores, as well as phosphate solubilization and nitrogen fixation. Indirectly, they act in the antagonism to phytopathogenic microorganisms (producing antibiotics and phenazines) promoting tolerance to biotic and abiotic stresses and inducing systemic resistance. Saline stress is one of the abiotic stresses that most compromises crop yields, such as maize. The excess of salts in the soil results in osmotic and ionic stress, which causes nutritional and metabolic damages to the plant. PGPR may reduce plant saline stress by mechanisms of induced systemic tolerance, which implies hormonal changes, antioxidant defense stimulus, osmotic adjustment and the expression of stress response genes. The objective of this work was to characterize the isolates of rhizobacteria from the genus *Streptomyces* spp. as PGPR and to evaluate the role that these bacteria play in the growth and in the attenuation of the effects of the saline stress in maize. The characterization of the isolates was performed by the quantification of the metabolites (indole compounds, siderophores and phenazines), the tolerance to salinity and the promotion of maize vegetative growth. For the growth experiments, maize seeds (*Zea mays* L.) were bacterized with the *Streptomyces* spp. and cultivated in a greenhouse. Growth was evaluated through the parameters of shoot and root length and dry matter, 45 days after sowing (stage V5). The tolerance of maize plants to salinity was evaluated by determining growth at the concentrations of 0, 50, 100, 150, 200 and 300 mM of NaCl. Sub lethal concentrations of NaCl were determined and used in the following experiments. The tolerance of *Streptomyces* spp. isolates to the salt stress was analyzed by the growth of the bacterial suspensions in the presence of different concentrations of NaCl. Four isolates of *Streptomyces* spp. exhibiting PGPR traits and salinity tolerance were selected and their action in attenuating the effects of saline stress on maize plants was evaluated in plants from bacterized seeds and submitted to 100 and 300 mM of NaCl. The growth parameters were evaluated 20 days after the onset of salinity stress. The activity of antioxidant ascorbate peroxidase and catalase was analyzed at 0, 6, 12 and 24 hours after treatment with NaCl. Results showed that all *Streptomyces* spp. isolates were capable to produce of indolic compounds and

siderophores, with CLV178 being the most productive of these two compounds. Phenazines were found in CLV186 and CLV194. The isolates were tolerant to salinity, growing at concentrations up to 300 mM. Growth of maize plant was favored by the interaction with rhizobacteria *Streptomyces*. CLV 95 promoted root and shoot growth, while seed treatment with CLV179 resulted in plants with a significant dry matter increase, as 40% root and 120% shoot when compared to non-bacterized seeds. Treating seeds with CLV179 resulted in significant promotion of growth under salt stress, regardless the concentration of NaCl used. An increase of 61.3% and 73% in shoot growth of the maize plants was recorded at 100 and 300 mM NaCl, respectively. Overall, APX activity was more intense in roots than in shoots of maize plants. The contrary result was observed with CAT. Specifically, CAT activity was modulated in the roots by CLV97, CLV178 and CLV179 at 100 mM NaCl, whereas the effect on APX activity was more expressive in the shoots at the same concentration.

**Key words:** Actinomycetes, Antioxidant systems, PGPR, Rhizobacteria, Salt stress.

**LISTA DE ABREVIATURAS E SIGLAS**

**ABA -** Ácido abscísico

**AIA -** Ácido indol-3-acético

**AJ** - Ácido jasmônico

**ACC –** Ácido 1-aminociclopropano-1-carboxílico

APX – Ascorbato peroxidase

**AS** - Ácido salicílico

**EROs** - Espécies reativas de oxigênio

CAT - Catalase

**FAO –** Organização das Nações Unidas para Agricultura e Alimentação (do inglês, *Food and Agricultural Organization*);

**ISP** – Meio de cultura ISP para *Streptomyces* (do inglês, *International Streptomyces Project*)

**ISR -** Resistência sistêmica induzida (do inglês, *Induced systemic resistance*)

**IST** - Tolerância sistêmica induzida (do inglês, *Induced systemic tolerance*)

**PCR –** Reação em cadeia da polimerase (do inglês, *Polymerase Chain Reaction*)

**PCA -** Fenazina 1-ácido carboxílico (do inglês, *Phenazine-1-carboxylic acid*)

**PGPR –** Rizobactérias promotoras do crescimento vegetal (*do inglês,* Plant Growth Promoting Rhizobacteria**)**

**1-OH-PHZ** – 1-Hidroxifenazina (do inglês, *2-Hydroxy-phenazine-1-carboxylic acid*)

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# Capítulo I: Introdução e Objetivos

## 1 Rizosfera e Rizobactérias

A camada de solo que circunda a zona das raízes e é intensamente influenciada pelo sistema radicular e por diversos microrganismos é denominada rizosfera. Neste ambiente, o sistema radicular está em contato direto com uma ampla gama de populações microbianas do solo (Vacheron et al., 2013; Noumavo et al., 2016). As características biológicas e físico-químicas da rizosfera dependem, em grande parte, da natureza dos compostos liberados pela raiz da planta na rizosfera. Esses exudados atraem populações de microrganismos, especialmente aqueles capazes de metabolizar esses compostos e de se proliferar nesse habitat (Vacheron et al., 2013). Algumas bactérias colonizadoras das raízes são caracterizadas como Rizobactérias Promotoras do Crescimento Vegetal (PGPR). Dentre estas, destacam-se os gêneros *Pseudomonas, Azospirillum, Azotobacter, Arthrobacter, Bacillus, Enterobacter, Serratia e Streptomyces* (Bhattacharyya e Jha, 2012; Noumavo et al., 2016).

Devido às suas características benéficas, as PGPRs têm sido apontadas como uma alternativa sustentável na agricultura (Kuan et al., 2016). O cultivo de espécies como milho (Jarak et al., 2012; Arruda et al., 2013; Kuan et al., 2016), o tomate (Ahirwar et al., 2015; Anitha e Kumudini, 2014), a canola (Grobelak et al., 2015) e o grão de bico (Gopalakrishnan et al., 2015) tem sido favorecido pela associação com PGPRs.

## 2 Mecanismos de ação de PGPRs

As PGPRs podem afetar o desenvolvimento das plantas de forma direta ou indireta. A produção de fitormônios e sideróforos, a capacidade de solubilização de fosfatos, e a fixação de nitrogênio consistem em mecanismos diretos de ação sobre as plantas. Os mecanismos indiretos incluem a capacidade de antibiose ou antagonismo a patógenos, a produção de enzimas líticas, a produção de fenazinas, e a indução de resistência sistêmica (ISR, do inglês, *Induced Systemic Resistance*) (Das et al., 2013; Kuan et al., 2016).

### 2.1 Produção de fitormônios

A produção de ácido indol-3-acético (AIA) por rizobactérias é uma característica muito comum (Kumar et al., 2012; Glick, 2014; Salla et al., 2014; Anwar et al., 2016). O aumento de AIA na rizosfera promove o crescimento da raiz e, por isso, aumenta indiretamente a capacidade de absorção de nutrientes (Ahemad et al., 2014). Bactérias como *Azospirillum* spp., *Pseudomonas* spp. e *Bacillus thuringiensis* são exemplos de PGPR produtoras de AIA (Arzanesh et al., 2011; Armada et al., 2014; Tank e Saraf, 2010). Além de auxinas, as rizobactérias podem produzir citocininas, as quais apresentam efeitos na iniciação da

raiz, na divisão e alongamento celular, e no aumento da área de superfície radicular de plantas cultivadas (Salamone et al., 2005).

## 2.2 Produção de Sideróforos

O ferro é um nutriente essencial para praticamente todas as formas de vida. No ambiente aeróbio, o ferro encontra-se na forma $Fe^{+3}$ e tende a formar hidróxidos insolúveis, tornando-se indisponível aos microrganismos (Rajkumar et al., 2009). Os sideróforos (*do grego*: "portador de ferro") são compostos quelantes de baixo peso molecular, com altíssima afinidade por ferro (Dimkpa, 2016; Tank e Saraf, 2010; Raza e Shen, 2010). Rizobactérias como *Bacillus*, *Enterobacter*, *Klebsiella*, *Pseudomonas*, *Rhodococcus* podem produzir esses compostos sob condições de estresse de ferro. Essas bactérias têm a capacidade de interagir com o $Fe^{+3}$, transportá-lo para dentro de suas células e reduzi-lo para a forma $Fe^{+2}$, que é biologicamente utilizável pelos vegetais. Sendo o ferro um nutriente essencial para as plantas, a produção de sideróforos por microrganismos como rizobactérias é importante para o desenvolvimento vegetal.

Existem mais de 500 biomoléculas que são classificadas como sideróforos. Dentre elas estão catecóis, fenóis, hidroxamato e carboxilato. Esta ampla variedade de compostos sideróforos evidencia a existência de diversos genes e reguladores que estão envolvidos na sua biossíntese, transporte e importação para célula (Visca et al., 2007).

## 2.3 Produção de fenazinas

As fenazinas são compostos heterocíclicos nitrogenados que são produzidos naturalmente por diversos microrganismos, incluindo *Pseudomonas* spp., *Streptomyces* spp., *Pelagiobacter* v*ariabilis*, *Pantoea agglomerans,* e *Vibrio* sp. (Abdelfattah et al., 2010). Atualmente, mais de 6.000 fenazinas são conhecidas, das quais cerca de 100 são produzidas por bactérias e são as mais estudadas devido à sua importância na interação com outros microrganismos, plantas e animais. A produção de fenazinas bacterianas ocorre no período de maior crescimento bacteriano, acumulando-se na fase estacionária (Sakhtah, 2013). Servem como doadores e aceptores de elétrons (Pierson e Pierson, 2010), podendo modificar o potencial redox das células, atuar como sinais que regulam a expressão gênica e contribuir para a formação de biofilme, aumentando a sobrevivência bacteriana. Em alguns casos, as fenazinas podem induzir a tolerância vegetal a estresses bióticos além de inibir diretamente organismos patogênicos (Audenaert et al., 2002; Pierson e Pierson, 2010).

A produção destes compostos heterocíclicos nitrogenados é mediada pela via do corismato, a qual produz a fenazina 1-ácido carboxílico (PCA) e a fenazina 1,6-ácido dicarboxílico (PDC), que originam uma ampla variedade de outras fenazinas (Seeger et al., 2011). A biossíntese de fenazinas pode variar em uma mesma espécie bacteriana em resposta response a alterações ambientais, nutricionais e populacionais, sugerindo que elas sejam sensíveis a variações ambientais e de hospedeiros (Pierson e Pierson, 2010;

Sakhtah et al., 2013).

