MARIA PAULA PARADA PINILLA

CARACTERIZAÇÃO DE BACTÉRIAS HALOTOLERANTES ISOLADAS DO BIOMA CAATINGA E AVALIAÇÃO DA PRODUÇÃO DE BIOPOLÍMEROS

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

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RESUMO

PARADA M. P. P. **Caracterização de bactérias halotolerantes isoladas do bioma caatinga e avaliação da produção de biopolímeros.** 2016. 115 f. Dissertação (Mestrado em Microbiologia) – Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, 2016.

Os organismos extremófilos são considerados atualmente reservatórios de novas biomoléculas de interesse biotecnológico. Dentro deste grupo encontram-se os microrganismos que requerem altas concentrações de sal para crescer, denominados halófilos. Também existem os halotolerantes que são aqueles microrganismos que não precisam de sal para proliferar, mas toleram altas concentrações de NaCl. Os ambientes salinos provaram ser uma fonte rica de microrganismos halotolerantes produtores de novos compostos naturais e, portanto, a pesquisa nestes ambientes torna-se de grande importância. No Brasil, na região salina de Areia Branca no bioma caatinga, foram isoladas bactérias halotolerantes que foram estudadas com o objetivo de avaliar a produção de novos biopolímeros de interesse biotecnológico. Acredita-se que os polímeros naturais desses microrganismos extremos podem ter aplicações inovadoras ou características diferentes às tradicionais. Neste estudo, os isolados foram identificados em nível de gênero com base na análise da sequência do gene 16s rRNA. Os isolados foram principalmente bactérias Gram-positivas atribuídas às famílias *Bacillaceae*, Staphylococcaceae, *Microbacteriaceae* e uma bactéria *Incertae Sedis* do filo firmicutes, afiliadas aos gêneros *Bacillus*, *Staphylococcus*, *Curtobacterium* e *Exiguobacterium*, respectivamente. Apenas um isolado Gram-negativo foi identificado e atribuído como membro da família *Pseudomonadaceae*, incluso no gênero *Pseudomonas.* Avaliou-se a tolerância ao sal dos isolados em meio TSB suplementado com 5, 35, 60 e 120 g/L de NaCl. Todos os isolados apresentaram a capacidade de crescer nas quatro concentrações de NaCl avaliadas, com exceção do isolado *Exiguobacterium* sp. sac36 que não cresceu na concentração de 120 g/L de NaCl no meio. Realizaram-se ensaios de acúmulo de polihidroxialcanoatos (PHA) e evidenciou-se que quatro isolados do gênero *Bacillus* são capazes de acumular 3-hidroxibutirato (3HB) a partir de glicose, xilose, e alguns destes em glicerol. Adicionalmente, confirmou-se que quando há altas concentrações de NaCl no meio, o acúmulo de 3HB dos isolados produtores diminui. Observou-se também que doze isolados halotolerantes são produtores de exopolissacarídeos (EPS). Testes realizados indicaram que os mesmos podem ter efeitos imunoestimulantes em macrófagos. Finalmente, avaliou-se a produção de ácido hialurônico (AH) pelos isolados halotolerantes. Segundo sugere o método de *Alcian blue*, todos os isolados foram capazes de produzir AH, mostrando que a maior parte deles acumulou o biopolímero em concentrações maiores ou semelhantes ao controle. Os resultados obtidos evidenciam que os isolados halotolerantes avaliados são uma fonte rica em compostos com atividades promissoras para as diferentes indústrias. O presente trabalho contribui no estudo do potencial biotecnológico de microrganismos isolados no bioma caatinga, destacando sua biodiversidade, versatilidade e a necessidade de continuar explorando esses ambientes extremos pouco estudados.

Palavras-chave: Microrganismos halotolerantes. Biopolímeros. Polihidroxialcanoatos. Exopolissacarídeos. Atividade imunoestimulatória. Ácido hialurônico. Ambientes extremos.

ABSTRACT

PARADA M. P. P. **Characterization of halotolerant bacteria isolated from Caatinga and evaluation of biopolymers production.** 2016. 115 p. Dissertation (Master thesis in Microbiology) – Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, 2016.

