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Exsudatos radiculares como reguladores da colonização da planta por *Methylobacterium* spp. e *Methylorubrum extorquens*

Dissertação apresentada ao Programa de Pós-Graduação em Microbiologia do Instituto de Ciências Biomédicas da Universidade de São Paulo, para obtenção do título de Mestre em Ciências.

Área de Concentração: Microbiologia

Orientador: Prof. Dr. Welington Luiz de Araújo

Versão corrigida

São Paulo
2019

RESUMO

MANTILLA GALINDO, M. A. **Exsudatos radiculares como reguladores da colonização da planta por *Methylobacterium* spp. e *Methylorubrum extorquens.*** 2019. 158f. Dissertação (Mestrado em Microbiologia) – Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, 2019.

O gênero *Methylobacterium* pode estabelecer associações epifíticas ou endofíticas com diferentes espécies vegetais. Para o estabelecimento dessa interação, ocorre uma comunicação química coordenada entre as partes, em que os exsudatos da planta hospedeira provavelmente desempenham um papel fundamental. Estudos anteriores, mostraram que a linhagem *M. mesophilicum* SR1.6/6 apresenta maior crescimento na presença de exsudatos vegetais, induzindo uma alteração no padrão da expressão de genes possivelmente envolvidos na colonização (atividade catalítica, formação de biofilme, metabolismo primário e genes envolvidos em resposta antioxidante). No entanto, o papel dos exsudatos vegetais, durante a interação com *Methylobacterium,* ainda não é bem entendido. Portanto, o objetivo deste trabalho foi avaliar a influência de exsudatos radiculares de plântulas de soja (*Glycine max*) e milho (*Zea mays*) no estabelecimento da interação com *Methylobacterium* spp. e *Methylorubrum extorquens.* Assim, foram desenhados *primers* específicos para 3 linhagens de *Methylobacterium* e uma linhagem de *Methylorubrum extorquens*, com a finalidade de quantificar cada uma das linhagens no solo e na raiz de plântulas de soja e milho por qPCR. Além disso, foi feita a identificação dos exsudatos vegetais durante a interação com *Methylobacterium* por GC-MS e a influência dos exsudatos vegetais na formação de biofilme também foi avaliada. Deste modo, foi observado que as linhagens AR1.6/2 (*M. extorquens*) e MP2-3 (*M. hispanicum*), quando inoculadas individualmente, sobreviveram no solo por até 90 dias. Enquanto as linhagens SR1.6/6 (*M. mesophilicum*) e R16E (*M. fujisawaense*) apresentaram menor taxa de sobrevivência no mesmo período avaliado, ademais foi visto que no consórcio bacteriano, só a linhagem MP2-3 sobreviveu no solo. Nos experimentos de interação, foi observado que a linhagem SR1.6/6, inoculada individualmente apresentou maior abundância nas raízes de plântulas de soja, e quando inoculada no consórcio, apresentou menor abundância. Em raiz de milho, a abundância das bactérias não diferiu significativamente. A partir das análises de GC-MS, foi observado que plântulas de soja e milho apresentaram perfis diferenciados, sendo que plântulas de milho mostraram maior porcentagem de carboidratos (16%) e ácidos orgânicos (7%), em relação aos exsudatos de plântulas de soja, que apresentaram 14% de carboidratos e 2% de ácidos orgânicos. Durante a interação, houve um aumento na abundância relativa de carboidratos, ácidos orgânicos e aminoácidos tanto em plântulas de soja como de milho. Por último, foi evidenciado que os exsudatos de plântulas de milho induziram a formação de biofilme bacteriano. Os resultados obtidos neste trabalho, evidenciam a influência dos exsudatos na seleção de linhagens específicas de *Methylobacterium* e ou *Methylorubrum,* mediante a disponibilização de fontes específicas de carbono. Além disso, é possível sugerir que o sucesso da colonização da planta por *Methylobacterium* é dependente do metabolismo dessas fontes de nutrientes.

Palavras chave: Exsudatos radiculares. Biofilmes. Interação planta-bactéria. Colonização. Ecologia de interações.

ABSTRACT

MANTILLA GALINDO, M. A. **Root exudates as regulators of plant colonization by *Methylobacterium* spp. and *Methylorubrum extorquens*** 2019, 158f. Master thesis (Microbiology) – Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, 2019.

The genus *Methylobacterium* can establish epiphytic or endophytic associations with different plant species. For the establishment of interaction, chemical communication between plant and bacteria take place, in this communication the root exudates probably play a key role. Previous studies showed that the strain *M. mesophilicum* SR1.6/6 increased their development in root exudates and the expression of colonization related genes (catalytic activity, biofilm formation, primary metabolism and antioxidant genes). However, the importance and functions of plant root exudates during interaction with *Methylobacterium* are not well understood. Therefore, this work aims to determinate the role of soybean (*Glycine max*) and corn (*Zea mays*) seedling root exudates in the establishment of interaction with *Methylobacterium* spp. and *Methylorubrum extorquens*. Thus, specific primers were designed for three *Methylobacterium* spp. strains and one *Methylorubrum extorquens* strain. These primers were used for the strain's quantification in soil and seedlings roots (soybean and corn) by qPCR. Additionally, the composition of seedlings roots in interaction with *Methylobacterium* were identified by GC-MS and the influence of exudates in biofilm formation were evaluated. Thus, it was observed that the strains AR1.6/2 (*M. extorquens*) and MP2-3 (*M. hispanicum*) when inoculated individually survived in the soil for up to 90 days, while the strains SR1.6/6 (*M. mesophilicum*) and R16E (*M. fujisawaense*) showed a low survival. In bacterial consortia, only MP2-3 strain was survived for 90 days in soil. In the interaction experiments was evidenced that SR1.6/6 strain when inoculated individually had the biggest abundance in soybean roots but in consortia had the lowest. In corn root, the abundance of bacteria didn't differ significantly. From GC-MS analyzes, was observed that soybean and corn seedlings presented different profiles, and corn seedlings showed a higher percentage of carbohydrates (16%) and organic acids (7%), in comparison with soybean, which presented 14% of carbohydrates and 2% of organic acids. During interaction, carbohydrates, organic acids and amino acids percentages increased in soybean and corn seedlings. Finally, it was evidenced that corn seedling exudates induced the bacterial biofilm formation. The results obtained in this work show the influence of exudates in the selection of specific strains of *Methylobacterium* and or *Methylorubrum*, by providing specific carbon sources, and it's suggested that the success of plant colonization by *Methylobacterium* is dependent on the metabolism of these nutrient sources.

