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**Análise da diversidade genética por MLSA e
avaliação da atividade antitumoral de linhagens de
Chromobacterium sp.**

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RESUMO

MENEZES, C. B. A. **Análise da diversidade genética por MLSA e avaliação da atividade antitumoral de linhagens de *Chromobacterium* sp.** 2009. 162 f. Dissertação (Mestrado) – Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, 2009.

Chromobacterium violaceum é uma β -Proteobactéria pertencente à família Neisseriaceae, abundante em águas escuras, como as do Rio Negro (Amazonas, Brasil), e em amostras de solo das regiões tropicais e subtropicais de diversos continentes. A coloração violeta das colônias é devido à produção do pigmento violaceína, que apresenta grande potencial biotecnológico. A diversidade genética dos isolados de *Chromobacterium* sp foi avaliada por *Multilocus Sequence Analysis* (MLSA) com base nas análises dos genes conservados *rpoA*, *lepA*, *gyrB*, *fusA* e rRNA 16S, concatenados. As análises do gene RNA ribossomal 16S e MLSA agruparam todos os isolados no gênero *Chromobacterium*, entretanto, cinco dos isolados estão distantes filogeneticamente das linhagens tipo, *Chromobacterium violaceum* e *Chromobacterium subtsugae*, sugerindo duas novas espécies. Resultados estes confirmados por hibridação DNA-DNA. A produção de compostos derivados do metabolismo com propriedades antitumorais foi avaliada através análise dos extratos obtidos por soxhlet em células tumorais humanas, MCF-7 (mama), NCI-ADR (mama com fenótipo de resistência a múltiplas drogas), UACC-62 (melanoma), NCI460 (pulmão), PCO3 (próstata), HT29 (cólon), OVCAR (ovário) e 786-0 (rim). Os extratos brutos dos isolados de *Chromobacterium* apresentaram atividades antitumorais potenciais e seletivas para determinadas linhagens celulares. Os extratos brutos de duas linhagens foram selecionadas para o fracionamento visando a identificação dos metabólitos secundários envolvidos na atividade antitumoral por pertencerem a grupos distintos, de acordo com a análise do gene rRNA 16S. Os extratos brutos e frações foram analisados por HPLC-DAD para avaliação da presença ou ausência da violaceína. Além disso, outros metabólitos secundários que não a violaceína podem estar relacionados à atividade antitumoral.

Palavras-chave: *Chromobacterium* sp.; *Multilocus Sequence Analysis* (MLSA); Violaceína; Atividade antitumoral.

ABSTRACT

MENEZES, C. B. A. **Genetic diversity analysis by MLSA and antitumor activity evaluation of *Chromobacterium* sp. strains.** 2009. 162 f. Master Thesis – Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, 2009.

Chromobacterium violaceum is a β -proteobacteria belonging to Neisseriaceae family, abundant in dark waters, such as the Rio Negro (Amazonas, Brazil), and in soil samples of tropical and subtropical regions of different continents. The violet color of the colonies is due to the production of the pigment violacein, which shows great biotechnologic potential. The genetic diversity of strains of *Chromobacterium* sp was evaluated by *Multilocus Sequence Analysis* (MLSA) based on the analysis of concatenated conserved genes *rpoA*, *recA*, *lepA*, *gyrB*, *fusA* and 16S rRNA. The analysis of 16S ribosomal RNA gene and MLSA grouped all isolates in the genus *Chromobacterium*, however, five isolates are phylogenetically distant of type strain *Chromobacterium violaceum* and *Chromobacterium subtsugae*, suggesting new species. These results confirmed by DNA-DNA hybridization. For the assessment of the production of secondary metabolites with antitumor properties, the extracts were obtained by soxhlet and used in trials of antitumor activity in human tumor cells, MCF-7 (breast), NCI-ADR (breast with phenotype of resistance to multiple drugs), UACC-62 (melanoma), NCI460 (lung), PCO3 (prostate), HT29 (colon), OVCAR (ovarian) and 786-0 (kidney). The crude extracts of the isolates from *Chromobacterium* showed potential and selective anti-cancer activities for certain cell lines. Two of the crude extracts were selected for fractionation to identification of secondary metabolites involved in antitumor activity. The extracts and fractions were analyzed by HPLC-DAD to assess the presence or absence of violacein. The results derived from this study suggest the existence of two new species for the genus *Chromobacterium*. In addition, other secondary metabolites than violacein may be related with antitumor activity.

