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ANÁLISE GENÔMICA DE *STREPTOMYCES OLINDENSIS* DAUFPE 5622 E DE SUAS VIAS CRÍPTICAS PARA A OBTENÇÃO DE NOVOS METABÓLITOS SECUNDÁRIOS DE INTERESSE BIOTECNOLÓGICO

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RESUMO

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A caracterização de novos clusters biossintéticos tem se tornado uma prioridade dentro da pesquisa com actinobactérias devido ao potencial biotecnológico para produzir novas moléculas com possível uso na indústria e farmácia. Estes produtos naturais têm readquirido interesse pelas características particulares que possuem, por exemplo: biodisponibilidade, especificidade de alvo e diversidade de estruturas químicas. Ademais, a maioria das vias biossintéticas para a produção destas moléculas, descobertas no genoma de bactérias do gênero *Streptomyces,* permanecem crípticas ou silenciadas em condições de cultura no laboratório. Isto tem levado ao desenvolvimento de diversas técnicas e metodologias para a expressão destas vias metabólicas. Uma destas estratégias é a super-expressão de genes reguladores específicos dentro destas vias, como as proteínas reguladoras de antibióticos em *Streptomyces* (SARP, pelas siglas em Inglês). O laboratório de Bio-Produtos no Departamento de Microbiologia no Instituto de Ciências Biomédicas, na Universidade de São Paulo, tem trabalhado com a linhagem *Streptomyces olindensis* DAUFPE 5622, que produz uma molécula do grupo das antraciclinas chamada Cosmomicina D.Esta possui uma alta atividade antitumoral e tem sido de grande interesse devido ao padrão de glicosilação. Utilizando sequenciamento *Shotgun*, foram obtidos 120 *contigs* representando o genoma de *S. olindensis*, os quais foram anotados e submetidos ao NCBI sob o número de acesso JJOH00000000. Utilizando o software antiSMASH e anotação manual, foram identificados trinta e três clusters envolvidos na produção de metabólitos secundários. Dentro destes, foram encontrados clusters gênicos para a produção de metabólitos previamente descritos, com diversas atividades biológicas, como a cosmomicina D, melanina, pigmento de esporos, fator-A, geosmina, albaflavenona, hopanoides, amiquelina, hidroxiectoina, desferrioxamina e fosacetamicina. Os outros vinte dois clusters possivelmente produzem derivados de compostos já conhecidos ou moléculas novas, motivo pelo qual, foi realizada uma análise de genômica comparativa para identificar, caracterizar e anotar cada via biossintética achada em *S. olindensis*. Subsequentemente, foram encontrados diversos reguladores específicos de vias metabólicas, tomando especial interesse os SARP. Foram escolhidos dois clusters biossintéticos para a super-expressão (Aminociclitol e um Policetídeo Tipo I) destes genes, levando a detecção do composto sob condições de cultura. Estudos posteriores permitirão identificar a estrutura química destes compostos e propor diferentes metodologias para a obtenção de produtos naturais de interesse biotecnológico, sintetizados por *Streptomyces olindensis*.

Palavras-chave: *Streptomyces*. *Genome mining*. Anotação de genoma. *Cluster* biosintético. Cosmomicina D. Super-expressão de genes reguladores

ABSTRACT

FERREIRA-TORRES M.A. **Analysis of S*treptomyces olindensis* DAUFPE 5622 genome and its cryptic pathways to obtain new secondary metabolites of biotechnological interest.** 2015. 103 p. Master thesis (Microbiology) - Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, 2015.