## 3. *Streptomyces* spp. como PGPR

As rizobactérias do gênero *Streptomyces* têm sido cada vez mais estudadas. Elas fazem parte do grupo das Actinomycetales, que são bactérias Gram-positivas, filamentosas e produtoras de esporos. Estas bactérias produzem e secretam diversos metabólitos secundários, incluindo hormônios, antibióticos e enzimas, que podem interferir no desenvolvimento vegetal. Os estreptomicetos são abundantemente representados na análise metagenômica da rizosfera e têm sido descritos como PGPR (Viaene, 2016). Além da promoção do crescimento, isolados de *Streptomyces* spp. têm sido relacionados ao aumento da resistência a doenças em diversas espécies. Plantas de *Araucaria angustifolia* tiveram seu crescimento promovido quando tratadas com *Streptomyces* spp. (PM1, PM4 e PM9) (Dalmas et al., 2011). A caracterização bioquímica de seis isolados de *Streptomyces* spp. (PM1, PM3, PM4, PM5, PM6 e PM9) demonstrou que estes isolados são capazes de produzir AIA (ácido 3-indolacético), sideróforos, solubilizar fosfato, além de apresentar antibiose contra *Pectobacterium carotovorum* var. *brasiliensis* (Dias et al., 2017). Plantas de *Eucalyptus globulus* e *E. grandis* apresentaram modulação do metabolismo secundário quando tratadas com *Streptomyces* spp. PM5 e PM9 (Salla et al., 2014), além de apresentar redução da doença mofo cinzento quando pré-tratadas com *Streptomyces* spp. e desafiadas por *Botrytis cinerea* (Salla et al., 2016). A inoculação de *Streptomyces* (CAI-21, CAI-26 e MMA-32) garantiu crescimento significativo de plantas de grão-de-bico em 30 dias após semeadura, além de resultar em aumento da expressão dos genes da β-1,3-glucanase e sideróforo sintase (Gopalakrishnan et al., 2015). Crescimento de *Triticum aestivum* foi promovido pelo tratamento de sementes com isolados de *Streptomyces* spp. (Anwar et al., 2016).

## 4. Os estresses ambientais e a ação das rizobactérias

As plantas estão constantemente expostas a estresses bióticos e abióticos, por isso, precisam lidar com tais adversidades para conseguirem sobreviver. Ambos os tipos de estresses atuam em nível celular, alterando vias de sinalização que influenciam na produção de espécies reativas de oxigênio - EROs (radicais superóxido e hidroxila, peróxido de hidrogênio, dentre outros), ácido abscísico (ABA), ácido salicílico (AS), ácido jasmônico (AJ) e etileno (Forieri et al., 2015; Jha e Subramanian, 2015). Para amenizar os estresses bióticos, as plantas contam com um sistema de defesa complexo que as auxilia na percepção e combate de microrganismos patogênicos. Ao contrário do estresse biótico, o abiótico envolve uma ampla variedade de alterações ambientais. Os estresses abióticos são causados por oscilações na quantidade de $CO_2$, por alterações na temperatura, no pH, na disponibilidade de água e luz, e pela presença de sais no solo.

A salinidade é um dos estresses abióticos que mais compromete o crescimento e a produtividade

das culturas em todo o mundo (Vaidyanathan et al., 2003). A escassez de água e a salinização do solo são os dois principais fatores limitantes para a agricultura sustentável em regiões áridas e semiáridas no mundo (Kang et al., 2017). O aumento da população mundial representa um desafio para as práticas agrícolas existentes, tendo em vista que a demanda na produção de alimentos é cada vez maior e ocasiona a superexploração de áreas agrícolas (Bharti et al., 2016). Estima-se que até 2050 a população sofra um aumento de 29%, e com isso a produção de alimentos deverá aumentar em 70% (FAO, 2017). Além disso, baixos índices pluviométricos e alta evaporação em regiões áridas e semiáridas contribuem com o aumento na salinização do solo (Mahajan and Tuteja, 2005; Porcel et al., 2012).

Concentrações elevadas de sais podem causar estresse osmótico, toxicidade, além impedir a captação de nutrientes pelas plantas (Läuchli e Grattan, 2014). Os solos são classificados como salinos quando a condutividade elétrica do saturado é igual ou maior que 4 dS/m, o que é equivalente a aproximadamente 40 mM de NaCl, e gera uma pressão osmótica de 0,2 MPa (Munns e Tester, 2008). Solos com condutividade elétrica igual ou menos que 2 dS/m são considerados neutros (Paul e Lade, 2014).

### 4.1. Efeitos celulares da salinidade e mecanismos de tolerância vegetal

Para entender os mecanismos fisiológicos para a tolerância a salinidade é necessário determinar se o crescimento da planta está sendo limitado pelo efeito osmótico do sal presente no solo ou pelo efeito tóxico da absorção de sal pela planta (Munns et al., 2016). De forma geral, as consequências deletérias de altas concentrações de sais nas plantas são o aumento da síntese de etileno e ácido abscísico (ABA) pelas raízes, choque hiperosmótico e desbalanço iônico (Nadeem et al., 2007; Geng et al., 2013).

O estresse osmótico afeta o crescimento da planta imediatamente após o aumento da concentração de sal ao redor das raízes (Munns et al., 2016). O acúmulo excessivo de sais no solo causa uma redução no potencial osmótico da solução do solo, restringindo a absorção de água pelas raízes e promovendo a desidratação da planta (Bartels e Sunkar, 2005). Além disso, o estresse reduz a taxa fotossíntetica e gera EROs, que danificam os componentes celulares, como membranas, pigmentos e ácidos nucleicos (Ibrahim, 2016; Islam et al., 2016). Geng et al. (2013) demonstraram que o crescimento das raízes de *Arabidopsis* foi rapidamente afetado pelo estresse salino, passando por estágios de parada no crescimento, quiescência e por fim recuperação e homeostase. O componente osmótico do estresse salino impediu o alongamento celular imediatamente após o tratamento de 140 mM NaCl, embora a recuperação parcial do crescimento tenha ocorrido 8 horas após esse tratamento. Os autores atribuem a recuperação do crescimento à absorção de íons orgânicos e síntese de osmólitos pela planta, que reduzem o potencial hídrico das células até que o alongamento celular pudesse ser retomado.

A absorção de íons através das raízes em solos salinos pode levar ao desbalanço iônico. Plantas que crescem sob altas concentrações de NaCl acumulam $Na^+$ e $Cl^-$ simultaneamente. Altas concentrações

de $Cl^-$ reduzem a capacidade fotossintética devido à degradação da clorofila e ao comprometimento do fotossistema II. Altas concentrações de $Na^+$ interferem no influxo de $Ca^{+2}$ e $K^+$ afetando a regulação estomática e reduzindo a fotossíntese e o crescimento (Tavakkoli et al., 2010).

A fim de ajustar o estado redox celular e reduzir o efeito tóxico de salinidade, o sistema antioxidante da planta deve ser ativado. Para proteger contra o estresse oxidativo, as células vegetais produzem enzimas antioxidantes, como superóxido dismutase (SOD), peroxidase (POX), catalase (CAT) e ascorbato peroxidase (APX), e ainda, antioxidantes não enzimáticos, tais como o ascorbato, glutationa, e α-tocoferol (Jha e Subramanian, 2015). O aumento da atividade das enzimas antioxidantes atua como um sistema de controle de danos, e promove proteção contra o estresse oxidativo, que de outra forma poderia causar peroxidação de lipídeos, resultando em danos a membrana celular e organelas, a proteínas e a estrutura do DNA, inibir a fotossíntese e a atividade de outras enzimas. A enzima SOD é uma enzima antioxidante presente em todos os organismos aeróbicos e compartimentos subcelulares propensos a uma explosão oxidativa (Nascimento e Barrigossi, 2014). A SOD catalisa a dismutação intracelular do superóxido em oxigênio e peróxido de hidrogênio, diminuindo o risco de formação de radicais hidroxila, que são extremamente tóxicos para a célula (Gill e Tuteja, 2010). A catalase é indispensável para a desintoxicação das células das plantas em condições de estresse, pois é responsável pela dismutação direta do peróxido de hidrogênio em água e oxigênio (Gill e Tuteja, 2010). A enzima POX catalisa a oxidoredução entre peróxido de hidrogênio e vários redutores, participa de processos fisiológicos vegetais, como a lignificação, suberização, formação e reticulação de componentes da parede celular, catabolismo de auxinas, senescência, proteção contra o ataque de patógenos, insetos e estressores abióticos. Suas funções enzimáticas têm sido relacionadas com muitos processos de desenvolvimento de defesa da planta em respostas a estresses bióticos e abióticos (Gulsen et al., 2010). A APX catalisa a conversão de $H_2O_2$ em água, sendo que o ascorbato atua como doador de elétrons. Em geral, as atividades de APX aumentam em plantas expostas a vários estresses ambientais (Wang et al., 2005).

## 4.2 Rizobactérias na tolerância ao estresse

O microbioma associado às raízes diminui o estresse vegetal por vários mecanismos (Berg et al., 2013). As PGPR podem induzir tolerância ao estrese salino através da elicitação do chamado processo de tolerância sistêmica induzida (IST, do inglês, *Induced Systemic Tolerance*), o qual envolve alterações como modulação dos níveis hormonais, defesa antioxidante, ajuste osmótico, expressão de genes de resposta ao estresse, produção de exopolissacarídeos e de compostos orgânicos voláteis (revisado por Kaushal e Wani, 2016; Yang et al., 2009).

Bactérias PGPR podem aliviar o estresse salino de plantas sensíveis ao sal por meio da indução de enzimas antioxidantes. Plantas de alface submetidas a severo estresse salino, inoculadas previamente com PGPR, apresentaram aumento da atividade das enzimas POX e catalase, bem como maior tolerância à salinidade (Kohler et al., 2009). Plantas de arroz (*Oryza sativa L.*), inoculadas com *B. pumilus* e *P.*

*pseudoalcaligenes* e submetidas a diversas concentrações de sais apresentaram aumento da atividade das enzimas antioxidantes POX, CAT e SOD (Jha e Subramanian, 2013).