Key words: *Chromobacterium* sp.; *Multilocus Sequence Analysis* (MLSA); Violacein; Antitumor activity; Secondary metabolites.

1 INTRODUÇÃO

Os produtos naturais vêm sendo cada vez mais imprescindíveis na busca por metabólitos secundários com diversas atividades biológicas e os microrganismos desempenham um importante papel como produtores de metabólitos secundários com grande potencial biotecnológico. Compostos derivados de bactérias e fungos são alvos potenciais na indústria farmacêutica. Dentre os atuais fármacos comercialmente disponíveis, cerca de 60% são provenientes direta ou indiretamente de produtos naturais, obtidos de microrganismos, plantas e animais.

A *Chromobacterium violaceum* é uma β -Proteobactéria pertencente à família Neisseriaceae, abundante em águas escuras, como as do Rio Negro (Amazonas, Brasil), e em amostras de solo das regiões tropicais e subtropicais de diversos continentes. A coloração violeta das colônias é devido à produção do pigmento violaceína, que apresenta atividades biológicas contra os patógenos da tuberculose, leishmaniose e Mal de Chagas, além das atividades antifúngica, antibacteriana, antioxidante e antitumoral.

Devido o potencial farmacêutico e biotecnológico dos produtos metabólicos de *C. violaceum* foi realizado em 2001 sequenciamento completo de seu genoma pela Rede Nacional do Projeto Genoma Brasileiro, no intuito de explorar a sua complexidade molecular. A linhagem tipo *Chromobacterium violaceum* ATCC 12472^T foi escolhida para o sequenciamento por ter sido utilizada em diversos estudos no Brasil nas últimas três décadas. Foram identificados vários genes putativos que apresentam potencial biotecnológico e farmacêutico para diferentes aplicações como: síntese de compostos de relevância médica, detoxificação ambiental, recuperação do ouro, aplicações biotecnológicas como: biocontrole de insetos, fungos e nematódeos; síntese de plástico biodegradável e biossíntese de celulose.

Pouco se conhece sobre a diversidade genética desta bactéria, sendo necessário o estudo com diferentes isolados para explorar, ainda mais, o potencial biotecnológico do gênero. A metodologia de *Multi Locus Sequence Analysis* (MLSA) pode ser uma ferramenta importante no estudo taxonômico, uma vez que este gênero comporta além da *C. violaceum*, as espécies *C. subtsugae*, *C. aquaticum* e *C. haemolyticum* descritas recentemente.

2 CONCLUSÕES

Análise do gene RNA ribossomal agrupou todos os isolados no gênero *Chromobacterium*, e sugere a existência de duas novas espécies para o gênero.

Análise de MLSA dos genes *rpoA*, *gyrB*, *fusA* e *lepA*, isolados e concatenados, corroboram com a análise de seqüências do gene RNA ribossomal 16S.

Os experimentos de hibridação DNA-DNA confirmaram os dados de rRNA 16S e MLSA.

Os extratos obtidos diretamente das células das linhagens de *Chromobacterium* sp. apresentaram diverso espectro de atividade antitumoral nas células tumorais humanas em testes *in vitro*.

A violaceína não é o único metabólito secundário responsável pelas atividades antitumorais apresentadas. Devido à seletividade apresentada na atividade antitumoral, e às diferentes respostas frente às linhagens tumorais avaliadas. Sugere-se a presença de outros compostos com potencial atividade antitumoral.

A purificação dos extratos brutos de duas das linhagens de *Chromobacterium* sp. analisadas resultou em frações potentes, seletivas e frações que não apresentaram atividade antitumoral. Uma das frações obtida pela purificação do extrato bruto etanólico de uma das linhagens merece destaque, pois foi seletiva e extremamente potente para a linhagem NCI-ADR/RES (ovário resistente a múltiplas drogas padrões), sendo, portanto promissora para futuros estudos.

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