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AVALIAÇÃO DA ATIVIDADE QUITINOLÍTICA POR BACTÉRIAS DO GÊNERO *Aeromonas* ISOLADAS DO ECOSSISTEMA MARINHO

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RESUMO

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Bactérias quitinolíticas são autóctones e extremamente importantes no ecossistema marinho, pois desenvolvem um papel fundamental no ciclo de nutrientes através da degradação da quitina na coluna d'água. A hidrólise da quitina é realizada por enzimas denominadas quitinases, as quais possuem diversas funções na natureza. Diferentes quitinases bacterianas, tais como endoquitinases, quitobiosidases e *N*-acetil-glicosaminidases têm sido descritas na literatura. Estas enzimas possuem diferentes mecanismos de hidrólise e podem ser utilizadas com diversos fins biotecnológicos. Com base na grande importância das bactérias quitinolíticas no ecossistema marinho e à procura de bactérias capazes de produzir quitinases para aplicação em processos biotecnológicos, o presente estudo teve como objetivo selecionar as melhores condições para o cultivo de 12 bactérias selecionadas do gênero *Aeromonas* a partir de três variáveis (temperatura, concentração de quitina e pH), avaliar o comportamento desses isolados na degradação de quitina durante 96 horas nas condições de cultivo pré-determinadas através de três metodologias (medida do halo de hidrólise de quitina, cromatografia líquida de alta eficiência e o teste de Antrona) e pela atividade de três enzimas quitinolíticas (*N*-acetil-glicosaminidase, quitobiosidase e endoquitinase). Além disso, caracterizá-las através do sequenciamento total do gene 16S rRNA e por *Multilocus Sequence Analysis* (MLSA) utilizando três *housekeeping* genes (*rec*A, *rpo*B e *rpo*D). Observou-se que os isolados podem representar duas espécies do gênero *Aeromonas* pelo sequenciamento dos *housekeeping genes* utilizados e que identificações baseadas no gene 16S rRNA não são apropriadas para a caracterização desses isolados em nível de espécie. Os isolados comportaram-se de diferentes maneiras em relação às condições de cultivo avaliadas e não necessariamente aqueles que apresentaram maior crescimento nas condições selecionadas foram aqueles que apresentaram maior atividade quitinolítica. O estudo mostrou que as bactérias atuaram na degradação de quitina durante as 96 horas de cultivo, mas que cada isolado possui sua particularidade, seja na tolerância às condições de cultivo, na degradação de quitina ou nos níveis e diversidade de quitinases expressos. No entanto, observou-se que as quantificações dos produtos de hidrólise de quitina por cromatografica líquida de alta eficiência e dos açúcares solúveis totais não são apropriadas para avaliação da degradação de quitina, pois não permitem o monitoramento desse processo devido à insolubilidade da quitina.

Palavras-chave: *Aeromonas*. Quitina. Quitinase. Bactérias Quitinolíticas. Ecossistema Marinho.

ABSTRACT

CARDOZO, F. A. **Chitinolytic activity evaluation by *Aeromonas* genus strains isolated from the marine ecosystem.** 2012. 111 f. Dissertation (Masters thesis in Biotechnology) - Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, 2012.

Chitinolytic bacteria are autochthonous and extremely important in the marine ecosystem. They have key role in nutrient cycling through the chitin degradation in the water column. The hydrolysis of chitin is performed by enzymes called chitinases, which have many functions in nature. Different bacterial chitinases, such as endochitinases, chitobiosidases and *N*-acetyl-glucosaminidases have been described in the literature. These enzymes have different mechanisms of hydrolysis and may be used for various biotechnological purposes. Based on the great importance of chitinolytic bacteria in the marine ecosystem and looking for bacterial chitinase to use in biotechnological processes, this study aimed to select the best conditions for the cultivation of 12 selected chitinolytic bacteria of the *Aeromonas* genus using three variables (temperature, chitin concentration and pH), to evaluate the behavior of these isolates on chitin degradation during 96 hours of cultivation under pre-determined conditions by three methods (halo of chitin hydrolysis, high-performance liquid chromatography and Antrona test) and by chitinolytic activity of three enzymes (*N*-acetyl-glucosaminidase, chitobiosidase and endochitinase). Moreover, characterize them by 16S rRNA gene full sequencing and Multilocus Sequence Analysis (MLSA) using three housekeeping genes (*rec*A, *rpo*B, and *rpo*D). It was observed that the isolates may represent two species of the *Aeromonas* genus by housekeeping genes sequencing and that identifications based on 16S rRNA gene are not suitable for the characterization of these isolates to the species level. The isolates had different behavior in relation under conditions evaluated and not necessarily those with greatest growth under the conditions selected were those that showed higher chitinolytic activity. The study showed the bacteria acted about chitin degradation during the 96 hours of cultivation, but each isolate has own features, or tolerance to conditions of cultivation, on chitin degradation and levels or diversity of chitinases. However, it was observed that the measurements of chitin hydrolysis products by high-performance liquid chromatographic and soluble sugars are not suitable for evaluation of the chitin degradation, because it does not allow monitoring of the process due to the insolubility of chitin.