## 5. A cultura do milho

O milho (*Zea mays* L.) é uma espécie da Família Poaceae, originada no México ou América Central e é uma das culturas mais antigas do mundo. Sua importância econômica é caracterizada por suas diversas formas de sua utilização, que incluem desde a alimentação animal até a indústria de alta tecnologia, incluindo a produção de biocombustíveis. No Brasil, o milho em grão é utilizado principalmente para alimentação animal, embora seja bastante consumido por humanos, sendo encontrado em óleos, farinhas e cereais matinais.

A produção mundial deste cereal deve atingir 1.064.828toneladas em todo o mundo na safra de 2017/2018 e 95.000 toneladas no Brasil. Neste período, o consumo do cereal deve ficar em aproximadamente 1.038.796 toneladas no mundo e no Brasil em 61.500 toneladas (USDA, 2016).

Embora a salinidade nos solos possa ocorrer naturalmente, práticas inadequadas de cultivo também contribuem para a salinização da rizosfera (Mahajan e Tuteja, 2005), o que tem se tornado um sério problema a produção de milho, que é particularmente vulnerável à salinidade (Mejía, 2003). Estudos indicam que rizobactérias *Pseudomonas syringae*, *P. fluorescens*, *Enterobacter aerogenes* e *Azospirillum* podem amenizar os efeitos citológicos do estrese salino em plantas de milho (Dimpka et al., 2009; Nadeem et al., 2007).

Nesse sentido, a identificação de microrganismos *Streptomyces* que favoreçam o crescimento de plantas de milho e que sejam capazes de atenuar os efeitos deletérios da salinidade poderá representar a possibilidade de elaboração de um biofertilizante, com potencial para comercialização.

## 6. HIPÓTESES

- Os isolados de *Streptomyces* spp. testados apresentam características de PGPR em condições naturais e salinas;
- Os isolados de *Streptomyces* spp. são capazes de promover a tolerância de plantas de milho à salinidade.

## 7. OBJETIVOS

### 7.1 Objetivo geral

- Caracterizar isolados de rizobactérias *Streptomyces* spp. como PGPR e avaliar a ação dessas bactérias no crescimento e na atenuação dos efeitos do estresse salino em plantas de milho (*Zea mays* L.).

### 7.2 Objetivos específicos

- Obter isolados de *Streptomyces* spp. a partir de fragmentos de solos com raízes, coletados de diferentes estados brasileiros;
- Caracterizar os isolados de *Streptomyces* spp. como produtores de AIA, sideróforos e de fenazinas;
- Verificar a tolerância à salinidade dos isolados *Streptomyces* spp. submetidos ao estresse salino e a produção de AIA em tais condições;
- Avaliar o efeito da inoculação de isolados de *Streptomyces* spp. no crescimento de plantas de milho em casa de vegetação;
- Avaliar o efeito da inoculação de isolados de *Streptomyces* spp. no crescimento de plantas de milho, submetidas ao estresse salino, em casa de vegetação;
- Avaliar a atividade de enzimas antioxidantes APX e CAT em plantas de milho tratadas com *Streptomyces* spp. e submetidas ao estresse salino.

# Capítulo II: Manuscrito submetido a periódico científico

## *Streptomyces* spp. enhanced vegetative growth of maize plants under saline stress

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**Abstract**

Saline stress is one of the abiotic stresses that most compromises the yield of crops of interest, such as maize. Plant growth promoting rhizobacteria (PGPR) can reduce plant saline stress by mechanisms of induced systemic tolerance. This work characterized the isolates of rhizobacteria of the genus *Streptomyces* spp. as PGPR and evaluated their role on growth and alleviation of the effects of the saline stress in maize. The production of indolic compounds, siderophores and phenazines was determined. The ability to promote growth (root and shoot length and dry mass) was evaluated in maize (*Zea mays* L.) plants cultivated in greenhouse, 45 days after *Streptomyces*-treated seeds were sowed. Sublethal concentrations of NaCl were determined in maize plants by culturing plants at concentrations from 0 to 300 mM NaCl. Four isolates of *Streptomyces* spp. exhibiting PGPR traits and salinity tolerance were selected and their implication on tolerance of maize plants to saline stress was evaluated in plants obtained from bacterized seeds and submitted to 100 e 300 mM NaCl, 20 days after the onset of salinity stress. The activity of antioxidant enzymes catalase (CAT) and ascorbate peroxidase (APX) was analyzed at 0, 6, 12 and 24 hours after treatment with NaCl. All *Streptomyces* spp. were capable to produce indolic compounds and siderophores, being CLV178 the best producer of these two compounds. Phenazines were found only in CLV186 and CLV194, and all isolates were tolerant to salinity, growing at concentrations up to 300 mM. Evaluation of growth parameters showed that bacterization with the isolate CLV179 resulted in growth of leaves and roots, increasing 130% leaf dry mass. Although CLV127 also promoted root length, it negatively affected leaf growth by approximately 10%. The other isolates tested did not affected growth and the results were comparable to the non-bacterized plants. In the roots, CAT activity was modulated by CLV97, CLV178 and CLV179 at 100 mM NaCl. CLV95, CLV97, and CLV178 induced APX activity in leaves from plants treated with 100 mM NaCl.

**Key words: Abiotic stress,** Actinomycetes, Antioxidant systems, Salt stress, PGPR

## 1. Introduction

Plants are constantly exposed to biotic and abiotic stresses, and they must deal with such adversities to survive. Abiotic stresses such as soil salinization can compromise growth and productivity of crops worldwide (Maathuis, 2014; Bacilio et al., 2016). This process consists in increasing the concentration of salts in the soil, which affects the crop yield and quality. Salinization occurs due to low rainfall rates, coupled with high evaporation in arid and semi-arid regions and inadequate cropping practices (Mahajan and Tuteja, 2005; Porcel et al., 2012). In addition, the continuously growing world population poses a challenge to existing managing of agricultural practices. The need to produce more food is parallel to impressive losses of arable land due to the increasing severity of soil annihilation by abiotic environmental conditions such as salinity (Bharti et al., 2016).

Most crops are highly susceptible to saline soil (Ibrahim, 2016). Thus, plants that grow under high concentrations of NaCl have their development limited either by the osmotic effect of the salt present in the soil or by the toxic effect of salt absorption by the plant (Munns et al., 2016). Intracellular accumulation of $Na^+$ and $Cl^-$ causes ionic imbalance and promotes nutritional damages, such as the reduction of $Ca^{+2}$ and $K^+$ influx, metabolic damages to stomatal regulation and photosynthetic rate, as well as induced hyperosmotic stress (Bharti et al., 2016).

Salinity stress generates reactive oxygen species (ROS), namely, $H_2O_2$, $O^{-2}$, and $OH^-$ that disrupt normal metabolism through damaging the DNA, RNA, and proteins as well as causing lipid peroxidation (Miller et al., 2010). These ROS compounds also cause chlorophyll destruction and damage the root meristem activity (Foreman et al., 2003; Jaleel et al., 2009). Moreover, plants under saline stress have their ethylene levels increased, which may inhibit root and shoot growth, suppress leaf expansion, and promote epinasty (Glick, 2004).

Towards a sustainable agricultural vision, crops need to provide disease resistance, tolerance to salt, drought, and heavy metal stresses, as well as better nutritional value. For this to happen, one possibility is to use soil microorganisms that increase the nutrient uptake capacity and water use efficiency (Armada et al., 2014). The microbial population present in the rhizosphere is relatively different from that of its surroundings due to the presence of root exudates that function as a source of nutrients for microbial growth (Burdman et al., 2000). There is clear evidence that a diverse group of root-associated microbes is essential for promoting plant adaptation to salinity (Turner et al., 2013; Zelicourt et al., 2013; Munns and Gilliham, 2015; Tkacz and Poole, 2015). The term “plant growth promoting bacteria - PGPR” refers to bacteria that colonize the roots of plants and enhance plant growth. Among PGPR, the genera *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Arthrobacter*, *Bacillus*, *Enterobacter*, *Serratia* and *Streptomyces* stand out (Bhattacharyya and Jha, 2012; Gray and Smith, 2005; Noumavo et al., 2016). These microorganisms act by direct mechanisms such as biofertilization (growth stimulus) and rhizoremediation (plant stress control), and by indirect means which are related to biological control,

reducing the impact of diseases, including antibiosis and competition for nutrients and niches (Hra et al., 2014).

PGPR have been reported to induce tolerance to saline stress through the elicitation of the so-called Induced Systemic Tolerance (IST), which involves modulation of hormone levels, antioxidant defense, osmotic adjustment, expression of stress response genes, as well as production of exopolysaccharides and volatile organic compounds (reviewed by Kaushal and Wani, 2016). The most common form of auxin, the indolacetic acid (IAA), produced by rhizobacteria may help the plant to overcome the negative effects of saline stress by altering the root morphology, favoring its growth, which will assist the water uptake. On the other hand, cytokinins may help stomatal regulation and prevent loss of water by the leaf (Zaidi et al., 2009; Ahemad et al., 2014).

In addition, PGPR can also relieve saline stress from salt-sensitive plants by inducing antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POX) and catalase (CAT), and non-enzymatic antioxidants such as ascorbate, glutathione, and α-tocopherol (Jha and Subramanian, 2015). Ascorbate peroxidase (APX) has vital defensive role against ROS (Apel et al., 2004) and can catalyze the breakdown of $H_2O_2$ that is produced by SOD. Catalase reduces ROS levels by catalyzing the breakdown of $H_2O_2$ into $H_2O$ and $O_2$ (Mhamdi et al., 2010).

Despite all these potentialities, although the phenazines produced by rhizobacteria are recognized for their antimicrobial property, they also may aid plant development and mitigate stress, due to their ability to modify the redox potential of cells, regulate gene expression, and assist in biofilm formation, increasing bacterial survival (Audenaert et al., 2002; Guttenberger et al., 2017).

Maize (*Zea mays* L.) is one of the most important crops both for human and animal consumption. The world production of this crop was approximately 1,075 million metric tons of grain in the period 2016/2017 (USDA, 2018). Considering that salinization is a worldwide problem and that maize is vulnerable to salinity (Mejía, 2003), the identification of *Streptomyces* spp. that favor growth of maize plants and that are able to attenuate the deleterious effects of salinity may represent the possibility for formulation of a biofertilizer with application on environmentally sustainable agriculture systems.

