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**ECOLOGIA MICROBIANA DE RIACHOS INFLUENCIADOS PELA
EXPLORAÇÃO DE PETRÓLEO NO BRASIL**

Tese apresentada ao Programa de Pós-graduação em Microbiologia do Instituto de Ciências Biomédicas da Universidade de São Paulo para obtenção do título de Doutor em Ciências.

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RESUMO

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Atividades humanas podem causar a degradação de ecossistemas e interferir na disponibilização dos serviços ambientais que eles proporcionam à própria sociedade. Evitar que a degradação aconteça é a melhor estratégia para garantir a continuidade desses serviços e, para isso, é preciso que as atividades humanas sejam constantemente monitoradas. Uma das estratégias mais utilizadas para o monitoramento é a medição de indicadores ambientais que representem a homeostasia do ambiente e tenham sensibilidade para identificar os sinais iniciais de degradação. Neste estudo nós caracterizamos as comunidades microbianas de ambientes aquáticos continentais na Amazônia, Mata Atlântica, Cerrado e Caatinga e utilizamos esses dados para selecionar indicadores do impacto causado pela atividade de exploração de petróleo (campos de petróleo com poços ativos a mais de 25 anos e sem histórico de incidentes). Avaliamos a diversidade taxonômica e funcional, através do sequenciamento do gene 16S e da análise com o microarranjo de genes funcionais GeoChip 5.0M, e comparamos os resultados de amostras de água, sedimento, biofilme e solo das margens de riachos influenciados por campos de petróleo com áreas de referência minimamente impactadas. Além das análises microbianas, foram medidos os parâmetros físico-químicos da água, parâmetros inorgânicos em água, solo e sedimento e parâmetros orgânicos (n-alcenos e hidrocarbonetos policíclicos aromáticos) em água e sedimento. Todas as estações amostrais apresentaram comunidades microbianas com alta riqueza de espécies e mais de 80% dos genes funcionais detectados com o GeoChip. As comunidades microbianas apresentaram baixa similaridade taxonômica, compartilhando menos de 30% das espécies entre si. Por outro lado, apresentaram alta similaridade funcional, compartilhando mais de 70% dos genes. O principal impacto da atividade de exploração de petróleo foi o uso do solo, como pode ser evidenciado pelo aumento da concentração de íons metálicos, da condutividade elétrica, do número de genes relacionados com o equilíbrio metálico, da abundância de genes de resposta a estresse oxidativo causado por metais e do número de espécies de microrganismos do solo na água dos riachos influenciados pela atividade de exploração. Não foram encontrados indícios de contaminação por hidrocarbonetos nas estações amostrais avaliadas. Diversas espécies de microrganismos e genes funcionais foram identificados como promissores indicadores da atividade de exploração de petróleo nos riachos mas não foi possível atrelar esses indicadores a grupos taxonômicos específicos nem tampouco a atividades metabólicas preferenciais. Isso aponta para uma mudança de paradigma no uso de indicadores para o monitoramento ambiental, indicando que no lugar de espécies indicadoras ou de grupos funcionais indicadores, será mais promissor desenvolver um conceito relacionado à sequências genéticas indicadoras.

Palavras-chave: Comunidades Microbianas. Diversidade Taxonômica. Potencial Funcional. Indicadores Ecológicos. Indústria do Petróleo.

ABSTRACT

Jonck CR. Microbial ecology of streams influenced by petroleum exploration in Brazil. [PhD Thesis (Microbiology)]. São Paulo: Instituto de Ciências Biomédicas, Universidade de São Paulo; 2017.