Characterization of new metabolic biosynthetic clusters has become a priority among researchers in actinobacteria because of their biotechnological potential to produce new molecules with possible use in the pharmaceutical and industrial field. These natural products regain interest because of their particular characteristics, for example: bioavailability, target specificity and chemical structure diversity. Moreover, most of the biosynthetic pathways for the production of these molecules that have been discovered on the genomes of *Streptomyces* remain cryptic or silenced under standard laboratory conditions, which lead to the development of different technical approaches to express these new clusters. One of the strategies is the overexpression of the specific regulatory genes within these pathways, such as *Streptomyces* Antibiotic Regulatory Protein (SARP) to detect these new products in different culture media. The Bio products Laboratory, in the Microbiology Department in the Biomedical Science Institute at São Paulo's University, has been working with *Streptomyces olindensis* DAUFPE 5622, the producer of the anthracycline Cosmomycin D, which has an important antitumoral activity and has attracted interest because of its distinctive glycosylation pattern. Using Shotgun sequencing the genome was obtained, organized in 120 contigs, annotated and submitted in the NCBI under the accession number JJOH00000000, and by further prediction were identified thirty-three secondary metabolite clusters using the antiSMASH server and manual annotation. Among them, were found gene clusters for the production of known metabolites with different biological activity such as Cosmomycin D, Melanin, Spore pigment, A-factor, Geosmin, Albaflavenone, Hopene, Amychelin, Hidroxyectoine, Desferrioxamine B and Fosacetamycin. The other twenty-two clusters were likely to produce derivatives of known compound or new molecules, which is why, a comparative genomic study was carried out to identify, characterize and annotate every genome cluster found in *S. olindensis*. Subsequently, several pathway-specific regulators in the described biosynthetic clusters were found, arising special interest the SARP, choosing two different pathways (Aminocyclitol and Polyketide Type I), to over express the regulatory genes, in order to detect the biological product under culture conditions. Further studies will allow the identity of the chemical structure of these products to be found and propose different approaches to obtain other natural products of interest synthetized by *Streptomyces olindensis*.

Keywords: *Streptomyces*. Genome mining. Genome annotation. Biosynthetic *Cluster*. Cosmomycin D. Overexpression of regulator genes.

1 INTRODUÇÃO

Historicamente, os metabólitos secundários de origem microbiana têm proporcionado uma grande quantidade de moléculas de interesse industrial para uso farmacêutico e outros campos na biotecnologia. Até 2010, tinham sido descritos mais de 500.000 sendo bioativos o 20%. Desta porção bioativa, 35.000 são produzidos por micro-organismos e o 35% é produzida por actinobactérias, sendo o restante produzido por fungos e outras bactérias (BÉRDY, 2012). Embora o desenvolvimento de novos fármacos tenha diminuído na última década, o interesse por novas estruturas químicas se mantem aberto na indústria devido as propriedades intrínsecas dos produtos naturais como a biodisponibilidade das moléculas, a complexidade estrutural e a especificidade de alvo (HARVEY; EDRADA-EBEL; QUINN, 2015). A interdisciplinaridade na pesquisa tem proporcionado diversas ferramentas para a busca de novas biomoléculas, encontrando, assim, diferentes *clusters* biossintéticos no genoma de vários micro-organismos, incluindo actinobactérias (BENTLEY et al., 2002; BÉRDY, 2012). Pode-se detectar domínios conservados típicos de enzimas que produzem moléculas com atividade biológica, caracterizadas quimicamente como policetídeos (PKS), peptídeos não ribossomais (NRPS), terpenos, peptídeos de síntese ribossomal, sideróforos, etc. Estes descobrimentos indicam um grande potencial biotecnológico para obtenção de novos antibióticos, compostos antitumorais e moléculas com outra aplicação biotecnológica (BÉRDY, 2005, 2012; CHALLIS, 2014; NETT; IKEDA; MOORE, 2009).