Thus, the objective of the present study was to characterize ten isolates of rhizobacteria *Streptomyces* spp. as PGPR and investigate whether the selected isolates can promote growth and help maize plants to attenuate the effects of saline stress.

## 2. Material and Methods

### *2.1. Selection and identification of rhizobacteria isolates*

Rhizobacteria were isolated from soil samples collected from rhizospheres of maize (*Zea mays* L.) at Mato Grosso do Sul (22° 28' 19,33" S) and Paraná (54° 48' 30,83" W), of soybean (*Glycine max* L. Merril) at Mato Grosso (15º 33' 32" S, 54º 17' 46" W), and from melon (*Cucumis melo* L.) at Ceará (04º 33' 42" S, 37º 46' 11" W), comprising different regions of Brazil. Soil samples were oven-dried at 30 ºC for seven days and stored at -20 ºC. Dissociation of the microorganisms from the roots was carried out by agitation in HCN liquid medium (Nomura and Hayakawa, 1988) at 100 rpm, 42 °C for 30 min. A volume of 100 µL of the dilution (1:10, v/v) was plated on ISP2 or ISP4 medium (Shirling and Gottlieb, 1966), supplemented with the antibiotics cycloheximide (100 µg $mL^{-1}$) and nalidixic acid (50 µg $mL^{-1}$), and the antifungal nystatin (100 µg $mL^{-1}$), and maintained for 15 days at 28 °C (Schrey et al., 2012). Plates from each soil sample were monitored for approximately 15 days and actinobacteria were selected using stereoscope (magnification at up to 40-fold), based on typical morphology of the genus *Streptomyces*, such as colony morphology, mycelia color and microscopic traits (Dhanasekaran and Jiang, 2016). Isolates with *Streptomycetes* morphology were cultured again on ISP2 or ISP4 medium containing the same antimicrobial components. After 10 days, the selected bacteria were submitted to Gram staining to confirm the Gram-positive characteristics. The identified isolates were stored in 50% glycerol at -80 °C as part of the Collection of Microorganisms of the Plant Biotechnology Laboratory (CLV) - PUCRS.

### *2.2. Characterization of* Streptomyces spp. *isolates as PGPR*

The determination of PGPR traits such as production of indolic compounds, siderophore, ACC deaminase, and phenazines, as well as promotion of plant growth was conducted using bacterial suspensions. For all experiments, cultures were grown in 10 mL ISP2 or ISP4 liquid medium, depending on which the medium provided optimal growth for each isolate (data not shown). Culture conditions were 28 ºC and 100 rpm for three to five days. The bacterial suspension was centrifuged at 2,500 g, the pellet was washed and suspended in sterile distilled water and the optical density was set to 1 ($10^8$ cfu $mL^{-1}$).

The ability of the *Streptomyces* isolates to produce indolic compounds was analyzed by the Salkowski method, with modifications (Dalmas et al., 2011). To the supernatant obtained from the rhizobacteria culture, 1 ml of the Salkowski reagent was added. Reaction was incubated for 30 min in the dark and absorbances were determined at 530 nm. Culture medium was used as blank. Quantifications were carried out in at least 15 replicates. Fresh mass of the pellet was determined. Concentration of indolic compounds was determined based on the calibration curve of IAA at 5, 10, 20, 30, 40, 50, 100, 150, and 200 µg of IAA $mL^{-1}$ and expressed in µg of IAA $g^{-1}$ bacterial cells.

Production of siderophores by *Streptomyces* spp. was tested by culturing the isolates in into CAS-LB agar (Chrome Azurol S; Lakshmanan et al., 2015) following Dias et al., (2017). Briefly, aliquots of 100 µL of the bacterial suspension were inoculated into wells (5 mm) made in the CAS-LB agar

agar. Sterile distilled water was used as control. Each isolate was inoculated into three wells. Plates were incubated at 28 ± 2 °C. The change of the medium color from bluish to yellowish-orange after incubation of *Streptomyces* spp. indicates the presence of siderophores. After three days, the halos around the wells were measured. It was considered positive for siderophores production the isolate that produced halos of at least 2 mm of border. Data were expressed as mean of the halo border.

The production of the phenazines, 1-hydroxyphenazine (1-OH-PHZ) and phenazine 1- carboxylic acid (PCA) was evaluated in the supernatant of a culture. One mL of culture supernatant was dried under a stream of air to one third of the initial volume. Analysis was performed by high performance liquid chromatography (HPLC) using a Sikam Chromatography TM S 600 system, MetaSil ODS reverse phase column 5 µm, 250 x 4.6 mm), and UV/VIS detector Model 3345 DAD, set at 367 nm. The equipment was operated at 40 °C and the chromatographic data obtained and processed by the Clarity Chromatography Software data system. The solvents used were 2.5% formic acid in water (v/v) (eluent A) and 2.5% formic acid in acetonitrile (eluent B). The linear gradient of the mobile phase will consist of 0-15% of eluent B from 0 to 2 minutes; 15-83% B of 2-14 minutes; 83-0% B for 14-16 minutes, and 0% B for up to 20 min (modified from Kern and Newman, 2014). The flow was maintained in 1 mL $min^{-1}$ and injection volume was 20 µL. The chromatography was performed in duplicate. The areas obtained in the chromatograms were compared to the calibration curves established with phenazine standards (Sigma-Aldrich™). The concentration was expressed in µg of phenazine per g of bacteria cells.

### *2.3. Tolerance of* Streptomyces *spp. to salinity*

The *Streptomyces* spp. isolates were tested for tolerance to the saline stress. Isolates were cultured in ISP2 or ISP4 liquid medium supplemented with NaCl at the concentrations of 50, 100, 150, 200 and 300 mM. As a control, the ISP2 or ISP4 media without NaCl supplementation was used. The cultures were maintained at 28 °C for five days. Bacterial growth was evaluated by the pellet mass (g) and expressed as mean of eight replicates. The isolates were also evaluated for their ability to produce indolic compounds under saline solutions, as described above.

### *2.4. Promotion of plant growth by* Streptomyces

The experiment of plant growth promotion was carried out under greenhouse conditions (19 to 28 ºC and 800 µmol $m^{-2}$ $s^{-1}$ of photosynthetic photon flux density) using maize bacterized seeds. The isolates were grown in ISP2/ISP4 liquid medium, at 28 °C, for five days. After culturing, the bacterial suspension was centrifuged, rinsed, resuspended in water, and adjusted to approximately $10^8$ cfu $mL^{-1}$. Maize seeds (*Zea mays* L., Maximus Viptera 3; Syngenta, Brazil) were aseptically bacterized for 20 minutes under gentle manual shaking. Afterwards, seeds were briefly blotted-dried and sown in plastic bags (1000 $mm^3$) filled with commercial organic soil (13% clay, 7.7% organic matter and ground calcareous rock; pH 6.6 [measured in water 1:5 (w/v)]; 260 µS $cm^{-1}$). The control was conducted with seeds treated with sterile

water. Irrigation was carried out whenever necessary. Thirty seeds were used per treatment (*Streptomyces* isolate).

Plant growth analysis was performed at stage V5 (approximately 45 days after sowing), and growth parameters of dry mass (g) and length of root and shoot (cm) were evaluated. The dry mass was determined after oven-drying leaves and roots separately, at 70 °C, for five days. Data were expressed in increment (%) of growth related to the control treatment as average of 30 plants per treatment. Scanning electronic microscopy (SEM) was used to confirm the colonization of maize roots by rhizobacteria. Sample preparation followed Dalmas et al. (2011).

*2.5. Tolerance of maize plants to salt stress*

Tolerance and survival of maize plants to salt were evaluated to determine sublethal concentrations of NaCl. In greenhouse, maize plants at V5 stage were treated with NaCl at concentrations of 50, 100, 150, 200 and 300 mM (1.64, 2.98, 3.07, 2.70 and 5.66 µS $cm^{-1}$, respectively). Plants submitted to the concentrations of 100, 150 and 200 and 300 mM NaCl were gradually adapted, starting the irrigation with 50 mM solution, and every three days increasing the NaCl concentration until reaching the concentration established for the treatment (Cardinale et al., 2015). Water was supplied every two days at the base of the bag through the entire period of the experiment to avoid any drought effect. After 20 days, parameters of vegetative growth were analyzed. Twenty plants were used per treatment (NaCl concentration), consisting of two seeds per bag. From the data obtained, sub-lethal concentrations were determined and used in the following experiments.

*2.6. Effect of* Streptomyces *spp. on tolerance to salt stress of maize plants*

Four isolates were chosen to evaluate the effect of *Streptomyces* on reducing salt stress of maize plants. The criterion of choice was based on the isolate that presented at least two characteristics of PGPR, promoted plant growth and showed tolerance to salt stress. The selected isolates were cultured in ISP2/ISP4 liquid medium, in duplicate for five days. Bacterization was performed as described above. Plants were established for 45 days prior salinization to allow adequate plant growth and root colonization. Salt stress was established using the NaCl concentrations determined in previous experiment (0, 100 e 300 mM). The control plants were treated with water. Irrigation was performed every two days at the base of the bag. Plant growth and survival were evaluated after 60 days after treatment. Growth parameters such as fresh and dry mass (g), root and leaf length (cm) and stalk diameter (cm) were analyzed. Plants were also analyzed for the enzyme activities of the antioxidant system. Colonization of maize roots by rhizobacteria under saline stress was evidenced by SEM (Dalmas et al., 2011).

The oxidative stress caused by salinity was analyzed through the activity of the antioxidant enzymes catalase (CAT, EC 1.11.1.6) and ascorbate peroxidase (APX, EC 1.11.1.11). Leaf samples of *Streptomyces*-pretreated plants subjected to saline stress were collected separately at 0, 6, 12 and 24 hours after reaching the final concentration of NaCl and frozen in liquid $N_2$ until biochemical analyzes. For

extraction of the enzymes, 0.5 g of leaves was homogenized in 2.5 mL of 50 mM potassium phosphate buffer (pH 7.0) containing 1% PVP and 0.1 mM EDTA. The homogenate was centrifuged at 3,200 g for 20 min at 4 °C and the supernatants were used for enzyme assays. CAT activity was measured from 0.5 M potassium phosphate buffer pH 7, 12 mM hydrogen peroxide and 50 µL extract. The enzymatic activity was evaluated by the rate of decomposition of $H_2O_2$ at 240 nm every 7 seconds resulting in 8 readings. APX activity was measured according to Nakano and Asada (1981) by monitoring the oxidation rate of ascorbate at 290 nm in reaction medium containing 50 mM potassium phosphate buffer pH 7, 0.5 mM ascorbic acid, 0.1 mM $H_2O_2$ and 50 µL extract. The quantification was performed by spectrophotometry. The total protein content was quantified by the spectrophotometry using NanoDrop Lite (ThermoScientific, USA).

