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INFLUÊNCIA DO TIPO DE ADUBAÇÃO NA PRODUÇÃO DE AMINOÁCIDOS E DE ÁCIDO INDOL-3-ACÉTICO, ETILENO E POLIAMINAS POR BACTÉRIAS FIXADORAS DE NITROGÊNIO ISOLADAS DE CANA-DE-AÇÚCAR (*SACCHARUM* SP.)

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Área de concentração: Microbiologia de Solos

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RESUMO

FERRARA, F. I. S. **Influência do tipo de adubação na produção de aminoácidos e de ácido indol-3-acético, etileno e poliaminas por bactérias fixadoras de nitrogênio isoladas de canade açúcar (*Saccharum* sp.)**. 2010. 166 f. Tese (Doutorado em Microbiologia) – Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, 2010.

Para se avaliar a influência da adubação orgânica, a que foi submetida cana-de-açúcar, na fisiologia de bactérias diazotróficas endofíticas foram identificados e estudados 36 isolados de plantas submetidas a tratamentos orgânico e convencional ou sem tratamento (controle). Foi evidenciado predomínio da família *Enterobacteriaceae* (75%). Essas bactérias foram avaliadas quanto à excreção de substâncias promotoras de crescimento vegetal (aminoácidos, poliaminas, AIA e etileno), escolhidas como indicadoras da ação do tratamento. Os resultados mostraram que foram significativamente maiores: a excreção de aminoácidos por bactérias provenientes do tratamento orgânico assim como a produção de etileno por bactérias isoladas de plantas controle. Além das 36 linhagens acima citadas, foram identificadas sete linhagens de *Rhizobium* por sequenciamento dos genes 16S rRNA, *recA* e *gapA*. Três dessas linhagens foram identificadas como *Rhizobium trifolii*, *Rhizobium etli* e *Rhizobium hainanense*. O gênero *Rhizobium* ainda não havia sido descrito como endofítico de cana-de-açúcar.

Palavras-chave: Cana-de-açúcar (adubação). Fixação de nitrogênio. Promotores de crescimento vegetal. Aminoácidos. *Rhizobium.*

ABSTRACT

FERRARA, F. I. S. **Influence of the type of fertilization on the release of amino acids and indole-3-acetic acid, ethylene and polyamines by nitrogen fixing bacteria isolated from sugarcane (*Saccharum* sp.).** 2010. 166 p. PhD Thesis (Microbiology) – Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, 2010.

Aiming to evaluate the influence of the organic fertilization, applied to sugarcane, on the physiology of endophytic nitrogen-fixing bacteria, 36 isolates of plants submitted to organic and conventional fertilization or not fertilized (control) were identified and studied. Results showed that the major part of the strains belong to the *Enterobacteriaceae* family (75%). The excretions of amino acids, polyamines, IAA and ethylene – substances chosen as indicators of the action of the treatment – were evaluated in the 36 strains. The release of amino acids was significantly higher in cultures of bacteria isolated from organic treated plants and the production of ethylene was produced in greater amounts by strains isolated from control plants. Seven isolates, different from the 36 tested for the release of plant growth promoters, were identified as *Rhizobium* strains through 16S rRNA, *recA* and *gapA* sequences. Three strains were identified as *Rhizobium trifolii*, *Rhizobium etli* and *Rhizobium hainanense*. This genus was not described as endophytic of sugarcane previously.

Keywords: Sugarcane (fertilization). Nitrogen fixation. Plant growth promoters. Amino acidos. *Rhizobium.*

INTRODUÇÃO

Bactérias fixadoras de nitrogênio podem ser encontradas livres no solo, em associação simbiótica nas raízes de plantas ou endofíticamente, em todos os órgãos do vegetal (ROSENBLUETH; MARTINEZ-ROMERO, 2006).

A associação simbiótica, estabelecida entre o gênero *Rhizobium* e plantas leguminosas em nódulos localizados na raiz do vegetal, é extremamente estudada. Essa relação é baseada na troca de nitrogênio, fixado pelo microrganismo e transferido para a planta e carbono, fornecido à bactéria pelo vegetal. A fixação biológica de nitrogênio realizada por bactérias simbióticas contribui grandemente para uma agricultura sustentável uma vez que provê os agrossistemas com consideráveis quantidades de N (MARINO et al., 2006).

