UNIVERSIDADE FEDERAL DO PARÁ
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM GENÉTICA E BIOLOGIA MOLECULAR

# ANÁLISE DA COMUNIDADE MICROBIANA INTESTINAL DE OSTRAS E DA ÁGUA PROVENIENTES DE RESERVAS EXTRATIVISTAS MARINHAS DO ESTADO DO PARÁ

SÁVIO DE SOUZA COSTA

Belém
2024

UNIVERSIDADE FEDERAL DO PARÁ
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM GENÉTICA E BIOLOGIA MOLECULAR

# ANÁLISE DA COMUNIDADE MICROBIANA INTESTINAL DE OSTRAS E DA ÁGUA PROVENIENTES DE RESERVAS EXTRATIVISTAS MARINHAS DO ESTADO DO PARÁ

SÁVIO DE SOUZA COSTA

Tese submetida ao Programa de Pós-Graduação em Genética e Biologia Molecular da UFPA como requisito parcial para obtenção do grau de Doutor em Genética e Biologia Molecular.

Orientador: Prof. Dr. Rafael Azevedo Baraúna

Belém
2024

## INSTITUIÇÕES PARTICIPANTES E FONTES FINANCIADORAS

### Instituições Participantes

- Laboratório de Genômica e Bioinformática (UFPA);
- Centro de Genômica e Biologia de Sistemas (UFPA);
- Instituto de Estudos Costeiros, Bragança (UFPA);
- Parque de Ciência e Tecnologia Guamá (UFPA);
- Laboratório de Engenharia Biológica (UFPA).

### Fontes Financiadoras

- Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES);
- Fundação de Amparo a Pesquisa do Pará (FAPESPA).

***“Eu sou a continuação de um sonho***
***Da minha mãe do meu pai***
***De todos que vieram antes de mim”***

*BK* – Continuação de um sonho

## AGRADECIMENTOS

Quatro anos. Eu olho pra trás e nunca imaginei que chegaria até aqui, mas aqui estou, em um dos momentos mais importantes da minha vida acadêmica. Mas ninguém chega a lugar nenhum sozinho. Sem cada uma das pessoas presentes na minha vida, este caminho teria sido bem mais difícil.

Primeiramente, quero agradecer aos meus pais, Severino Costa e Sidônia Souza, que me educaram no caminho da justiça, da lealdade e da busca do melhor através do estudo. Vocês são meus exemplos, amo vocês. Minha irmã, Suellen Costa, tu és meu pilar, a flor mais bonita desse jardim e a voz que me aconselha. Obrigado por existir.

Meus irmãos de outra mãe, Marcinho, obrigado por nunca arredar o pé do meu lado, we still standing! Sempre! Sophya, obrigado por crescer comigo. Minha cunhada, Yanne, obrigado por me curar e cuidar de mim. E aquela que, além de tia, considero minha amiga, Tia Sônia, obrigado por se orgulhar de mim. Gostaria de agradecer também à Tia Solange e ao Tio Amélio. Obrigado, Kaká, Tio Will e Caio por ressignificarem nossa família no caminho de Deus.

Minha avó, é pela senhora, sempre será. Obrigado por me amar todos os dias. Obrigado pelos conselhos, pelos abraços. Por ser o porto onde vou sempre que preciso recuperar meus pedaços. Te amo pra todo o sempre.

Aos meus bichinhos de estimação, Zequinha ou Zé, obrigado por miau miau. E Vader, obrigado por Au Au.

Eu sempre digo que considero meus amigos minha segunda família, a família que escolheu ficar. Quero agradecer ao John, pelas conversas e por me ajudar a levantar tantas vezes. Ao Olavo, Brendo e Léo, as noites de jogos que, durante a pandemia, foram nosso escape. A Marly, pelos conselhos e puxões de orelha. Ao Gustavo e Bianca, por shows, pizzas e abraços. A Nívia, por ouvir e guiar. A Fernanda, Jhonatas, Bonnie e Emely, pelas companhias nas noites de estudo. Para Jaqueline, Eduardo, Mayumi e Mateus, a confiança e amor que nasceu desde a graduação. Iago Blanco, meu amigo e irmão. A Fabiana, por filosofias e conselhos. Ryanne, Luis Vinicius, Gilmour, Raphael, Marcello, Lany, Becky e Bruno, pelas cervejas e conforto nas noites de Belém.

Aos amigos que me acompanharam no laboratório durante esses anos de

pesquisa, café e estudo. Em especial, Carol e Johnes, obrigado pela lealdade e amizade que segue para além da vida científica. Ao Adan, obrigado pelos códigos e conversas. Dr. Daniel, Anne, Ian, Eli, Naiana, Alyne, Ciro, Adna, Carol, Suellen, Bea e Gio, obrigado por todo o aprendizado compartilhado.

