

**LUCIANA APARECIDA AVILA**

**DIVERSIDADE E POTENCIAL BIOTECNOLÓGICO DE  
*Pseudomonas* spp. DE SEDIMENTOS DE MANGUEZAIS**

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## RESUMO

**AVILA, L. A. Diversidade e potencial biotecnológico de *Pseudomonas* spp. de sedimentos de manguezais.** 2012. 129 f. Tese (Doutorado em Biotecnologia). Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, 2012.

Os manguezais possuem condições ambientais únicas, sendo composto por espécies particulares de plantas, animais e micro-organismos, que interagem neste ambiente caracterizado pela interface entre o continente e o oceano em regiões intertropicais. O objetivo deste trabalho foi avaliar a diversidade e o potencial biotecnológico da comunidade de *Pseudomonas* spp. em sedimentos de manguezais do Estado de São Paulo. Para tanto, amostras de sedimentos de distintos manguezais foram avaliadas, sendo; *i*) manguezal contaminado com petróleo em Bertioga, *ii*) manguezal sem contaminação em Bertioga *iii*) manguezal preservado de Cananéia. Inicialmente foi realizada uma prospecção do gene *gacA* entre os isolados de sedimentos de manguezais e então, avaliação da atividade antimicrobiana e síntese de enzimas pelas linhagens selecionadas. A diversidade de *Pseudomonas* spp. foi avaliada por meio da detecção do gene 16S rRNA e do gene *gacA* de isolados de *Pseudomonas*, como também por métodos independentes de cultivo (eletroforese em gel com gradiente de desnaturação - DGGE e construção de biblioteca de clones). Estudos foram realizados para avaliar o potencial de *Pseudomonas* para promoção de crescimento de milho e redução dos efeitos do estresse salino sobre a planta. Dos 83 isolados obtidos, 55 (66,27%) foram positivos para o gene *gacA*. Com base no gene 16S rRNA e perfil de ácidos graxos da membrana celular somente 29 isolados puderam ser caracterizados em espécie e outros 13 caracterizadas como *Pseudomonas* sp. Extratos bruto de linhagens de *Pseudomonas* sp. demonstraram atividade antifúngica e antibacteriana de amplo espectro. Todas as linhagens selecionadas produziram as enzimas catalase, celulase, lacase e lipase. No estudo da estrutura da comunidade de *Pseudomonas* spp. pela análise do DGGE do 16S rRNA, foi possível observar perfis de bandas distintos em cada um dos manguezais e pontos amostrados, enquanto que para a análise baseada no gene *gacA*, as bandas mostraram-se distribuídas de maneira aleatória entre as amostras analisadas. De acordo com a análise da biblioteca de clones, os diferentes manguezais apresentam comunidades de *Pseudomonas* spp. bem distintas, corroborando com resultado da análise de RDA dos perfis de DGGE. Aproximadamente 60% dos clones de sedimentos de manguezais não puderam ser caracterizados pelo gene 16S rRNA. Entre as espécies caracterizadas, *P. nitroreducens* e *P. fluorescens* foram predominantes. *Pseudomonas* de sedimentos de manguezais halotolerantes produzem AIA, ACC deaminase, NH<sub>3</sub>, solubilizam fosfato e fixam nitrogênio. *Pseudomonas* sp. selecionadas promoveram o crescimento de milho, estimulando também o desenvolvimento das plantas cultivadas sob estresse salino. Este trabalho enfatiza a importância de manguezais para obtenção de bactérias com potencial agrícola, industrial e biotecnológico.

**Palavras-chave:** Manguezal, *Pseudomonas*, Regulador global de resposta *gacA*, DGGE, Biblioteca de clones, Biofertilizantes, Estresse salino.

## ABSTRACT

**AVILA, L. A. Diversity and biotechnological potential of *Pseudomonas* spp. from mangrove sediments.** 2012. 129 p. Ph. D. thesis (Biotechnology). Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, 2012.

Mangroves possess unique environmental conditions, harboring particular species of plants, animals and microorganisms that interact in this environment that is characterized by the interface of continent and ocean in intertropical regions. The objective of this study was to evaluate the diversity and biotechnological potential of *Pseudomonas* spp. community in mangrove sediments of São Paulo state. Sediment samples from different mangroves were evaluated as follows; *i*) oil-contaminated mangrove in Bertioga, *ii*) uncontaminated mangrove in Bertioga, *iii*) preserved mangrove in Cananéia. Initially, the prospection of *gacA* gene was performed between the isolates of mangrove sediments and then the evaluation of antimicrobial activity and enzyme synthesis by the selected strains. *Pseudomonas* spp. diversity was analyzed through the detection of 16S rRNA gene and *gacA* gene of *Pseudomonas* isolates, as well as through unculturable methods (Denaturing Gradient Gel Electrophoresis - DGGE and clone library construction). Studies were conducted to analyze the potential of *Pseudomonas* for maize growth promotion and reduce the effects of salt stress on the plant. Of the 83 isolates, 55 (66,27%) were positive for *gacA* gene. Based on 16S rRNA and fatty acid profile of the cell membrane, only 29 isolates could be characterized at species level and 13 were characterized as *Pseudomonas* sp. Crude extracts of *Pseudomonas* sp. strains demonstrated broad spectrum antifungal and antibacterial activities. All selected strains produced the enzymes catalase, cellulase, laccase and lipase. In the study of community structure of *Pseudomonas* spp. by DGGE using 16S rRNA, it was possible to observe different band profiles in each of the mangroves and sample points, while for the *gacA* gene-based, the bands were randomly distributed within samples. According to clone library analysis, the different mangroves showed very distinct *Pseudomonas* spp. communities, corroborating with the results of RDA analysis of DGGE. Approximately 60% of the clones from mangroves sediments could not be characterized by 16S rRNA gene. Among the characterized species, *P. nitroreducens* and *P. fluorescens* were dominant. *Pseudomonas* mangrove halotolerant produce IAA, ACC deaminase, NH<sub>3</sub>, solubilize phosphate and fix nitrogen. Selected *Pseudomonas* sp. were able to promote maize growth, also stimulating the development of plants cultivated under salt stress. This work emphasizes the importance of mangroves for the search of bacteria with agriculture, industrial and biotechnological potential.