Human activities can cause degradation of ecosystems and interfere with the availability of the environmental services they provide to society itself. Preventing degradation from happening is the best strategy to ensure the availability of these services, and for this to happen, human activities have to be constantly monitored. One of the most used monitoring strategies is the measurement of indicators that represent the homeostasis of the environment and have the sensitivity to identify the initial signs of degradation. In this study we characterize the microbial communities of continental aquatic environments in the Amazon, Atlantic Rainforest, Cerrado and Caatinga and use this data to select indicators of oil exploration activity impacts (oil fields operating for at least 25 years with no history of spills). We evaluated the taxonomic and functional diversity through 16S gene sequencing and GeoChip 5.0M functional gene microarray. Results from water, sediment, biofilm and stream banks soil samples influenced by oil fields were compared to minimally impacted reference areas. In addition to the microbial communities, we analyse physical-chemical parameters of water, inorganic parameters in water, soil and sediment and organic parameters (n-alkanes and polycyclic aromatic hydrocarbons) in water and sediment. All sampling stations showed microbial communities with high species richness and more than 80% functional genes detected with GeoChip. The microbial communities presented low taxonomic similarity, sharing less than 30% of the species with each other. On the other hand, they showed high functional similarity, sharing more than 70% of the genes. The main impact of the oil exploration activity was land use, as evidenced by the increase in the concentration of metallic ions, the electrical conductivity, the number of genes related to the metal homeostasis, the abundance of metal related oxidative stress response genes and the number of soil microbial species in the water of the streams influenced by the exploration activity. No evidence of hydrocarbon contamination was found at the sample stations evaluated. Several microbial species and functional genes were identified as promising indicators of oil exploration activity in streams but it was not possible to link these indicators to specific taxonomic groups nor to preferential metabolic activities. This points to a paradigm shift in the use of indicators for environmental monitoring, indicating that instead of indicator species or indicator functional groups, it will be more promising to develop a concept related to indicator genetic sequences.

Keywords: Microbial Communities. Taxonomic Diversity. Functional Diversity. Ecological Indicators. Petroleum Industry.

Capítulo 1

INTRODUÇÃO

O desenvolvimento da nossa sociedade exerce uma pressão considerável sobre os sistemas naturais[?]. Devido ao progressivo crescimento demográfico, mesmo o atendimento das demandas básicas da civilização humana acaba gerando degradação ambiental[?]. No entanto, diversos recursos utilizados por essa mesma sociedade têm sua origem nos sistemas naturais[?], o que leva à necessidade de restaurar os ambientes que foram degradados[?].

Para quebrar esse ciclo vicioso, é necessário aumentar a eficiência dos processos industriais e evitar que as atividades humanas causem degradação além da capacidade de suporte do ambiente[?]. Para evitar que esse limiar ecológico seja ultrapassado, é necessário compreender o ambiente onde a atividade ocorre, monitorar os efeitos da atividade e mitigar os impactos que tem potencial para danificar o ambiente, um conjunto de ações conhecida como gestão ambiental[?].

Um dos passos mais importantes desta gestão reside no alvo do monitoramento, ou seja, onde o monitoramento ocorrerá e quais parâmetros do ambiente serão monitorados. Essa definição é altamente dependente do tipo e da escala da atividade que será realizada[?] e erros de dimensionamento podem acarretar em severos prejuízos para o ambiente ou para a atividade.

A produção de petróleo em áreas terrestres, por exemplo, requer que várias atividades que causam impactos no ambiente sejam realizadas para que o produto possa ser bombeado do subsolo. As mais corriqueiras são a remoção da cobertura vegetal com a consequente exposição do solo, terraplanagem, perfuração com a produção de resíduos, construção de estradas, faixas de dutos e instalações de processamento, tráfego de veículos e presença humana[?].

Esse tipo de atividade, por ser realizado em grande escala, tem alto potencial de causar impactos com efeitos difusos. Nestes casos, os locais mais adequados para avaliar seus efeitos são os ecossistemas relacionados com a bacia de drenagem^{???}.

Os rios são interconectados com toda a paisagem[?] e qualquer distúrbio que ocorra na bacia de drenagem irá influenciar as características dos corpos hídricos[?]. Essa característica faz com que o rio integre as alterações que ocorrem em seu entorno, o que o torna a melhor sentinela das

condições ambientais de um ecossistema[?].

O uso de componentes dos ecossistemas lóticos em gestão ambiental já está bastante difundido. Em geral, avaliações ambientais utilizam características físico-químicas da água, concentrações de elementos químicos orgânicos e inorgânicos em água e sedimento e também os componentes biológicos dos rios como indicadores de sua condição[?].

Diversas comunidades já foram testadas como bioindicadores de integridade ou de poluição em ambientes lóticos^{?????}, mas as mais frequentemente utilizadas são as comunidades do perifíton[?], dos macroinvertebrados bentônicos[?] e dos peixes^{??}.