Key words: Root exudates. Biofilms. Plant-bacteria interaction. Colonization. Ecology of interactions.

INTRODUÇÃO

O gênero *Methylobacterium* pertence à classe Alfa-proteobacteria, ordem Rhizobiales e família Methylobacteriaceae (DONOVAN; MCDONALD; WOOD, 2014). Este gênero é constituído por 32 espécies descritas sendo *Methylobacterium organophilum* a espécie tipo do gênero (GREEN; ARDLEY, 2018). Membros deste gênero se caracterizam por apresentar a capacidade de metabolizar o metanol na ausência da biotina como cofator (GREEN; ARDLEY, 2018), e por desenvolver associações endofíticas ou epifíticas com diversas espécies de plantas (ARAÚJO et al., 2015; DOURADO et al., 2013). Estas associações, geralmente, apresentam um estímulo positivo na planta, promovendo o crescimento vegetal pela produção de fito-hormônios (citocininas e auxinas), disponibilizando nutrientes, inibindo patógenos e regulando os níveis de etileno (hormônio do estresse) (CHAUHAN et al., 2015; KUMAR et al., 2016; MADHAIYAN et al., 2006a).

Os mecanismos que permitem o estabelecimento da interação entre *Methylobacterium* e a planta hospedeira ainda não são bem compreendidos, mas acredita-se que os exsudatos vegetais desempenham um papel fundamental no processo (BERENDSEN; PIETERSE; BAKKER, 2012; SÁNCHEZ-CAÑIZARES et al., 2017; SASSE; MARTINOIA; NORTHEN, 2018). Os exsudatos vegetais, são compostos secretados pela raiz, que correspondem a 20-40% do carbono (BADRI; VIVANCO, 2009; SINGH et al., 2019) e 15% do nitrogênio fixado pela planta (SASSE; MARTINOIA; NORTHEN, 2018) e desempenham um papel fundamental na estruturação das comunidades microbianas da rizosfera (COMPANT et al., 2019; HAICHAR et al., 2014). Estes exsudatos, podem também estar envolvidos no favorecimento de espécies capazes de trazer algum benefício ecológico e evolutivo para a planta (HARDOIM; VAN OVERBEEK; ELSAS, 2008). No entanto, a espécie favorecida pelos exsudatos deve ser capaz de coexistir com outros micro-organismos e colonizar a planta hospedeira (SASSE; MARTINOIA; NORTHEN, 2018), sendo necessário portanto, não somente responder ao estímulo da planta, mas também competir com outros micro-organismos.

Estudos anteriores, mostraram que *M. mesophilicum* SR1.6/6 é capaz de colonizar plântulas de soja (*Glycine max*) após o reconhecimento inicial, sendo observadas células individuais após 24 h e produção de biofilme após 72 h (ARAÚJO et al., 2015). Também foi observado que os exsudatos das raízes de plântulas de milho (*Zea mays*) e de *Citrus sinensis* induzem um maior crescimento de *M. mesophilicum* SR1.6/6 (SALGUERO-LONDOÑO, 2015). Os exsudatos de plântulas de soja induzem a expressão de diferentes grupos de genes, incluindo aqueles relacionados com a atividade catalítica, adesão, atividade transportadora e

genes envolvidos em resposta antioxidante nesta linhagem (ARAÚJO et al., 2015), sugerindo que esta resposta possa estar associada à capacidade de colonizar a planta hospedeira.

Contudo, ainda pouco se conhece acerca de como os exsudatos vegetais afetam a interação de *Methylobacterium* com a planta hospedeira, ou selecionado diferentes genótipos microbianos. Por outro lado, as estratégias utilizadas por *Methylobacterium*, em resposta à planta, ainda não foram elucidadas. Dessa forma, o principal objetivo deste trabalho foi identificar compostos presentes nos exsudatos radiculares associados à especificidade e ao aumento da competitividade de *Methylobacterium* spp. e *Methylorubrum extorquens*, durante a interação com plântulas de milho (*Zea mays*) e soja (*Glycine max*), e avaliar a capacidade destas bactérias em competirem sob diferentes condições no ambiente. Visando a ter melhor entendimento da interação de *Methylobacterium* com a planta hospedeira. Para isso, nesse trabalho foi avaliada a sobrevivência em solo e a abundância de *Methylobacterium* spp. e *Methylorubrum* na raiz plântulas de soja e milho, bem como a identificação de compostos presentes nos exsudatos vegetais, envolvidos na interação.

CONCLUSÕES

a) As plântulas de soja (*Glycine max*) e milho *(Zea mays*) exsudam um conjunto de metabólitos específicos em resposta à presença de cada isolado inoculado;
b) O sucesso da colonização da planta hospedeira por *Methylobacterium* spp. e *Methylorubrum extorquens* depende da capacidade de responder aos exsudatos da planta e competir com outras bactérias associadas;
c) A sobrevivência no solo não está associada à capacidade de colonizar a planta hospedeira.

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