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**IDENTIFICAÇÃO DE GENES DE *Burkholderia* sp.  
ASSOCIADOS AO CONTROLE BIOLÓGICO DE  
*Pectobacterium carotovora***

Dissertação apresentada ao Programa  
de Pós-Graduação Interunidades em  
Biotecnologia USP/Instituto  
Butantan/IPT, para obtenção do  
Título de Mestre em Biotecnologia.

Área de concentração: Biotecnologia

Orientador:  
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São Paulo  
2011

## RESUMO

MANO, E. T. **Identificação de genes de *Burkholderia* SP. associados ao controle biológico de *Pectobacterium carotovora***, 2011. 99 f. Dissertação (Mestrado em Biotecnologia) – Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, 2011.

A bactéria *Pectobacterium carotovora* causa danos a diferentes hospedeiros por meio da produção de enzimas pectinolíticas que degradam o pectato de cálcio da lamela media próximo a parede celular, causando extravasamento do conteúdo celular e consequentemente a podridão mole. Em orquídeas, as lesões ocorrem inicialmente nas folhas e avançam sobre os tecidos até atingir o pseudocaule, causando a morte da planta. Sua virulência é dependente das interações com o seu hospedeiro, com outros microrganismos, e com o ambiente. Resultados recentes demonstraram que bactérias endofíticas do gênero *Burkholderia* são capazes de controlar a podridão mole em orquídeas, e tem sido observado em *Oncidium* que a aplicação da bactéria pode reduzir em até 100% os sintomas da podridão mole. No entanto, os aspectos moleculares envolvidos neste controle ainda não foram estudados. Neste trabalho, 602 em um total de 1788 transformantes foram caracterizados quanto a sua habilidade em inibir os sintomas da podridão mole causada pela *P. carotovora*. , onde foram observados 16 mutantes com alteração no padrão de inibição e/ou a perda da capacidade total em controlar a doença quando comparado à linhagem selvagem. Entre estes mutantes foram encontrados sete diferentes genes inativados pelo transponson, sendo estes: região intermediária de uma proteína semelhante à patatina; glicosiltransferase; proteína hipotética com sequências traço do 23S rRNA; glutamato sintase; proteína transportadora da família facilitadora principal; poli-beta-hidroxialcanoato depolimerase e ácido graxo desaturase. Estes genes podem estar envolvidos em processos de síntese de aleloquímicos, competição por nutrientes, adaptação a condições ambientais, e na interação com o hospedeiro e/ou entre microrganismos. No entanto, o envolvimento destes genes na perda da capacidade em controlar a podridão mole deve ser melhor estudado.

Palavras-chave: *Burkholderia*. Controle biológico. Transponson Tn5. Podridão mole. Clonagem gênica.

## ABSTRACT

MANO, E. T. **Identification of genes of *Burkhoderia* sp. associated with biological control of *Pectobacterium carotovora*.** 2011. 99 p. Master Thesis (Biotechnology) – Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, 2011.

The bacterium *Pectobacterium carotovora* cause damage to different hosts and by production of pectic enzymes that degrade calcium pectate of the middle lamella near of the cell wall, causing overflow of cell content and consequently the soft rot. In *Orchids*, the lesions occur initially in the leaves by tissues macerating , and reach the pseudo-stem causing the plant death. The virulence depend on the interactions the pathogen and the host plant as well as with other microorganisms, and the environment. Recent results has show that endophytic bacteria belonging to *Burkholderia* genus were able to control the soft rot in *Orchids*, and has been observed in *Oncidium* that the application of these bacteria reduce up to 100% the soft rot symptoms. However, the molecular aspects involved in the control have not been studied. In this work, 602 of a total 1788 transformants were characterized for their ability to inhibit soft rot caused by *P. carotovora*. We identified 16 mutants showing shifts in inhibition pattern or lost of the ablility to inhibit soft rot symptoms. Among these mutants, we identified 7 genes related to disease inhibition: phospholipase like patatin protein region intermediate; glycosiltransferase protein; hypothetical protein with 23S rRNA sequences traces; glutamate synthase; major facilitator transporter protein; poli-beta-hidroxyalkanoate depolymerase; and fatty-acid desaturase. These genes may be involved in process of allelochemicals synthesis, competition for nutrients, adapting to environmental conditions, and interaction between the host and microorganisms. However, the involvement of these genes in loss of ability to control the soft rot disease is being further studied in details.

Key words: *Burkholderia*. Biological control. Transposon Tn5. Soft rot. Cloning gene.

# 1 INTRODUÇÃO

As orquídeas constituem um dos mais apreciados grupos de plantas ornamentais exploradas comercialmente no Brasil, sendo que, dentre os setores agrícolas em fase de expansão, seu cultivo vem se destacando. No entanto, essa intensificação na produção visando atender a demanda no mercado, resultou em um aumento no ataque de várias doenças e pragas. A implantação de monoculturas, manejo inadequado, incluindo a reciclagem de água de irrigação, eliminação de inimigos naturais por aplicação de produtos químicos não seletivos, mudanças de temperatura e umidade, resultam em alterações físicas, químicas e biológicas, que podem tornar a cultura vulnerável ao ataque de patógenos.