### *2.7. Molecular identification of isolates*

All bacterial isolates used in this work were subjected to taxonomic identification. Isolates were cultured in 10 mL of ISP2/ISP4 liquid medium, incubated at 28 °C with 100 rpm shaking for 3 days. Bacterial genomic DNA was extracted by the Wizard® kit following manufacturer's instructions. DNA extracted from each isolate was used for PCR amplification of the complete 16S rDNA sequence using the following primer oligonucleotides: 9f (5'-AGAGTTTGATCCTGGCTCAG-3'), 1542r (5'-AGAAAGGAGGTGATCCAGCC-3'), MG3f (CAGCAGCCGCGGTAATAC) and 800r (TACCAGGGTATCTAATCC) which result in a fragment of approximately 1,500 bp. PCR conditions involved denaturation at 94 °C for 2 min, followed by 30 cycles at 94 °C for 45 s, 55 °C for 45 s and 72 °C for 60 s, and one last cycle at 72 °C for 6 min using the Taq Platinum Enzyme Kit (Invitrogen ™). The PCR product was sequenced by Myleus facility (Belo Horizonte, Brazil) and the obtained sequences were compared to those from the GenBank database of NCBI using the Nucleotide BLAST analysis tool (http://blast.ncbi.nlm.nih.gov/).

### *2.8. Statistical analysis*

Data from PGPR characterization were submitted to the homogeneity of variances test (Levene) and analyzed by ANOVA. Means were separated by the Duncan's multiple range test ($P$ = 0.05). Data from plant growth were analyzed by Student's T-test, $P$=0.05. ANOVA two-way was used for evaluating the interaction between the factors (time x salinity treatment), at $P$ = 0.05.

## 3. Results

### *3.1. Selection and identification of rhizobacteria isolates*

Ten actinomycetes were isolated from the four rhizospheric soil samples. All of them showed typical morphology of *Streptomyces* spp. and were able to grow in either ISP2 or ISP4 at 28 ºC. All selected isolates were confirmed as belonging to the genus *Streptomyces* based on the 16S rDNA gene partial sequences. The *Streptomyces* sp. 16S rDNA sequences were deposited in GenBank database and accessions numbers were assigned as: CLV95 - MN461005, CLV97- MN461006, CLV127-MN461007, CLV178-MN461008, CLV179-MN461009, CLV18-MN461010, CLV188-MN461011, CLV193- MN461012, CLV194-MN461013, CLV205-MN461014.

### *3.2. Characterization of Streptomyces spp. isolates as PGPR*

The rhizobacteria isolates with morphology of *Streptomyces* spp. showed characteristics that allow them to be considered PGPR. All isolates were able to produce indolic compounds, siderophores and phenazines, although variations among the isolates were recorded. Quantitative analysis of the culture supernatant of the *Streptomyces* spp. isolates showed variable production of indolic compounds (IC) (Table 1). The isolate CLV178 produced the highest concentration of IC, followed by isolate CLV186. CLV95 and CLV194 also produced IC over 100 μg $g^{-1}$ cells. The other isolates produced less than 25 μg $g^{-1}$ cell, and the lowest concentration of IC was obtained with CLV127 (Table 1). Similar to the production of IC, CLV178 showed a highlighted production of siderophores, reaching a halo border of 1.91 cm (Table 1; Fig. 1). The isolates CLV97, CLV193 and CLV194 were also effective on siderophore production. CLV127 and CLV205 were the isolate with the lowest production of siderophores (Table 1).

Phenazines were only detected in the supernatant obtained from CLV194 and CLV186 cultures. CLV194 produced showed 1-OH-PHZ and CLV186 produced both phenazines analyzed, and the concentration of 1-OH-PHZ was 2.2 -fold compared to CLV194 (Table 1).

### *3.3. Tolerance of Streptomyces* spp. *isolates to salinity*

All isolates were able to grow in culture medium supplemented with 50 to 300 mM of NaCl. Most of isolates were not affected by high NaCl concentrations. Indeed, NaCl at 200 and 300 mM promoted growth of CLV178 and CLV127, respectively (Table 2). The only isolate that had its growth impaired by 300mM NaCl was CLV205 (Table 2). Surprisingly, seven out of 10 isolates showed similar growth at 200 and 300 mM NaCl when compared to the control cultures, indicating tolerance of those *Streptomyces* to salt stress.

Once tolerance to salinity was proven for these isolates, the capability of producing indolic compounds under such stress was evaluated. Most of the isolates responded to salt stress producing indolic

compounds (Table 3). Concentrations of 200 and 300 mM NaCl induced IC in the CLV95, CLV97, CLV127, CLV179, and CLV205, with concentrations ranging from 2.1 to 4.2-fold when compared to the culturing without NaCl (Table 3). Surprisingly, CLV188 and CLV194 showed markedly increase concentrations of IC at 200 mM, reaching 8.1 and 55.5-fold the IC concentration found in the control treatment, respectively (Table 3). Both isolates also showed high level of IC from 50 to 150 mM NaCl, although at 300 mM the IC production was significantly reduced. CLV178 and CLV186 were affected by salt stress and had reduced production of IC in all NaCl concentrations tested.

### *3.4. Promotion of plant growth by* Streptomyces *spp.*

Treating maize seeds with *Streptomyces* spp. isolates affected the plant growth (Fig. 2 (a)). CLV95 promoted an increase in root and shoot length of 25% and 15%, respectively. The CLV97 and CLV205 increased root length by approximately 30%. The CLV127 promoted approximately 40% of increase in root length, although it negatively affected the growth of the shoot by approximately 10%. The isolate that showed the greatest interference in the growth parameters was CLV179, which promoted the increase of the aerial part and the dry mass of the roots in 50%, and the dry mass of the shoot in 130% (Fig. 2 (a) and (b)). The remaining isolates maintained the growth parameters similar to the control and were not deleterious for plant development (Fig. 2 (a) and (b)).

### *3.5. Tolerance of maize plants to salt stress*

The saline tolerance of maize plants was evaluated by the growth of root and leaves of maize plants treated with different NaCl concentrations (Table 4). Plants showed some decrease of leaf growth at 50 mM NaCl. However, the negative effect of salinity on growth of maize plants was seen from 100 mM NaCl (Table 5) and the most affected parameters were root length and dry mass of the root and leaves. NaCl at 300 mM greatly affected the development of the plant, resulting in reduction of approximately 31% in root length, 16% in leaves' length, as well as 30% and 38% in the root and leaf dry mass, respectively.

### *3.6. Effect of* Streptomyces *spp. on tolerance to salt stress of maize plants*

Based on the results obtained with PGPR traits, promotion of growth of maize plants and tolerance to salinity of both bacteria and plants, four isolates were selected for evaluating whether they help attenuating the effects of salt stress during growth of maize plants. The isolates CLV95, CLV97, CLV178 and CLV179 were used to bacterize maize seeds and then, plants treated with 100 and 300 mM of NaCl were analyzed based on growth parameters (length, fresh mass and dry mass of roots and leaves and the stalk diameter). Moreover, the activity of antioxidant enzymes was investigated.

All four *Streptomyces* spp. isolates affected in some extent the growth of maize plants subjected to saline stress (Table 5). The CLV95, CLV97 and CLV178 had increased root length by 17%, 13% and 8%, respectively, at 100 mM NaCl. Furthermore, CLV178 showed a 36% increase in the length of leaves at 300 mM NaCl and also promoted a discrete increase in the parameters of fresh mass and dry mass of the root and aerial part, and diameter of the stem when compared to non-bacterized plants (Table 5). On the other hand, treating seeds with CLV179 isolate resulted in significant promotion of growth under salt stress, regardless the concentration of NaCl. An increase of 61.3% and 73% in shoot growth of the maize plants was recorded at 100 and 300 mM NaCl, respectively (Table 5). The stalk diameter was significantly improved (Table 5).

Antioxidant enzymes APX and CAT in roots and leaves of maize plants showed variation in their activities in response to salt stress (Fig. 3 and 4). Data were analyzed according to the time of exposure to the salt concentrations. However, the factor time showed no statistical significance and data were then analyzed independently of time of exposure to salt.

CLV95 promoted the increase of APX activity in the leaves and roots of plants submitted to 100 and 300 mM NaCl, respectively (Fig. 3(a) and 4(a)). APX activity in the leaves was also induced in CLV97 and CLV178 at 100 mM NaCl (Fig. 4(a)). However, at 300 mM all isolates induced a decrease in APX activity in leaves when compared to non-bacterized plants (Fig. 4(a)). Interestingly, CLV178 significantly induced APX activity in the leaves when no salt was added to the plants (Fig. 4 a).

CAT activity was induced by CLV97, CLV178 and CLV179 in maize roots submitted to 100 mM NaCl (Fig. 3b)). CLV 97 also increased CAT activity in the leaves of plants subjected to 100 mM NaCl (Fig. 4(b)). Overall, activity of APX in the roots was more intense than in the leaves and the opposite result was registered with CAT activity (Fig. 3 and 4).