Em *Streptomyces coelicolor*, a actinobactéria modelo, foram encontrados 29 *clusters* biossintéticos dentro do seu genoma dedicados à produção de metabólitos secundários de interesse industrial, e em *Streptomyces avermitilis* detectaram-se 37 *clusters* para a produção de novas biomoléculas (NETT; IKEDA; MOORE, 2009). Estes dados são um indício do potencial codificado no genoma das actinobactérias, sendo candidatas à busca de novos produtos metabólicos, inicialmente pelo uso de ferramentas de bioinformática para sua caracterização e expressão. A maioria destes novos *clusters*, encontrados em diversos microrganismos, não é detectada em condições de fermentação padrão em laboratório, por isso são chamados de *clusters* ou vias crípticas (BÉRDY, 2012; NETT; IKEDA; MOORE, 2009; OCHI;

HOSAKA, 2013). Este fato leva à busca de novas estratégias para a expressão destes compostos, como a diversificação de meios de cultura e condições de crescimento para a obtenção de novas moléculas (OSMAC approach) (BODE et al., 2002) ou a produção em hospedeiros heterólogos (BALTZ, 2010; STARCEVIC et al., 2012).

No laboratório de Bioprodutos vem se trabalhando com a bactéria *Streptomyces olindensis*, isolada da região de Olinda, no estado de Pernambuco, Brasil. Esta bactéria produz o agente antitumoral Cosmomicina D, amplamente estudada, já que a molécula possui um padrão de glicosilação pouco comum, que potencializa a sua atividade contra diversas linhas celulares tumorais. Além disso, possui uma maior quantidade de genes de resistência que ajudam na saída do composto e na sobrevivência da bactéria (FURLAN et al., 2004; GARRIDO et al., 2006).

A sequência do genoma foi descrita previamente por Rojas e colaboradores em 2014, para ganhar um maior entendimento sobre o cluster gênico da Cosmomicina D e expandir as possibilidades na área de biossíntese combinatória para, assim, modificar a atividade biológica da molécula. Neste estudo é apresentada uma análise completa do genoma como também a comparação com outras espécies de *Streptomyces* relevantes no campo dos compostos bioativos. Finalmente é revelado o potencial codificado para uma variedade de novos produtos naturais, além do agente antitumoral Cosmomicina D, o que levará ao desenvolvimento de diferentes estratégias para a expressão e obtenção de novas moléculas.

5 CONCLUSÕES

- A descrição das características físicas permitiu comparar a topologia do cromossomo de *S. olindensis* com genomas de referência, atribuindo à linearidade *in-silico* e identificando proteínas envolvidas na plasticidade e receptividade do cromossomo, encontrando a possível inserção do *cluster* do metabólito híbrido entre melanina e nucleosídeo.

- A classificação funcional das proteínas codificadas no genoma de *Streptomyces olindensis* DAUFPE 5622, atribui mais do 50% das regiões codificadoras à funções do metabolismo primário e 2.8% das proteínas foram relacionadas com metabolismo secundário.

- A análise de genômica comparativa, utilizando a linhagem *S. olindensis* DAUFPE 5622 e quatro espécies próximas altamente conhecidas, permitiu elucidar a conformação do core genoma em bactérias deste gênero, relatando vias de metabolismo primário, e alguns metabólitos secundários amplamente distribuídos em actinobactérias como sideróforos e exopolisacarídeos.

- Utilizando os grupos de ortólogos da base de dado do KEGG, conseguiu-se determinar as funções dos elementos únicos presentes em *S. olindensis*, destacando-se as proteínas envolvidas na produção de metabólitos secundários como policetídeo sintases, NRP sintases, terpeno sintases entre outros.

- A ferramenta antiSMASH permitiu a identificação da maioria dos *clusters* biossintéticos, facilitando a caracterização das vias metabólicas visando a produção de novos produtos naturais bioativos.

- Realizando a anotação funcional e caracterização dos *clusters* metabólicos, logrou-se realizar uma comparação com vias metabólicas já conhecidas para assim, evitar o redescobrimento de moléculas descritas previamente.

- A identificação e anotação das 34 vias biossintéticas permitiu determinar a presença de genes reguladores, selecionando os clusters biossintéticos a serem ativados.

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