Agora, chegar aqui sem o meu orientador seria impossível. Dr. Rafael Baraúna, que me acompanha desde a minha graduação, és um exemplo de professor, pesquisador e amigo. Obrigado por toda a confiança em todos esses anos! Junto dele, tenho professores incríveis que me aconselham e guiaram minha vida acadêmica, como o Dr. Diego Assis e Dr. Allan Veras. Sem vocês, esse caminho teria sido bem mais difícil. Agradeço também ao Dr. Artur Silva e à Dra. Paula Schneider, pela confiança e apoio nestes anos. Ao Dr. Dioniso, por todo esforço e conhecimento depositado neste projeto.

Por fim, à CAPES, Fapespa e ao Governo do Brasil, por financiar o projeto e a bolsa de doutorado.

Sumário

**RESUMO**

O presente estudo investigou detalhadamente a dinâmica do pan-bacterioma de ostras de mangue (*Crassostrea gasar*) e seus habitats amazônicos, utilizando uma abordagem inovadora de metabarcode com alta resolução filogenética, possibilitada pela metodologia de sequenciamento de leituras longas por nanoporos. As ostras, que são nativas da costa atlântica do Brasil e amplamente cultivadas na região amazônica, especialmente no estado do Pará, desempenham um papel crucial tanto na subsistência de comunidades locais quanto na ecologia de manguezais. Coletas de ostras e amostras de água foram realizadas nas estação seca e na estação chuvosa em quatro municípios do Nordeste Paraense: Santo Antônio de Urindeua, Nova Olinda, Pereru de Fátima e Lauro Sodré. Além disso, o estudo também focou em compreender como fatores abióticos, como salinidade e pH, influenciam a estrutura e composição do microbioma das ostras e das localidades. A diversidade alfa e beta do microbioma foi significativamente maior nas amostras de água em comparação com as amostras de ostras, refletindo a heterogeneidade e complexidade do ambiente aquático. Diferenças sazonais significativas na diversidade beta foram observadas entre as amostras de ostras de todos os locais. Os filos mais abundantes nos moluscos foram *Actinomycetota* e *Pseudomonadota*, principalmente nas regiões Santo Antônio de Urindeua e Nova Olinda. O bacterioma incluí gêneros como *Sphaerochaeta* e *Crinalium*, que estão envolvidos em funções ecológicas cruciais, como a fixação de nitrogênio e a digestão de matéria orgânica complexa, proporcionando assim benefícios nutricionais ao hospedeiro. A abundância de *Salmonella* em períodos de poucas chuvas evidencia a necessidade de monitoramento contínuo em toda a cadeia produtiva. Este estudo pioneiro é um passo fundamental para a compreensão das interações ecológicas entre ostras e seus microbiomas em ecossistemas amazônicos, com implicações diretas para a gestão ambiental e a segurança alimentar.

**ABSTRACT**

The present study thoroughly investigated the dynamics of the pan-bacteriome of mangrove oysters (*Crassostrea gasar*) and their Amazonian habitats, using an innovative high-resolution phylogenetic metabarcoding approach, enabled by long-read nanopore sequencing technology. Oysters, native to Brazil's Atlantic coast and widely cultivated in the Amazon region, particularly in the state of Pará, play a crucial role in both the subsistence of local communities and the ecology of mangroves. Oyster and water samples were collected during the dry and rainy seasons in four municipalities in Northeastern Pará: Santo Antônio de Urindeua, Nova Olinda, Pereru de Fátima, and Lauro Sodré. Additionally, the study focused on understanding how abiotic factors, such as salinity and pH, influence the structure and composition of the oyster microbiome and their aquatic habitats. Alpha and beta diversity of the microbiome were significantly higher in water samples compared to oyster samples, reflecting the heterogeneity and complexity of the aquatic environment. Significant seasonal differences in beta diversity were observed among oyster samples from all locations. The most abundant phyla in the mollusks were *Actinomycetota* and *Pseudomonadota*, mainly in the regions of Santo Antônio de Urindeua and Nova Olinda. The bacteriome included genera such as *Sphaerochaeta* and *Crinalium,* which are involved in crucial ecological functions, such as nitrogen fixation and the digestion of complex organic matter, thus providing nutritional benefits to the host. The abundance of Salmonella during dry periods highlights the need for continuous monitoring throughout the production chain. This pioneering study is a crucial step toward understanding the ecological interactions between oysters and their microbiomes in Amazonian ecosystems, with direct implications for environmental management and food security.

## 1 INTRODUÇÃO

As ostras são moluscos bivalves que se alimentam através da filtração de partículas em suspensão na água, sendo estas: microalgas, bactérias, fitoplânctons, microzooplânctons e matéria orgânica. As ostras estão presentes em diversos estuários da costa brasileira, onde destaca-se a presença no nordeste do estado do Pará, onde o cultivo de ostras da espécie *Crassostrea gasar* é comum. Devido ao processo de alimentação por filtração, a colonização bacteriana do seu intestino está relacionada ao ambiente que habitam.