**4. Discussion**

PGPR are the microorganism present in the rhizosphere that promote growth and help plants to cope with biotic and abiotic stresses (Shaikh et al., 2018). Identification of effective PGPR initiates with a screening of microorganisms followed by pure culture on solid medium to determine traits of plant growth promotion. These characteristics include production of phytohormones (Anwar et al., 2016; Hayat et al., 2010) and siderophores (Patel et al., 2016), hydrolytic enzymes (Dubey et al., 2014; Kumar et al., 2012) and antibiotics (Ahanger et al., 2014; Sindhu et al., 2009). Among these, the production of indolic compounds seems to be the most common trait found among plant growth promoting rhizobacteria (Kumar et al., 2012; Anwar et al., 2016). All the rhizospheric *Streptomyces* spp. isolated in this work were capable of producing indolic compounds, quantified based on the 3-indol acetic acid (IAA) standard. Although variation in concentration was observed, it was possible to select isolates with great capacity of IC production, such as CLV178 and CLV186. Production of indolic compounds by rhizobacteria is a

desirable trait when these microorganisms are to be used to formulate biofertilizers. IAA is a molecule signal, involving in the regulation of plant development, specifically in organogenesis, cell division, cell differentiation and genes regulation (Ryu and Patten, 2008). Increasing IAA in the rhizosphere promotes root growth and expanded root surface, positively affecting water acquisition and nutrient uptake, which can directly result in plant growth (Ahemad et al., 2014; Dimpka et al., 2009). However, the most indolic-productive *Streptomyces* isolate did not promote either root or shoot growth of maize plants, likely due to the inhibitory effect on root growth resulting from high concentrations of auxin. For example, an IAA overproducing mutant of the bacterium *Pseudomonas fluorescens* BSP53a inhibited the development of roots in cherry cuttings (Dubeikovsky et al., 1993). Contrastingly, some of *Streptomyces* isolates that produced concentration of indolic compounds below 20 µg g-1 of cells, namely CLV97, CLV127 and CLV179, were efficient on enhancing root length of maize plants, suggesting that very high productive IAA-isolates may not be appropriated for biofertilizer formulation. However, it is important to consider that growth promotion may not be associated with a single metabolic characteristic of the rhizobacterium (Bhattacharyya and Jha, 2012). The profile of PGPR traits of each isolate may result in different effect on plant growth.

Besides IAA, all *Streptomyces* isolates were also able to produce siderophores, and remove iron from Fe-CAS complex, although the formed halos were different in extent. Siderophores are molecules of low molecular weight that chelate iron with very high affinity (Dimkpa, 2016), and are produced and secreted under iron stress to sequester this element from the external environment or from the host. They are able to interact with $Fe^{+3}$, transport it into their cells and reduce it to $Fe^{+2}$, which is biologically usable by plants. Significant production of siderophores was recorded for isolates CLV97, CLV178, and CLV194. The production of siderophores by microorganisms such as rhizobacteria is important for plant development. *Streptomyces* spp. have been reported as producers of IAA and siderophores (Anwar et al., 2016; Bhattacharyya and Jha, 2012; Tamreihao et al., 2016).

Phenazine production was also observed in two of the ten isolates investigated in this work. CLV186 was capable to produce both 1-carboxylic acid and 1-hydroxyphenazine phenazine, and CLV194 produced only 1-hydroxyphenazine. These compounds are important because they serve as electron donors and receptors (Pierson and Pierson, 2010) and can modify the redox potential of cells, acting as signals that regulate gene expression and contributing to biofilm formation, which affects bacterial survival. In some cases, phenazines may still induce plant tolerance to biotic stresses and inhibit pathogenic organisms (Audenaert et al., 2002; Pierson and Pierson, 2010).

Promotion of growth of maize plants by bacterial isolates was also analyzed. Most of the PGPR traits reported in *Streptomyces* are related to the promotion of plant growth. *Streptomyces* spp. are recognized as PGPR in several species such as tomato (El-Tarabily 2008; Dias et al., 2017), sunflower (Ambrosini et al., 2012) and chickpea (Gopalakrishnan et al., 2015), Araucaria (Dalmas et al., 2011) and Eucalyptus (Salla et al., 2014). Our results showed that the isolates tested either promoted or showed neutral effect on maize vegetative growth. None isolate was significant deleterious to growth of maize

plants. It is noteworthy that CLV179 promoted leaf growth by 50%, even though it produced low amounts of indolic compounds. Seed bacterization with this isolate resulted in increased root and leaf dry mass, evidencing enhanced plant development. Contrary, CLV95, one of the largest producers of indolic compounds, promoted only 25% of root growth and approximately 15% of leaf growth. Experimental evidence suggested that plant growth stimulation is a net result of multiple mechanisms that may be activated simultaneously (Martinez-Viveros et al., 2010).

The hypothesis that *Streptomyces* spp. were capable of attenuating the effect of salt stress in maize plants was tested. Salinity may affect both plants and microorganisms in the soil. Microorganisms can be classified according to their salinity tolerance and may be non-tolerant (tolerate a small salt concentration, about 1% w/v), slightly tolerant (tolerating up to 6-8% w/v), moderately tolerant (up to 18-20% w/v), and extremely tolerant (grow at all salt concentrations from zero to saturation) (Larsen 1986). Studies on salt-tolerant PGPR indicate that under saline conditions, these microbes have developed complex physiological and biochemical mechanisms which maintain their survival and multiplication in saline conditions, (Bharti et al., 2016; Omar et al., 2009; Vaishnav et al., 2016; Habib et al., 2016). *Streptomyces* are reported as salt-tolerant bacteria, and tolerance was previously determined as varying from 1 to 2 M NaCl (Palaniyandi et al., 2014; Tresner et al., 1968). Most of the isolates of *Streptomyces* spp. tested in this study were tolerant to NaCl (50 to 300 mM), maintained the ability to produce indolic compounds under such stress, and thus they could be cited as halotolerantes. Indeed, it has been reported that halotolerant plant-growth-promoting rhizobacteria (PGPR) alleviate salt stress and help plants to maintain growth (Shukla et al., 2011).

Salinity causes severe reduction of growth in plants. This stress prevents plant from capturing nutrients by lowering the water potential and creating osmotic stress. The plant strategy to overcome this negative effect is to uptake $Na^+$ and $Cl^-$, leading to another level of stress, which is a substantial increase in cellular ion contents, negatively affecting cellular biochemistry (Maathuis 2014). The use of rhizobacteria may alleviate salt stress in several species, including maize (Estrada et al., 2013). In order to investigate the potentiality of *Streptomyces* isolates on attenuating the negative effects of salinity on growth of maize plants, four isolates were chosen. The isolates capable of grow in saline conditions, produce high and low amounts of indolic compounds, in combination with production of siderophores, were selected (CLV95, CLV97, CLV178 and CLV179). Although CLV194 was also efficient on IC production and phenazines, when it was grown under salt stress the production of IC was markedly increased, reaching levels that would had negatively affect root growth.

The growth of maize plants bacterized and submitted to saline stress of 100 and 300 mM NaCl was analyzed by means of the evaluation of the length, the fresh and dry mass of the root and the leaves, and the diameter of the stalk. The isolates CLV95, CLV97, CLV178 and CLV179 promoted growth of maize plants when exposed to salt stress. Most significant results were observed with isolates CLV178 and CLV179, in which most of the analyzed parameters were improved at both NaCl concentrations. Between these two isolates, CLV179 was the one with the lowest amount of indole compounds produced

under salt stress, however, this isolate was capable of siderophore. Tank and Saraf (2010) reported PGPR strains showing siderophore production in saline conditions, suggesting that the production of this molecule is not significantly affected by salinity. Several studies reported that the seeds inoculated with PGPR showed increased plant height, leaf size, root length and dry matter of tomato (Mayak et al., 2004), groundnut (Saravanakumar and Samiyappan 2007), red pepper (Siddikee et al., 2011) and rice (Nautiyal et al., 2013; Nakbanpote et al., 2014) in saline soils.

Salt tolerance is a complex phenomenon that involves morphological, physiological and biochemical processes. Although the phenotype of enhanced growth of maize plants subjected to saline stress has been observed in the presence of *Streptomyces*, plants need strategies to minimize the deleterious effects of NaCl. Many plants deal with salinity by minimizing the direct effect of $Na^+$ ions through salt exclusion, salt sequestration or salt excretion, although these mechanisms are often not sufficient to confer tolerance (Wang et al., 2016). Plants employ a system of detoxifying enzymes, as SOD, APX e CAT to combat oxidative stress induced by salinity (Jaleel et al., 2009; Wang et al., 2016). These enzymes have the ability to scavenge the ROS ($O_2^{-}\cdot$, $H_2O_2$, and hydroxyl free radical OH·) and maintain them at low levels (Habib et al., 2016). Some PGPR are able to induce the activity of these enzymes to minimize the effects generated by the abiotic stress. Thus, the activity of CAT and APX enzymes in bacterized plants and submitted to saline stress were evaluated. Isolates CLV97, CLV178 and CLV179 induced enhanced activity of CAT at 100 mM NaCl in the roots, while in the shoots the highest activity was induced by CLV97 at the same concentration of salt. The CAT is one of the front line in the fight against free radicals and it is essential at the beginning of stress, helping the action of the other enzymes of the antioxidant system, reducing the action of ROS (Mhamdi et al., 2010). The induction of CAT activity in the roots of plants bacterized by CLV97, CLV178 and CLV179, within 24 h after salt treatment, strengthens the hypothesis of early action of CAT in salt stress. In this 24-hour period, CLV97 was able to induce CAT in the maize leaves.

On the other hand, CLV95 induced APX activity at 300 mM NaCl in the roots and a discrete enhancement of APX activity was noted in the shoots with CLV95, CLV97 and CLV178. APX enzymes play a key role in the ascorbate-glutathione cycle, detoxifying hydrogen peroxide, using ascorbate as a specific electron donor (Caverzan et al., 2012). In the light of these results, we can hypothesize that PGPR *Streptomyces* may be triggering the systemic salt tolerance on maize plants, since metabolic responses were seen in both roots and shoots of the plants. There have been evidences showing that plants in association with PGPR show better adaptation against salinity stress (Rodriguez and Redman 2008; Tkacz and Poole, 2015; Turner et al., 2013; Zelicourt et al., 2013).

In conclusion, the isolates of *Streptomyces* spp. obtained in this study were characterized as PGPR and show potential to improve growth of maize plants under natural and saline soils. The isolates helped maize plants to cope with the salinity stress, especially the CLV179. *Streptomyces* spp. also modulated the detoxifying enzymes CAT and APX either in the roots or in the leaves of maize plants, indicating a

systemic response to the interaction plant-microorganisms. CLV179 would be a candidate for formulation of a commercial biofertilizer.