**Key words:** Mangrove, *Pseudomonas*, Global response regulator *gacA*, DGGE, Clone library, Biofertilizers, Salt stress.

## 1.1 INTRODUÇÃO

O ecossistema de manguezal possui condições ambientais únicas, incluindo a interface de aerobiose e anaerobiose causada pelo regime de marés e a salinidade do solo que se situa entre 5 à 30%, podendo atingir até 90%. Os manguezais apresentam uma composição faunística e florística características, assim como uma microbiota adaptada as condições impostas pelo ambiente de transição de águas doces e salgadas.

Neste ambiente, os micro-organismos de sedimentos são abundantes e desempenham um importante papel na decomposição e mineralização da matéria orgânica, contribuindo para a produtividade do ecossistema de manguezal (NEDWELL et al., 1994; ALONGI, 1996; HOLGUIN et al., 2001) e ainda degradação de poluentes, em áreas impactadas. Além da importância ecológica e ambiental, os micro-organismos de manguezais podem ser importantes alvos biotecnológicos para a descoberta de produtos naturais.

Ambientes extremos, como os manguezais, são propícios para a bioprospecção de novos micro-organismos, suas atividades, produtos metabólicos ou genes. Os micro-organismos dos manguezais ainda são pouco estudados, apesar das particularidades desse ambiente. Muitos dos organismos de manguezais apresentam adaptações e especializações que lhes permitem sobreviver neste ambiente de águas salgadas. As comunidades bacterianas em ambientes salinos podem ser halofílicas ou halotolerantes (ZAHARAN et al., 1992), com diversa funcionalidade, como fixação de carbono, fixação de nitrogênio, degradação de celulose, metanogênese, produção de antibióticos e enzimas (HOLGUIN et al., 2001).

Bactérias do gênero *Pseudomonas* apresentam uma versatilidade metabólica e nutricional, com habilidade de se adaptar a diferentes ambientes, como os manguezais. As *Pseudomonas* são há muito tempo estudadas, no entanto, novas espécies e novos metabólitos produzidos por este grupo continuam sendo descritos. Na interação com espécies vegetais, *Pseudomonas* podem favorecer o desenvolvimento da planta conferindo maior resistência às condições de estresse (SGROY et al. 2009), fornecendo nutrientes como nitrogênio e fosfato solúvel e produzindo reguladores de crescimento vegetal. Dentre o potencial dessas bactérias está à capacidade de promoção de crescimento de plantas, o controle biológico de

fitopatógenos, a degradação de xenobióticos, a produção de biossurfactantes, a produção de enzimas e a produção de antibióticos.

Muitos dos antibióticos e enzimas produzidas por *Pseudomonas* sp. são regulados pelo gene *gacA*, assim este gene pode ser utilizado para bioprospecção neste grupo de bactérias. O uso de técnicas de biologia molecular para estudo de diversidade e *screening* funcional de comunidades microbianas pode permitir o acesso a novos micro-organismos, possibilitar a descoberta de novos produtos naturais, além de possibilitar o conhecimento da ecologia desses micro-organismos em seu ambiente natural.

Dessa forma, no presente estudo foram usados métodos dependentes e independentes de cultivo para avaliar a diversidade de comunidades de *Pseudomonas* de sedimentos de manguezais do Estado de São Paulo, utilizando o gene *gacA* para selecionar linhagens produtoras de antibióticos e enzimas. Adicionalmente, também foi avaliado o potencial de linhagens de *Pseudomonas* para promoção de crescimento de planta e tolerância a estresse salino.

## CONCLUSÕES

Com base nos resultados obtidos pode-se concluir:

- A triagem com base no isolamento seletivo de *Pseudomonas* spp. de sedimentos de manguezais e detecção do gene *gacA* foi eficiente para seleção de linhagens produtoras de compostos de interesse biotecnológico;
- Os extratos brutos das linhagens de *Pseudomonas* sp. demonstraram atividade antimicrobiana de amplo espectro. Assim, podemos considerar o ambiente de manguezal uma importante fonte de bactérias produtoras de metabólitos antifúngicos e antibacterianos;
- As espécies de *Pseudomonas* estudadas apresentam grande potencial para produção das enzimas lacase, celulase e lipase;
- A produção de antimicrobianos e enzimas pelas *Pseudomonas gacA* positivas sugerem o envolvimento deste gene na síntese desses compostos;
- As técnicas independentes de cultivo demonstraram diferenças na composição das comunidades de *Pseudomonas* dos diferentes manguezais amostrados;
- Conforme análise de DGGE, a comunidade de *Pseudomonas* é modulada pelas condições ambientais (características físico-químicas e contaminação com óleo cru) e não pelo potencial competitivo dessas bactérias;
- As linhagens de *Pseudomonas* spp. avaliadas são halotolerantes, apresentam atividade de ACC deaminase, produzem altos níveis de AIA, são capazes de fixar nitrogênio, solubilizar fosfato e produzir amônia, demonstrando grande potencial dessas bactérias em promover o crescimento de plantas para uso em solos salinos;
- A linhagem 103, *P. putida*, se destacou entre as linhagens halotolerantes para promoção do crescimento de milho e para redução dos efeitos do estresse salino sobre planta.

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