A podridão mole causada pela bactéria *Pectobacterium carotovora* é citada como uma das principais doenças encontradas em orquídeas. Estas bactérias infectam a planta hospedeira geralmente sobre alta umidade, onde produzem enzimas que degradam os pectados de cálcio da lamela média junto à parede celular, resultando em danos celulares com derramamento do conteúdo celular e necrose do tecido. Esta bactéria é um patógeno oportunista, e a sua virulência é dependente das interações com seu hospedeiro e microrganismos que ocupam o mesmo nicho, além da interação com o ambiente, como nutrientes e quantidade de água livre disponíveis, temperatura e tensão de oxigênio.

Atualmente, somente o controle químico, com custo elevado, tem apresentado resultados positivos na prevenção da podridão mole, mas é importante ressaltar o agravamento a longo prazo do uso intensivo de agroquímicos, tanto no aspecto da saúde humana como ao meio ambiente. No contexto de alternativas mais naturais para a contenção de patógenos, o controle biológico tem sido uma alternativa, principalmente quando empregado em conjunto com outros métodos, pois apresenta um menor custo e agressividade ao ecossistema comparado ao tratamento químico.

Experimentos em campo mostram que bactérias do gênero *Burkholderia* são capazes de colonizar uma variedade de plantas, aumentando significativamente o seu crescimento, além de reduzir a presença de patógenos. O controle biológico utilizando burkholderias poderia substituir parcialmente a utilização de pesticidas químicos comuns, visto que já foi relatada uma grande variedade de compostos com atividade antimicrobiana produzidos por *Burkholderia* sp., tais como cepacinas, pirrolnitrinas, cepaciamicinas, cepacidinas, alteridinas, quinolonas, fenazinas, sideróforos e lipopeptídeos. Entretanto, o envolvimento destes metabólitos, assim como os mecanismos de controle não são bem conhecidos para esta

bactéria.

O controle de *P. carotovora* em *Oncidium flexuosum* foi demonstrado em estudo utilizando isolados endofíticos, onde apenas isolados de *Burkholderia* obtidos de eucalipto e cana-de-açúcar foram capazes de inibir o aparecimento dos sintomas da podridão mole em fragmentos foliares. Testes *in planta*, com a inoculação conjunta de *P. carotovora* e *Burkholderia* sp. resultaram no controle de 100% dos sintomas. Apesar do cenário favorável, existe uma baixa adoção do controle biológico em campo, em parte, por incerteza de sua eficácia, sendo necessário avaliar, por meio de estudos apropriados, os fatores que determinam o sucesso do agente de biocontrole. A supressão de doenças é resultado de uma interação complexa entre o antagonista, o patógeno, a planta hospedeira e a comunidade associada, além de fatores ambientais, sendo, portanto de grande interesse a sua compreensão.

Deste modo, este trabalho tem o intuito de identificar, por meio da obtenção e análise de uma biblioteca de mutantes gerados por mutagênese aleatória com o transponson Tn5, quais os possíveis mecanismos envolvidos no controle da podridão mole de *P. carotovora* por uma linhagem endofítica de *Burkholderia* sp. Os resultados obtidos poderão permitir uma melhor compreensão dos mecanismos envolvidos na complexa interação da tríade patógeno-hospedeiro-agente de controle biológico.

## **6 CONCLUSÃO**

Por meio dos resultados obtidos no presente estudo, foram identificados 7 genes associados ao controle da podridão mole, com isso, foi possível concluir que:

- Isolados de *Burkholderia* spp. obtidos de cana de açúcar são capazes de controlar a podridão mole de *Oncidium* sp. causada por *P. carotovora*,
- A mutagênese aleatória por transposon constitui uma abordagem eficiente na identificação de genes envolvidos no controle da podridão mole de *Pectobacterium carotovora* pela linhagem de *Burkholderia cepacia* TC3.4.2R3 (O SEQUENCIAMENTO DO GENE Rec MOSTROU ISSO), permitindo gerar uma biblioteca de mutantes com perda da expressão de diferentes genes;
- O controle deste patógeno por *B. cenocepacia* TC3.4.2R3 em *Oncidium* ocorre por meio da produção de compostos aleloquímicos, competição por nutrientes, adaptação às condições ambientais, interação com a planta hospedeira, e entre microrganismos.
- Como perspectiva futura, um estudo mais aprofundado, a partir de uma análise mais específica e pontual para cada gene interrompido, auxiliarão no entendimento dos processos alterados nestes mutantes e a sua importância nas interações entre microrganismos em sistemas biológicos.

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