Apesar de reconhecerem que as comunidades microbianas tinham um papel preponderante na dinâmica dos ecossistemas lóticos[?], era comum elas serem desconsideradas em estudos limnológicos clássicos[?]. Quando consideramos estudos microbiológicos dos rios, no entanto, vemos que essa disciplina é, na verdade, o berço da própria microbiologia.

O reconhecimento dos microrganismos como causadores de doenças^{????} e o entendimento de que a água era o meio de sua veiculação^{??} fez com que os primeiros estudos microbiológicos fossem também os primeiros esforços na identificação de indicadores microbianos^{???}.

No entanto, a grande discrepância entre o número de células contadas a partir de uma amostra com o número de células viáveis em cultivo[?] impedia a identificação de uma robusta relação de causa e efeito da comunidade microbiana com as atividades avaliadas[?]. A utilização da comunidade microbiana ficou limitada a indicadores de contaminação fecal por mais de um século devido a essa limitação metodológica[?].

O desenvolvimento de métodos moleculares baseados na leitura das sequências genéticas propiciou um grande salto de conhecimento para a ecologia microbiana[?], principalmente devido à sua capacidade de quantificar e qualificar os organismos que não eram recuperados pelas técnicas de cultivo[?]. A utilização de técnicas “meta-ômicas” (genômica, proteômica, metabolômica) para identificação de indicadores é o caminho natural desta linha de pesquisa[?].

Para tanto, há a necessidade de identificarmos as melhores métricas relacionadas às comunidades microbianas que possam ser utilizadas como indicadores para cada tipo de atividade. A exploração de petróleo onshore é realizada em larga escala em várias partes do mundo e ferramentas mais robustas e expeditas para a apropriada gestão ambiental dessas atividades pode trazer diversos benefícios tanto para a melhoria das condições dos ecossistemas impactados quanto para a sustentabilidade da própria atividade econômica.

O objetivo principal desta pesquisa é identificar, dentro da hierarquia ecológica dos riachos e

com ênfase na biologia das comunidades microbianas (ou seja, desde parâmetros abióticos até características moleculares), elementos que respondem aos impactos das atividades de exploração de petróleo em diferentes domínios morfoclimáticos e que, portanto, possam ser utilizados como indicadores na gestão ambiental dessas atividades. Para atender esse objetivo, as seguintes atividades foram executadas:

- Identificamos ambientes lóticos influenciados por campos de petróleo nos quatro maiores domínios morfoclimáticos brasileiros e, para cada um deles, definimos uma estação amostral de referência;
- Determinamos as características físicas e químicas de cada estação amostral, analisando parâmetros físico-químicos, orgânicos e inorgânicos em cada compartimento dos riachos;
- Identificamos, qualitativa e quantitativamente, as OTUs microbianas (Bacteria e Archaea) em cada estação amostral, as quais utilizamos como uma aproximação do número de espécies em cada ambiente;
- Levantamos o potencial funcional das comunidades microbianas em cada estação amostral;
- Comparamos os resultados das áreas influenciadas pela exploração de petróleo com as áreas de referência e verificamos quais variáveis se mostram consistentemente diferentes entre os diferentes domínios morfoclimáticos.

Capítulo 2

CARACTERIZAÇÃO MICROBIANA DE RIACHOS INFLUENCIADOS PELA EXPLORAÇÃO DE PETRÓLEO NO BRASIL

2.1 CONCLUSÕES

Com base nas análises dos parâmetros abióticos, constatamos que os impactos mais relevantes da atividade de exploração de petróleo nos ambientes aquáticos continentais estão relacionados com o uso do solo. Isso fica evidente quando vemos valores significativamente maiores de condutividade e concentração de íons metálicos na água das áreas influenciadas pelos campos de petróleo (tabela ??).

A indústria do petróleo apresenta um grande potencial de causar impactos e esse potencial está relacionado com o fato de que o próprio produto alvo da indústria é um elemento que pode causar severos danos ambientais. Quando não ocorrem vazamentos, no entanto, a instalação e desenvolvimento de um campo de petróleo causam impactos que resultam na lixiviação de elementos do solo para os rios. Como todos os impactos dessa indústria, sua magnitude é potencial, podendo ser baixa em áreas com boa gestão ambiental, como é o caso da Amazônia, ou mais alta se ocorrer a sinergia de atividades, como foi visto no Cerrado.