Keywords: *Aeromonas*. Chitin. Chitinase. Chitinolytic Bacteria. Marine Ecosystms.

INTRODUÇÃO

As bactérias quitinolíticas são extremamente importantes no ecossistema marinho, pois possibilitam a restauração dos níveis de carbono e nitrogênio através da degradação da quitina na coluna d'água. As bactérias envolvidas nesse processo mostram-se altamente eficientes, já que apenas vestígios de carapaças de artrópodes e outros organismos que possuem quitina em sua estrutura são encontrados nos sedimentos oceânicos. No entanto, a atividade antropogênica tem atingido níveis agressivos nos últimos anos, podendo afetar de maneira negativa ou positiva a sobrevivência dessas bactérias, ou seja, essas bactérias podem ser eliminadas de seus habitats em um curto período de tempo ou adquirir resistência às pressões seletivas do ambiente tornando-se capazes de realizar de maneira cada vez mais eficiente suas funções.

Diante da importância das bactérias quitinolíticas para a manutenção da vida no ecossistema marinho, da ausência de trabalhos científicos sobre a diversidade dessas bactérias no litoral brasileiro, e ainda, buscando selecionar espécies bacterianas com capacidade de produzir quitinases em níveis que pudessem ser empregados em processos biotecnológicos, iniciou-se em 2005 no Laboratório de Ecologia Microbiana Molecular do Instituto de Ciências Biomédicas da Universidade de São Paulo, um trabalho sobre a diversidade de bactérias quitinolíticas no litoral do Estado de São Paulo. Através desse trabalho realizado por Souza (2009), onde foram coletadas amostras de água do mar e plâncton no Canal de São Sebastião, Ubatuba e Baixada Santista, foi possível conhecer a diversidade de bactérias quitinolíticas em ambientes naturais com diferentes níveis de atividade antropogênica, obter um total de 492 bactérias quitinolíticas e ainda selecionar aquelas que apresentavam maior capacidade de degradação de quitina.

As 492 bactérias quitinolíticas obtidas foram analisadas quanto a sua capacidade de degradação de quitina em meio mineral contendo quitina como única fonte de carbono após 96 horas de incubação a 28 °C. A capacidade de degradação de quitina foi verificada a partir da medida do diâmetro dos halos de hidrólise formados ao redor das colônias, as quais indicavam o potencial de produção de quitinases pelas bactérias quitinolíticas.

Diante dos trabalhos realizados pelo grupo do nosso laboratório com bactérias quitinolíticas desde 2005, de todo o conhecimento adquirido durante esses anos e do crescente interesse biotecnológico por quitinases e bactérias quitinolíticas, 12 bactérias quitinolíticas do gênero *Aeromonas* parcialmente sequenciadas pelo gene 16S rRNA e com maior capacidade de degradação de quitina (SOUZA, 2009) foram selecionadas para o presente projeto de

mestrado, visando selecionar as melhores condições de cultivo a partir de três variáveis selecionadas (temperatura, concentração de quitina e pH) e avaliar o comportamento desses isolados na degradação de quitina durante 96 horas de cultivo.

CONCLUSÕES

- Os isolados podem representar duas espécies do gênero *Aeromonas* pelo sequenciamento dos *housekeeping genes* utilizados e identificações baseadas no gene 16S rRNA não são apropriadas para a caracterização desses isolados em nível de espécie.

- O método de s*pread plate* associado à utilização de pérolas de vidro ofereceu uma boa altenativa para contagem de bactérias quitinolíticas viáveis.

- Os halos de hidrólise de quitina, produzidos pelas bactérias quitinolíticas, não estão relacionados à atividade enzimática ou aos produtos de hidrólise de quitina quantificados.

- A quantificação dos produtos de hidrólise de quitina por cromatografica líquida de alta eficiência e dos açúcares solúveis totais não são apropriadas para avaliação desse processo, pois não permitem monitorar a degradação devido à insolubilidade da quitina.

- Todas as bactérias foram capazes de expressar as três quitinases avaliadas. No entanto, maiores atividades foram observadas em 24 horas de incubação para as enzimas quitobiosidases e endoquitinases e em 96 horas para *N*-acetil-glicosaminidases.

- Os perfis enzimáticos diferem entre os isolados e os perfis de cada isolado também diferem entre eles ao longo do cultivo, mostrando a diversidade de enzimas existente entre eles e envolvidas no processo de degradação de quitina.

- Os isolados comportaram-se de diferentes maneiras em relação às condições de cultivo e não necessariamente aqueles que apresentaram maior crescimento foram aqueles que apresentaram maior atividade quitinolítica.

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