Microrganismos endofíticos colonizam ativamente tecidos vegetais, estabelecendo relações variáveis com o hospedeiro (SCHULZ; BOYLE, 2006). Bactérias e fungos são os endófitos mais comuns. Cada indivíduo pertencente a uma das cerca de 30 mil espécies de plantas existentes, é hospedeiro de um ou vários organismos endofíticos (STROBEL; DAISY, 2003). Geralmente, as densidades populacionais de bactérias endofíticas são menores que as de bactérias patogênicas, indicando que são reconhecidas diferentemente pelo vegetal e que se estabelecem de forma mais controlada. Evolutivamente, os endófitos parecem ser intermediários entre saprófitas e fitopatógenos, uma vez que obtém abrigo e nutrientes do hospedeiro sem lhe causar danos (HALLMANN et al., 1997). As bactérias endofíticas colonizam espaços intercelulares e vasos xilemáticos, podendo daí promover o crescimento da planta direta ou indiretamente (WEYENS et al., 2009) de diferentes formas. Dentre elas, estão o possível estímulo pela transferência de nitrogênio fixado (HAN et al., 2005),

solubilização de nutrientes (RODRIGUEZ; FRAGA, 1999; VERMA et al., 2001), liberação de hormônios de crescimento e vitaminas (PIRTTILA et al., 2004) produção de enzimas regulatórias (CORREA; BARRIOS; GALDONA, 2004) e antagonismo a organismos patogênicos (RENWICK; CAMPBELL; COE, 1991; PAL et al., 2001; KARTHIKEYAN et al., 2005; COMPANT et al., 2005).

Diferentes gêneros bacterianos, dentre eles *Pseudomonas*, *Xanthomonas*, *Azotobacter, Klebsiella, Azospirillum, Acinetobacter, Bacillus, Burkholderia, Enterobacter, Erwinia, Rhizobium, Serratia* e *Herbaspirillum*, são capazes de fixar N_2 atmosférico e de sintetizar substâncias com potencial uso na promoção de crescimento vegetal (KLOEPPER et al., 1986; FRANKENBERGER; ARSHAD, 1995; ARSHAD; FRANKENBERGER, 1998; REINHOLD-HUREK; HUREK, 1998; GUTIÉRREZ-MAÑERO et al., 2001; TILAK et al., 2005; VIKRAM et al., 2007).

Ainda não está demonstrado como ocorre a transferência de nitrogênio fixado ao hospedeiro pelas bactérias endofíticas. No entanto, Urquiaga; Cruz e Boddey (1992) mostraram que diferentes cultivares de cana-de-açúcar obtiveram grandes quantidades de nitrogênio fixado por bactérias endofíticas. Bactérias diazotróficas podem contribuir para o crescimento vegetal independente da fixação de N_2. Estudos com mutantes *nif*$^-$ evidenciaram maior desenvolvimento de plantas inoculadas pela produção de PGPs (HUREK et al., 1994; HALLMANN et al., 1997).

A liberação de substâncias promotoras de crescimento vegetal (SPCV) nitrogenadas, sintetizadas a partir do N_2 é vantajosa, pois implica que a planta produza menos quantidade dessas substâncias e a fixação de N_2 impede a competição, por outras fontes de N-

combinado. *Herbaspirillum seropedicae e A. diazotrophicus* sintetizam ácido indol-3-acético (AIA) e as giberelinas A_1 e A_3 em meios quimicamente definidos (BASTIÁN et al., 1998) e o gênero *Azospirillum* produz substâncias do tipo citocininas (STRZELCZYK; KAMPERT; LI, 1994). *Azospirillum* sp, isolado do interior de raiz de mandioca liberou, no meio de cultura, hormônios como AIA e etileno, reguladores de crescimento como poliaminas e outras substâncias nitrogenadas, como aminoácidos (THULER et al., 2003a).