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Table 1– PGPR characteristics of *Streptomyces* spp. isolates

| ***Streptomyces* Isolates** | **Indolic compounds µg g$^{-1}$ cells** | **Production of siderophore (cm)[a]** | **Phenazines µg g$^{-1}$ cells[b]** | |
|---|---|---|---|---|
| | | | PCA | 1-OH-PHZ |
| **CLV 95** | 168.19 c[c] | 1.11 de | - | - |
| **CLV 97** | 12.89 e | 1.72 ab | - | - |
| **CLV 127** | 8.62 e | 0.89 e | - | - |
| **CLV 178** | 414.63 a | 1.91 a | - | - |
| **CLV 179** | 18.58 e | 1.46 bcd | - | - |
| **CLV 186** | 301.60 b | 1.21 cde | 574.4 | 573.2 |
| **CLV 188** | 11.53 e | 1.07 de | - | - |
| **CLV 193** | 18.76 e | 1.52 bc | - | - |
| **CLV 194** | 100.10 d | 1.84 ab | - | 255.2 |
| **CLV 205** | 23.90 e | 1.05 e | - | - |

[a] Values are the average width of the halo border (cm) of at least three replicates.

[b] Phenazines: average of duplicates; - : not detected. PCA: phenazine 1- carboxylic acid; 1-OH-PHZ: 1-hydroxyphenazine

[c]Means followed by the different letters in the columns indicate significant difference according Duncan's multiple range test ($P$ = 0.05).

Table 2- Growth of *Streptomyces* spp. isolates under different NaCl concentrations (0 to 300 mM). Data were evaluated as pellet mass (g)

| ***Streptomyces* spp. isolates** | **NaCl (mM)** | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 50 | 100 | 150 | 200 | 300 |
| **CLV 95** | 1.02 ab | 0.49 b | 0.60 ab | 0.54 b | 0.61 ab | 1.09 a |
| **CLV 97** | 0.94 a | 0.64 ab | 1.03 a | 0.32 b | 0.74 ab | 0.87 ab |
| **CLV 127** | 1.00 b | 0.29 c | 0.44 c | 0.19 c | 0.30 c | 1.58 a |
| **CLV 178** | 0.47 b | 0.39 b | 0.87 ab | 0.44 b | 1.10 a | 0.50 b |
| **CLV 179** | 1.05 a | 0.88 a | 0.50 a | 1.00 a | 1.08 a | 0.98 a |
| **CLV 186** | 0.57 a | 0.33 b | 0.37 ab | 0.35 b | 0.37 ab | 0.43 ab |
| **CLV 188** | 0.54 ab | 0.53 ab | 0.62 ab | 0.64 a | 0.43 ab | 0.27 b |
| **CLV 193** | 1.92 a | 0.91 b | 0.67 b | 0.49 b | 0.64 b | 1.38 ab |
| **CLV 194** | 0.56 ab | 0.32 b | 0.32 b | 0.34 b | 0.38 b | 0.71 a |
| **CLV 205** | 1.36 a | 1.10 ab | 1.60 a | 1.02 ab | 1.02 ab | 0.54 b |

Values are the average of eight flasks. Means followed by different letters within the lines indicate significant difference according Duncan's multiple range test ($P = 0.05$).

Table 3 - Production of indolic compounds by rhizobacteria in the presence of different concentrations of NaCl. Data are expressed in µg $g^{-1}$ cells

| ***Streptomyces* spp. isolates** | **NaCl (mM)** | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 50 | 100 | 150 | 200 | 300 |
| **CLV95** | 128.5 b | 157.4 b | 155.6 b | 227.1 a | 270.8 a | 140.8 b |
| **CLV97** | 33.4 bc | 12.0 c | 54.3 bc | 22.8 bc | 111.7 a | 64.3 b |
| **CLV127** | 6.1 b | 3.7 b | 2.0 b | 11.0 ab | 11.4 ab | 17.9 a |
| **CLV178** | 329.3 a | 44.7 c | 101.73 bc | 199.08 b | 84.42 bc | 136.03 bc |
| **CLV179** | 12.3 c | 9.7 c | 23.57 b | 3.99 c | 14.86 bc | 43.09 a |
| **CLV186** | 256.8 b | 403.6 a | 202.1 bc | 109.8 bc | 65.4 c | 206.0 bc |
| **CLV188** | 355.4 c | 1544.4 b | 1589.9 b | 1517.93 b | 2883.0 a | 0.0 d |
| **CLV193** | 16.0 c | 25.8 c | 46.19 c | 243.37 a | 129.79 b | 47.91 c |
| **CLV194** | 75.2 c | 2088.2 b | 2205.4 b | 2361.7 b | 4164.7 a | 34.0 c |
| **CLV205** | 25.4 d | 37.0 cd | 72.8 b | 43.1 bcd | 105.0 a | 61.9 bc |

Values are the average of 15 to 20 bacterial suspensions. Means followed by different letters within the lines indicate significant difference according Duncan's multiple range test ($P = 0.05$).

Table 4– Growth of maize plants submitted to different concentrations of NaCl, cultivated for 20 days under salt stress.

| **NaCl (mM)** | **Root Length (cm)** | **Shoot Length (cm)** | **Root dry mass (mg)** | **Shoot dry mass (mg)** |
|---|---|---|---|---|
| **0** | 47.5 a | 51.0 a | 376.1 a | 395.6 a |
| **50** | 41.3 ab | 46.2 b | 334.9 ab | 343.1 ab |
| **100** | 33.3 c | 42.5 b | 346.1 ab | 318.1 bc |
| **150** | 29.2 c | 41.7 b | 272.3 c | 284.7 bcd |
| **200** | 35.5 bc | 43.9 b | 289.2 bc | 271.1 cd |
| **300** | 32.7 c | 42.6 b | 261.7 c | 241.7 d |

Values are the average of 20 plants. Means followed by different letters within the columns indicate significant difference according Duncan's multiple range test ($P = 0.05$).

Table 5 - Effect of *Streptomyces* spp. on tolerance to salt stress of maize plants treated with 100 and 300 mM NaCl

| Isolate | Treatment | Root length (cm) | Shoot length (cm) | Fresh mass of root (g) | Fresh mass of root (g) | Dry mass of root (mg) | Dry mass of shoot (mg) | Stalk (cm) |
|---|---|---|---|---|---|---|---|---|
| Control | 100 | 40.0 bc | 57.1 cd | 3.6 cd | 4.1 c | 0.4 c | 0.7 c | 2.3 c |
| | 300 | 33.3 d | 50.0 d | 3.0 d | 4.2 c | 0.4 c | 1.0 c | 2.5 c |
| CLV 95 | 100 | 46.8 a | 55.4 cd | 4.2 c | 4.6 c | 0.4 c | 0.8 c | 2.1 c |
| | 300 | 34.5 d | 53.3 cd | 3.4 cd | 4.7 c | 0.5 c | 1.1 c | 2.4 c |
| CLV 97 | 100 | 45.2 a | 56.5 cd | 3.4 cd | 4.1 c | 0.4 c | 0.7 c | 2.3 c |
| | 300 | 35.4 ab | 53.0 cd | 3.4 cd | 4.2 c | 0.4 c | 0.9 c | 2.3 c |
| CLV 178 | 100 | 43.2 ab | 59.3 c | 3.7 cd | 4.9 bc | 0.4 c | 0.9 c | 2.5 c |
| | 300 | 37.3 cd | 68.0 b | 4.1 cd | 7.1 b | 0.6 b | 2.6 b | 3.2 b |
| CLV 179 | 100 | 35.5 cd | 92.1 a | 6.3 b | 14.1 a | 0.8 a | 2.5 b | 5.1 a |
| | 300 | 37.8 cd | 86.3 a | 7.6 a | 13.6 a | 0.9 a | 4.7 a | 4.7 a |

Control: Non-bacterized seeds

Means followed by different letters within the columns indicate significant difference according Duncan's multiple range test ($P = 0.05$).

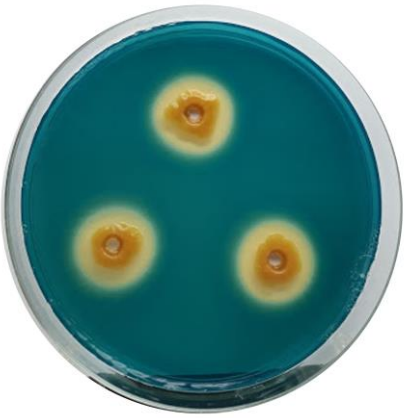

Fig. 1- Siderophore production of the selected rhizospheric *Streptomyces* spp. Bar = 1.8 cm.

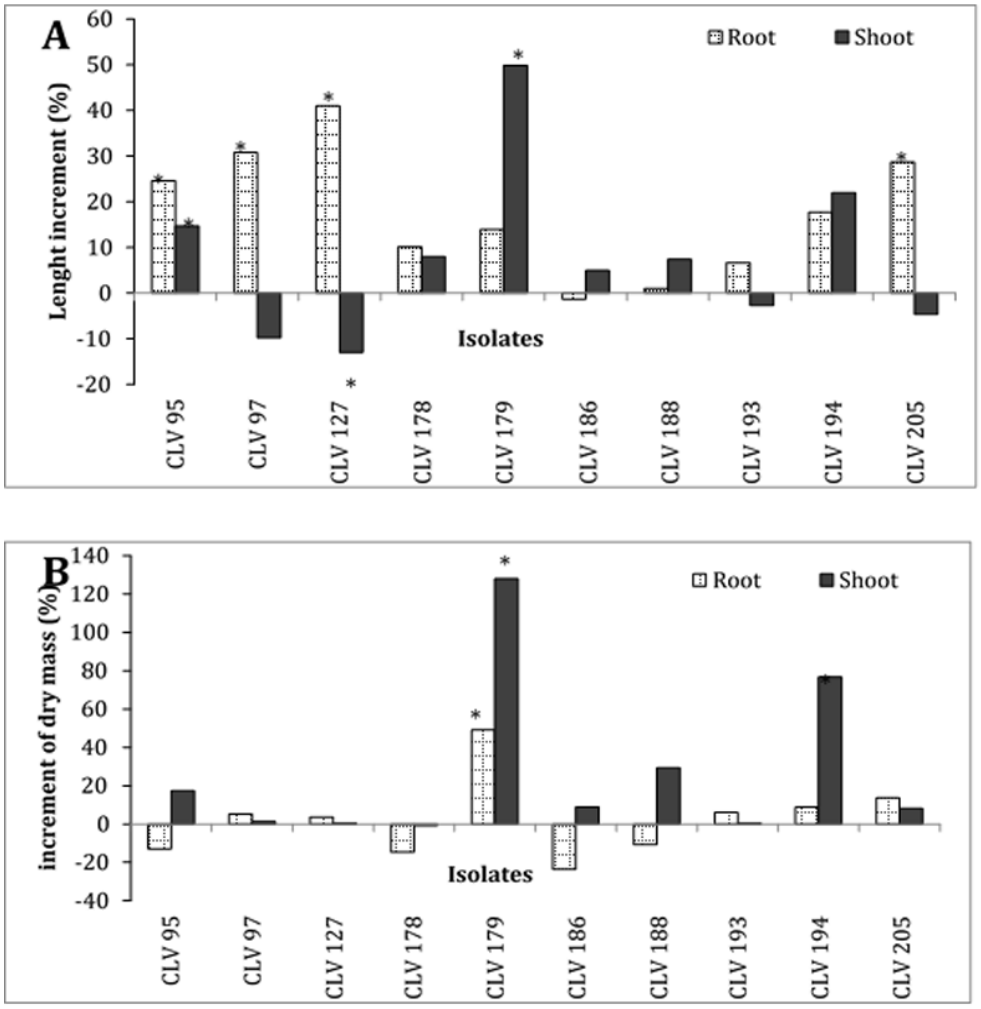


Fig. 2 - Growth of maize plants (root and aerial part) obtained from seeds bacterized with *Streptomyces* spp. isolates. (a) Increment of plant length; (b) Increment of dry mass. Values are the average of 30 plants. Bars with asterisk indicate significant difference according Student's T Test ($P$ = 0.05).