Os riachos de cabeceira se mostraram ambientes adequados para o monitoramento da atividade de produção dos campos de petróleo, sendo sensíveis ao principal impacto que ela causa. As estações amostrais da Caatinga, no entanto, sempre se distanciaram das demais nas análises de agrupamento k-means (figuras ?? e ??). Isso se deve principalmente à característica hidrológica do domínio morfoclimático, uma vez que, apesar de estarem estruturados como riachos de cabeceira, as estações da Caatinga raramente apresentam água durante o ano e, quando apresentam, localizam-se em depressões do leito do rio com praticamente nenhum fluxo. Isso nos leva à conclusão de que as estações amostrais da Caatinga não podem ser consideradas similares às demais áreas do estudo por apresentarem características de ambientes lênticos temporários.

A comunidade microbiana apresentou diversidade taxonômica variável enquanto a diversi-

dade funcional, pelo contrário, foi significativamente estável. Essa condição foi observada não obstante a ocorrência de impactos ou a diferença de domínios morfoclimáticos. Seja comparando duas áreas da Amazônia ou uma da Amazônia com uma da Caatinga, o padrão de dissimilaridade da diversidade taxonômica foi basicamente o mesmo, e foi bastante alto (aproximadamente 90%, considerando os dados de abundância relativa). Utilizando os dados do potencial funcional, a mesma comparação mostrou um padrão semelhante, mas invertido. Ou seja, o padrão de dissimilaridade continuava parecido entre os ambientes, mas, neste caso, foi muito baixo (cerca de 30%).

É importante adicionar que esse padrão não é corroborado pelo perfil de distribuição dos organismos no nível de filo, cuja estabilidade pode nos ter entorpecido para a realidade aparentemente caótica da distribuição de espécies microbianas. No nível de OTUs, a Proteobacteria de um ambiente não é, necessariamente, a mesma Proteobacteria do outro, e ainda que seja, tem poucas possibilidades de ter uma abundância parecida.

Podemos concluir, portanto, que um contingente altamente variável de microrganismos utiliza sempre as mesmas ferramentas (genes) para sobreviver em todas as estações amostrais avaliadas. Uma possível releitura desta conclusão, utilizando os genes e não os organismos como foco, é que um conjunto similar de genes está presente em todos os domínios morfoclimáticos e eles se rearranjam de diferentes maneiras (em diferentes organismos) para sobreviver. Esse entendimento nos levou a indagar se os genes não seriam melhores indicadores do que os organismos, questão que tentaremos responder no capítulo seguinte.

Capítulo 3

INDICADORES MICROBIANOS DA ATIVIDADE DE EXPLORAÇÃO DE PETRÓLEO EM RIACHOS BRASILEIROS

3.1 CONCLUSÕES

Em todos os parâmetros avaliados, tivemos a seleção de indicadores respondendo a um leve aumento de concentração de íons metálicos na água. É bastante conclusivo, por conseguinte, que o impacto com o qual as comunidades microbianas se deparam é decorrente do uso do solo. Mesmo o impacto apresentando baixa magnitude, todos os parâmetros apresentara seleção de indicadores, o que atesta a sensibilidade dessa metodologia para utilização em monitoramento ambiental.

Obviamente que essas metodologias precisarão passar por algum refinamento. Praticamente todos os indicadores taxonômicos e a grande maioria dos indicadores funcionais pertencem à biosfera rara de suas comunidades, indicando que futuros estudos nesta linha de pesquisa se beneficiarão da utilização de métodos com ampla cobertura de sequenciamento e que não utilizem amplificação do DNA para melhor caracterizar os indicadores. Após essa identificação, no entanto, microarranjos customizados com os indicadores descobertos podem se tornar uma robusta ferramenta de gestão ambiental.