Para a classificação taxonômica de microrganismos em geral tem sido amplamente usada a identificação molecular incluindo diazotróficos endofíticos e simbiontes (RIVAS et al., 2009; DELÉTOILE et al., 2009). Para esse tipo de estudo o gene mais utilizado é o que codifica o 16sRNA ribossômico, que se manteve conservado durante a evolução sendo, por isso, informativo a respeito da posição taxonômica das bactérias (ZEIGLER, 2003). Entretanto, justamente por ser bem conservado, seu sequenciamento permite obter informações apenas no nível de gênero. Há necessidade de se estudar outros genes para se estabelecer corretamente a filogenia bacteriana indicando a distância evolutiva entre os isolados (ZEIGLER, 2003).

O cultivo secular da cana-de-açúcar (*Saccharum* sp.) com a utilização de poucas quantidades de fertilizantes nitrogenados, sem que houvesse o esgotamento das reservas de N do solo, levou a sugestão de que bactérias diazotróficas associadas a planta poderiam ser a fonte de N para essas culturas (BODDEY et al., 2004). Estudos de interações entre planta e bactérias diazotróficas mostraram que a cana-de-açúcar pode obter quantidades substanciais de nitrogênio fixado biologicamente quando inoculada com estes microrganismos (MUTHUKUMARASAMY; REVATHI, 1999).

A preocupação com a fertilização nitrogenada, que é danosa ao meio ambiente, fez com que se implantassem alternativas para se adubar as culturas vegetais ao redor do mundo. Consistindo na aplicação de compostos orgânicos residuais de plantas já processadas A agricultura orgânica resulta em menor lixiviação de nutrientes e maior utilização de carbono para o crescimento, menos erosões, maior biodiversidade e menores níveis de pesticidas nos sistemas hídricos (REGANOLD et al., 1987; DRINKWATER et al., 1995; KREUGER; PETERSON; LUNGREN, 1999; MADER et al., 2002; KRAMER et at., 2006). Considerando que o tipo de adubação interfere inclusive em níveis moleculares por alteração da expressão de determinados genes bacterianos (PRAKAMHANG et al., 2009), pode-se supor que a produção de substâncias ativas em plantas seja alterada de acordo com o tipo de fertilização ou sua ausência.

O presente trabalho teve como intuito, mostrar se a adubação orgânica interfere positivamente em características relacionadas à promoção de crescimento vegetal de bactérias diazotróficas endiofíticas isoladas de cana-de-açúcar quando comparada à adubação convencional.

CONCLUSÕES

- ✓ BEFN isoladas de cana-de-açúcar são capazes de liberar ativamente aminoácidos e poliaminas produzidas a partir do N_2 atmosférico em meio de cultura sintético (MMS)
- ✓ Grande porcentagem de BEFN de cana-de-açúcar pode liberar os fitormônios AIA e etileno em meio contendo seus respectivos precursores
- ✓ As BEFN capazes de liberar aminoácidos e/ou poliaminas possuem também a capacidade de produzir AIA
- ✓ A porcentagem de BEFN excretoras de aminoácidos é maior em plantas adubadas organicamente e menor em plantas não adubadas
- ✓ O tipo de adubação aplicado a cana-de-açúcar não interfere na porcentagem de BEFN capazes de excretar poliaminas, AIA e etileno
- ✓ BEFN isoladas de cana-de-açúcar adubada organicamente podem excretar maior quantidade de aminoácidos que BEFN isoladas de plantas tratadas convencionalmente ou sem tratamento
- ✓ O etileno é produzido em maior quantidade em bactérias isoladas de cana-de-açúcar sem adubação
- ✓ O tipo de adubação não interfere na quantidade de poliaminas e AIA liberadas por BEFN
- ✓ A maioria de BEFN cultiváveis presentes em cana-de-açúcar pertence ao grupo das enterobactérias

✓ Pelo menos quatro espécies diferentes de *Rhizobium* são capazes de penetrar plantas de cana-de-açúcar e permanecer no interior do vegetal tempo suficiente para serem isolados após desinfecção externa da planta

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