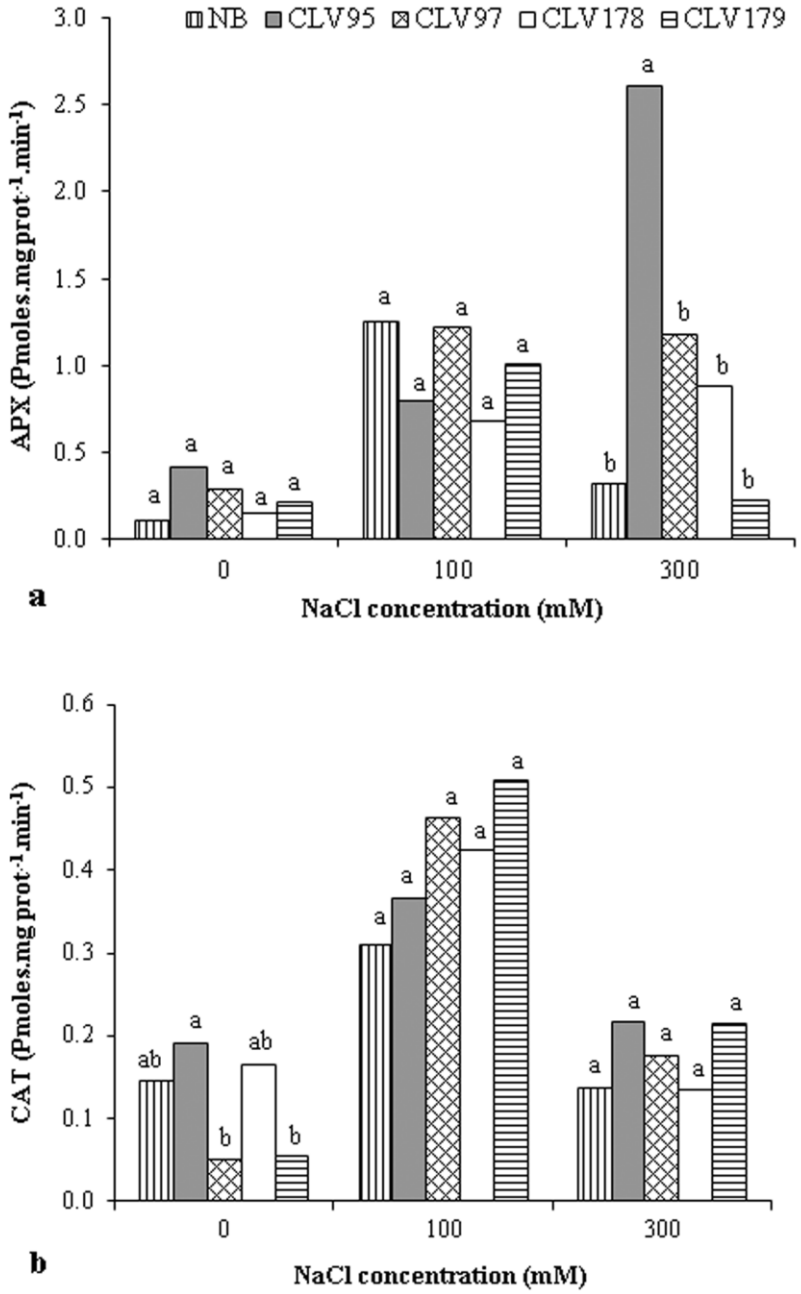


Fig. 3 – Activity of antioxidant enzymes (a) Ascorbate peroxidase -APX; (b) Catalase – CAT in maize roots treated with *Streptomyces* spp. isolates and submitted to salt stress (100 and 300 mM NaCl). Letters on the bars indicate significant difference amongst bacteria within the NaCl concentration according to Duncan's multiple range test ($P$ = 0.05). NB: non bacterized seeds.

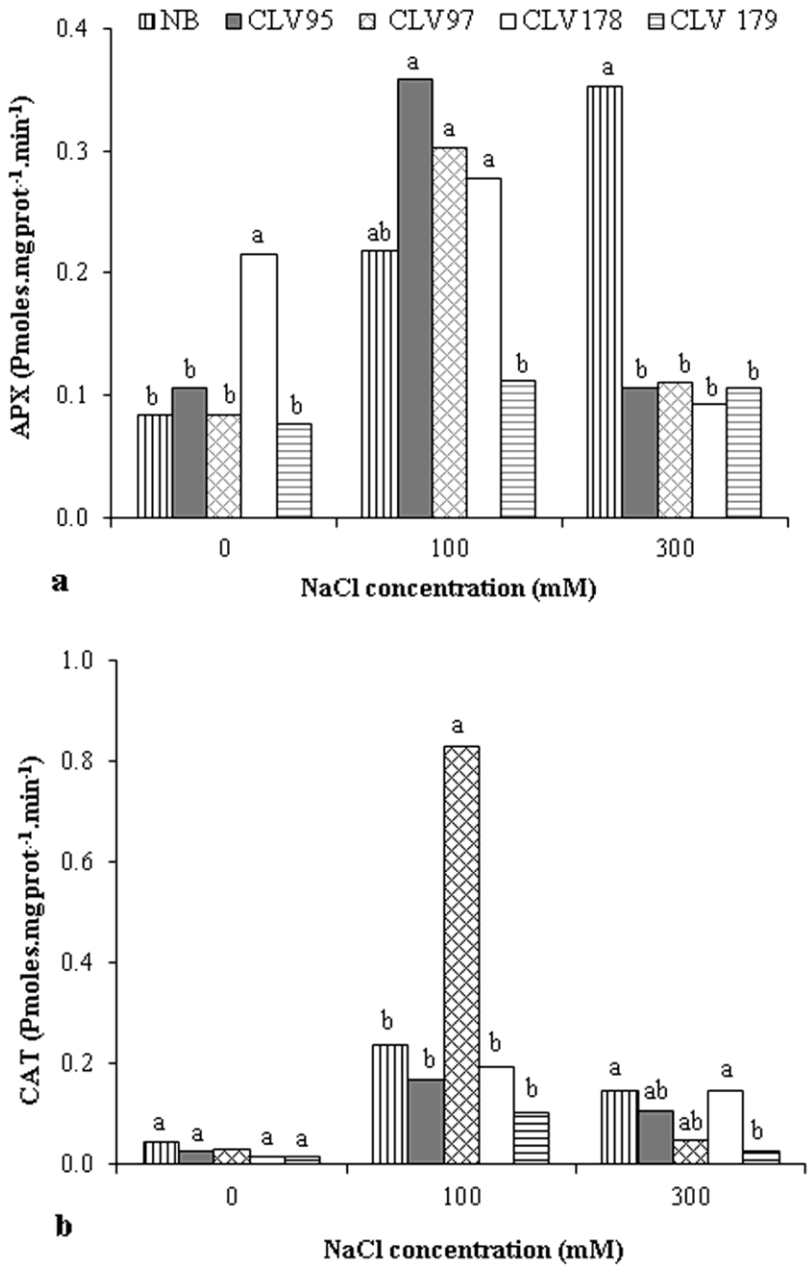


Fig. 4 – Activity of antioxidant enzymes (a) Ascorbate peroxidase -APX; (b) Catalase – CAT in of maize leaves treated with *Streptomyces* spp. isolates and submitted to salt stress (100 and 300 mM NaCl). Letters on the bars indicate significant difference amongst bacteria within the NaCl concentration according to Duncan's multiple range test ($P$ = 0.05). NB: non bacterized seeds.

## Capítulo III: Considerações finais

Os isolados de *Streptomyces* spp. avaliados apresentam características de PGPR e alguns deles podem aliviar os efeitos gerados pelo estresse salino em plantas de millho. Todos os isolados investigados foram produtores de compostos indólicos e de sideróforos, embora apenas os isolados CLV186 e CLV194 produziram fenazinas. Todos os isolados foram capazes de crescer em presença de NaCl e de produzir compostos indólicos nessas condições, apesar de variações no crescimento bacteriano e na quantidade desses compostos terem sido encontradas nas diferentes concentrações de NaCl testadas. O crescimento das plantas de milho foi afetado pela presença das rizobactérias. A bacterização de sementes com os isolados CLV95, CLV97, CLV127, CLV179 e CLV205 resultou na promoção do crescimento das plantas. Dentre estes isolados, o que apresentou resultado mais significativo foi o CLV179, promovendo incremento de 50% da parte aérea. Os isolados CLV95, CLV97, CLV178 e CLV179 promoveram a tolerância das plantas de milho à salinidade, estimulando o crescimento das plantas mesmo sob estresse salino. Além disso, a interação com os isolados de *Streptomyces* spp. resultou na modulação da atividade das enzimas CAT e APX, sugerindo que as rizobactérias isoladas nesse estudo podem estar contribuindo para a indução de tolerância sistêmica em plantas de milho sob estresse salino. Esses resultados sugerem que os isolados de *Streptomyces* CLV95, CLV97, CLV178 e CLV179 possam ser investigados para futura aplicação como biofertilizantes em solos afetados pela salinização.

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