Essa característica dos indicadores serem predominantemente encontrados na biosfera rara pode ser explicada pela estreita faixa de variação de dissimilaridade, tanto da diversidade taxonômica quanto do potencial funcional. A primeira mostrou que todas as comunidades apresentam alta dissimilaridade. Todos os valores, se considerarmos o dendrograma de abundância taxonômica (figura ??A) estão entre 70 e 90%. Isso significa que 10% da comunidade é similar, 70% é totalmente diferente e apenas essa faixa de 20% da comunidade apresenta variações que possam ser comparadas entre si para dar origem a indicadores. O potencial funcional, por outro lado, mostrou que as comunidades são altamente similares. A faixa de variação ficou entre 15 e 30% (figura ??A). Isso significa que, funcionalmente, a comunidade é 70% similar, 15% diferente e

tem apenas os restantes 15% de variação para dar origem aos indicadores. Essa constatação nos permite concluir como é possível que exista a dissociação entre a diversidade taxonômica e o potencial funcional, duas características de uma mesma comunidade microbiana.

A conclusão mais importante deste capítulo está relacionada com esse fato. Como mencionamos no exemplo sobre as Acidobacteria, os indicadores selecionados não foram atrelados a grupos taxonômicos específicos. O fenômeno, no entanto, se repete com os genes funcionais. Demos o exemplo dos dez genes relacionados com o metabolismo secundário selecionados como indicadores, mas o número total de genes relacionados ao metabolismo secundário é muito maior (tabela ??), indicando que vários genes, apesar de serem do mesmo grupo funcional, não responderam da mesma maneira.

Observamos que nem a taxonomia e nem os grupos funcionais podem ser considerados indicadores inequívocos do efeito dos poços de petróleo sobre a comunidade microbiana dos riachos, ainda que grupos de genes, não atrelados a grupos taxonômicos ou funcionais específicos, tenham respondido de forma bastante robusta aos impactos. Com isso, concluímos que as abordagens que utilizam espécies indicadoras ou mesmo funções metabólicas indicadoras não são adequadas para monitorar a resposta das comunidades microbianas às atividades que causam mudanças no uso do solo. A melhor estratégia, neste caso, é utilizar um conceito de sequências genéticas indicadoras, mesmo que elas não se adéquem às nossas artificiais formas de classificação.

Capítulo 4

COMPARAÇÃO DO POTENCIAL FUNCIONAL DE COMUNIDADES MICROBIANAS ENTRE DIFERENTES BIOMAS

4.1 CONCLUSÃO

A existência de um core microbiano, ou seja, um grupo de microrganismos com distribuição cosmopolita foi uma ideia aventada desde o princípio dos estudos microbiológicos. Essa era a ideia central de Baas-Becking com seu “*everything is everywhere*” (tudo está em todo lugar) e, para justificar a ausência de determinados grupos em diferentes locais ele complementou sua hipótese com o “*but the environment selects*” (mas o ambiente seleciona).

Quase um século e o desenvolvimento de novas metodologias foram necessários para confirmar que o adendo de Baas-Becking era, na verdade o padrão. Comunidades microbianas são extremamente diversas mesmo em ambientes semelhantes e próximos. Mesmo réplicas de um mesmo local podem apresentar consideráveis diferenças taxonômicas e atestam a ideia de que as comunidades microbianas são altamente variáveis do ponto de vista taxonômico.

Assim como ocorreu com os macro organismos, a microbiologia se voltou à ecologia baseada em características, impulsionando os estudos sobre o potencial funcional. Esta se mostrou bastante estável e semelhante entre ambientes bastante diversos.

A conclusão deste capítulo aponta para a existência de um core funcional nas comunidades microbianas, composto não apenas por genes pertencentes aos mesmos grupos, mas por grupos idênticos de genes, ou seja, um certo percentual de genes, redundantes em suas funções, aparece em todo lugar, resgatando parcialmente a hipótese de Baas-Becking (mas com foco em genes e não em espécies).

Obviamente que esse percentual precisa ser confirmado com a utilização de métodos capazes de suplantarem as limitações dos utilizados aqui (principalmente o baixo número de sequências imposto pelo GeoChip) mas, em se confirmando a existência de um core funcional tão robusto, podemos estar diante de um fenômeno que representa a própria assinatura da vida no planeta. O

reconhecimento deste fenômeno e o desenvolvimento de novas tecnologias capazes de avaliá-lo pode nos levar ao desenvolvimento de estudos ecológicos baseados em sequências genéticas, o que pode elevar sobremaneira a nossa capacidade de predição sobre as comunidades biológicas.

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