Extremophile organisms are considered reservoirs of new biomolecules of biotechnological interest. In this group there are microorganisms that require high salt concentration to grow, called halophiles, and halotolerant microorganisms, that do not need salt to proliferate but can tolerate high concentrations of NaCl. Saline environments proved to be a rich source of new natural compounds by halotolerant producers and therefore, research in these environments becomes of great importance. In Brazil, in the saline region of Areia Branca in the caatinga biome, halotolerant bacteria were isolated and studied in order to evaluate the production of new biopolymers of biotechnological interest. It is believed that the natural polymers of those extreme microorganisms could have innovative applications or different characteristics from the traditional biopolymers. In this study, the isolates were identified at the genus level based on 16S rRNA gene sequence analysis. Isolates were mainly Gram-positive bacteria from Bacillaceae, Staphylococcaceae and Microbacteriaceae families, and *Bacillus*, *Exiguobacterium*, *Staphylococcus* and *Curtobacterium* genera. One of the Gram-negative isolate was identified as member of the Pseudomonadaceae family, genus *Pseudomonas*. The evaluation of salt tolerance of the bacterial isolates on TSB medium supplemented with 5, 35, 60 and 120 g / L NaCl was performed. All the isolates showed the ability to grow in the four concentrations evaluated, except for *Exiguobacterium* sp. sac36, that did not grow at 120 g / L NaCl. Polyhydroxyalkanoate (PHA) accumulation assays were performed using glucose, xylose and glycerol as carbon source. The results showed that four strains of the genus *Bacillus* were able to accumulate 3-hydroxybutyrate (3HB) in all conditions. Additionally, it was confirmed that the presence of high concentrations of NaCl in the medium causes a decrease in 3HB accumulation in the cells. It was observed that twelve halotolerant bacteria produced exopolysaccharides (EPS). Tests performed indicated that those EPS could have immunostimulatory effects on macrophages. Finally, hyaluronic acid (HA) production was evaluated. According to Alcian blue method, all strains were able to produce HA, showing that most of the isolates accumulated the biopolymer in higher or similar concentrations to the control. The results showed that the halotolerant isolates are a rich source of compounds with promising activities for different industries. This study contributes to the knowledge of microorganisms from the caatinga biome and their biotechnological potential, highlighting their biodiversity, versatility and the need to continue exploring these poorly studied extreme environments.

Keywords: Halotolerant microorganisms. Biopolymers. Polyhydroxyalkanoate. Exopolysaccharides. Immunostimulating activity. Hyaluronic acid. Extreme environment.

1 INTRODUÇÃO

Os organismos que vivem nos extremos de pH (>pH8.5, <pH 5.0), temperatura (>45 °C, <15 °C), pressão (>500 atmosferas), salinidade (>58,4 g/L de NaCl) e em concentrações elevadas de compostos recalcitrantes ou metais pesados são conhecidos como extremófilos (PODAR; REYSENBACH, 2006). Graças às diferentes estratégias de adaptação que estes organismos desenvolveram para proliferar em ambientes extremos e também a capacidade de biossíntese de diferentes metabólitos, atualmente são considerados reservatórios de novas biomoléculas com potencial biotecnológico (DE CARVALHO; FERNANDES, 2010).

Dentro deste grupo, estão os microrganismos halófilos, que são aqueles que requerem altas concentrações de sal para crescer e podem ser classificados como: halófilos moderados, halófilos extremos limítrofes e halófilos extremos. No entanto, existe outra classificação para aqueles microrganismos que, embora não precisem de sal, toleram altas concentrações (≥ 58,4 g/L) de NaCl, sendo então chamados de halotolerantes (HAMEDI; MOHAMMADIPANAH; VENTOSA, 2013; JAIN; NAGAL; JAIN, 2012; OREN, 2010; VENTOSA; ARAHAL, 2011).

Estes microrganismos podem ser encontrados nos três domínios da vida; Archaea, Bacteria e Eukarya, e têm sido isolados de ambientes hipersalinos das diversas áreas geográficas da Terra, tais como lagos salinos, pântanos de água salgada, desertos e mares (DALY, 2010; JAIN; NAGAL; JAIN, 2012; VENTOSA et al., 2008, 2015). Por outro lado, destacam-se também por serem possíveis produtores de diversos compostos de importância médica, alimentar, ambiental e cosmética (YIN et al., 2014).

Em particular, os biopolímeros destes organismos são macromoléculas interessantes que têm sido utilizadas recentemente em aplicações biomédicas e agroindustriais devido às suas propriedades terapêuticas e biodegradáveis, como, por exemplo, a capacidade imunoestimuladora em células humanas de sangue e atividade antioxidante para terapia contra o câncer (GUGLIANDOLO et al., 2014; RAVEENDRAN et al., 2015; YE et al., 2016).

Do mesmo modo, muitos desses microrganismos são capazes de acumular biopolímeros, como os polihidroxialcanoatos (PHA), uma família de plásticos biodegradáveis que representam uma alternativa promissora para substituir os plásticos derivados do petróleo, usando diferentes substratos de baixo custo (HUANG et al., 2016; MOORKOTH; NAMPOOTHIRI, 2016).

Igualmente, existe um grande interesse nos exopolissacarídeos (EPS), polímeros de elevado peso molecular sintetizados e acumulados na superfície das células de bactérias halotolerantes, devido às suas atividades biológicas inovadoras tais como: antioxidantes, imunoestimulantes, antitumorais e antivirais (GUGLIANDOLO et al., 2014; SPANÒ; ARENA, 2016; YE et al., 2012). Além disso, produzem solutos compatíveis, como a ectoína e a hidroxiectoína, comercialmente utilizados como agentes de proteção para células de mamíferos e peles (YIN et al., 2014).

Como se pode constatar, os ambientes salinos são uma fonte rica de microrganismos produtores de novos compostos naturais de interesse biotecnológico e a pesquisa nestes ambientes tornou-se de grande importância nos últimos anos (ZHANG et al., 2005). No caso particular do Brasil, encontram-se regiões salinas no bioma caatinga que representam uma fonte única de recursos naturais pouco estudados.

A caatinga é um bioma único que cobre uma vasta área no nordeste do Brasil, que corresponde a quase 10 % do território brasileiro (DE ALBUQUERQUE et al., 2007). Este bioma, segundo a United Nations Environmental Programme (2007), tem um clima semiárido, ou seja, possui um índice de aridez de 0,2-0,5 (valores descritos para regiões desérticas), o qual considera tanto as precipitações em mm e temperaturas medias anuais, e apresenta duas estações bem definidas: chuvosa e seca, com predominância de um clima seco e quente.

Por outro lado, existem poucos estudos sobre o potencial biotecnológico de microrganismos nesta região (FERREIRA, 2014). Por esta razão, foram isoladas bactérias halotolerantes na região salina de Areia Branca no bioma caatinga, com o intuito de avaliar a produção de PHA e EPS de interesse biotecnológico. Acredita-

se que os polímeros naturais desses microrganismos extremos podem ter aplicações inovadoras ou características diferentes às tradicionais.

2 CONCLUSÕES

O presente trabalho buscou contribuir no estudo do potencial biotecnológico de microrganismos isolados no bioma caatinga, destacando sua biodiversidade e a relevância de continuar explorando ambientes extremos pouco estudados. Os resultados descritos ao longo do texto evidenciam que os isolados halotolerantes são uma fonte rica em compostos com atividades interessantes para as diferentes industrias. Consequentemente, os ensaios preliminares realizados indicaram que a maioria dos isolados são bactérias Gram-positivas, pertencentes a gêneros descritos frequentemente em ambientes extremos, que graças à capacidade que possuem de sintetizar diferentes compostos conseguem proliferar nessas condições adversas e até proteger outro tipo de organismos como espécies vegetais. Todos os isolados avaliados são microrganismos halotolerantes, que apresentam a capacidade de crescer em 120 g/L de NaCl, característica relevante para a aplicação destas bactérias em nível industrial em processos ambientalmente amigáveis onde a água do mar pode ser usada.

Este trabalho permitiu confirmar que quatro isolados do gênero *Bacillus* são capazes de acumular 3-hidroxibutirato (3HB) a partir de glicose, xilose e glicerol, adicionalmente, observou-se acúmulo de 3HB em experimentos se fornecendo altas concentrações de NaCl, o que levou a uma diminuição no acúmulo. Porém, os resultados sugerem o potencial uso destas bactérias pertencentes ao gênero *Bacillus* na produção de PHB, em processos onde fontes de carbono de baixo custo e meios com elevada concentração de sal podem ser usadas.

Alguns isolados halotolerantes foram capazes de produzir EPS, os quais não são citotóxicos e têm efeitos imunoestimulantes em macrófagos murinos, resultados que mostram a relevância desses compostos bacterianos no desenvolvimento de novas drogas e medicamentos para o tratamento de diferentes doenças como as geradas por virus e células cancerígenas. Os ensaios de produção de biopolímeros permitiram evidenciar dois grupos, membros do mesmo gênero *Bacillus*, diferenciados pelo composto sintetizado e posição filogenética: produção de PHB

(isolados próximos à espécie *B. endophyticus)*, e produção de EPS (isolados próximos à espécie *B. paralicheniformis*).

Todos os isolados halotolerantes foram capazes de produzir AH e 58% deles acumularam o biopolímero em concentrações maiores ou semelhantes ao controle. A produção deste biopolimero a partir das bactérias halotolerantes isoladas do bioma caatinga, apresentam uma vantagem sob as linhagens usadas atualmente nas industrias pois não são microrganismos patogênicos.

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