A maricultura é um dos setores que mais crescem no cenário global da produção industrial de alimentos. Atualmente, a China é líder em produção aquícola, detentora de 83% do total de ostras produzidas no mundo. No Brasil, o cultivo de ostras é uma atividade caracterizada por uma produção baseada na unidade familiar, que oferece alternativas de opção de renda e de dinamização econômica para as comunidades pesqueiras, com fixação nas áreas de origem, graças à geração de empregos (VALENTI *et al*., 2000).

As ostras são mundialmente consumidas, algumas são cultivadas e outras são coletadas nas áreas entre marés para atividade de subsistência ou comercial (AMADI *et al*., 2015). Os ambientes aquáticos como oceanos, rios, lagos, estuários são sistemas ecológicos que possuem uma vasta diversidade de microrganismos. Estas comunidades microbianas estão envolvidas em diversos processos ambientais e sua diversidade é diretamente ligada a influencias em condições ecológicas e pressões seletivas (KING *et al.*, 2020).

Os animais marinhos ligados a este ambiente estão expostos a uma diversidade microbiana, que podem colonizar a superfície externa destes animais e também passam a colonizar o trato digestivo. No caso das ostras, isto ocorre principalmente por serem organismos filtradores apartir da ingestão de alimentos e de água (THOMPSON *et al*., 1993; ASHA *et al.,* 2014). Desta forma, descrever a diversidade da microbiota gastrointestinal de ostras da espécie *Crassostrea gasar* oriundas de estuários paraenses é fundamental. Para isso a utilização de abordagens de metagenômica é de extrema importância para entender propriedades como riqueza, estrutura e a dinâmica das comunidades microbianas associadas ao trato digestivo das ostras, além de compreender como é a diversidade do ambiente nas quais as ostras estão inseridas, afim de desvendar os processos implícitos que

regulam a organização desses sistemas.

## 1.1 Morfologia e caracterização nutricional dos bivalves

Os bivalves pertencem ao filo *Mollusca*, sendo este o segundo maior filo animal em número de espécies, depois dos Antrópodes. O filo *Mollusca* inclui uma enorme diversidade de animais, tendo oito classes, sendo elas: Bivalvia, Caudofoveata, Cefalópodes, Gastrópodes, Monoplacophora, Poliplacóforos, Scaphopoda e Solenogastres. A classe Bivalvia representa cerca de 27% do filo *Mollusca* e é composta por cerca de 7.500 espécies de animais, que têm como caracteristica morfologica a presença de corpo mole protegido por uma concha achatada. Esta classe é de suma importância por ser a mais explorada como produto alimentar (GOSLING, 2003; DAME, 2011).

A presença da concha neste filo retrata um manto responsável pela secreção do esqueleto calcário, além de possuirem placas que têm como função a proteção do corpo mole. O sistema digestivo do filo Mollusca é rádula linguiforme, com dentes, além de possuem um celoma reduzido, ocupando quase a totalidade da cavidade pericárdica. Os indivíduos são majoritariamente marinhos, embora se distribuam por diversos habitats como areia, rocha ou solo (AMARAL *et al*., 2011; CLACK et al., 2013).

Como já citado, os moluscos bivalves são uma espécie majoritariamente marinha, sendo encontrados em água doce, possuindo distribuição mundial e por diversas profundidades (DUMBAULD *et al*., 2009). Contudo, preferem habitar locais onde haja um fluxo de água permanente, podendo ser sésseis, livres ou enterrados na areia. Estes organismos podem ser classificados como filtradores ou detritívoros. Alimentam-se das partículas em suspensão ou de detritos de matéria orgânica em decomposição. Importante ressaltar que os moluscos são extremamente sensíveis às alterações no meio ambiente, devido ao seu sistema vascular aberto, a hemolinfa, onde os hematócitos circulam atravessando a maioria dos tecidos e órgãos. Este sistema favorece a exposição direta dos moluscos bivalves ao ambiente onde se inserem (DUMBAULD *et al*., 2009).

Nos organismos adultos há uma grande variação do exoesqueleto, tendo os menores não mais que 5 mm, e os maiores podendo atingir mais de 20 cm de

comprimento. Os moluscos possuem valvas, que são compostas por um ou dois músculos adutores, dependendo da espécie, responsáveis pelos movimentos de abertura ou fecho da concha (Figura 1). A abertura da concha dá-se automaticamente após o relaxamento dos músculos adutores (DAME, 2011). Nos bivalves não é possível distinguir a cabeça do corpo, o corpo é constituído por um pé e pelas brânquias, estruturas especializadas na respiração e alimentação. As brânquias são constituídas por pequenas lâminas ciliadas cuja função é de conduzir a corrente de água para a cavidade onde se dá a digestão. A simetria dos moluscos bivalves é bilateral, podendo oscilar entre as formas alongadas e ovais. (GOSLING, 2003; DAME, 2011).

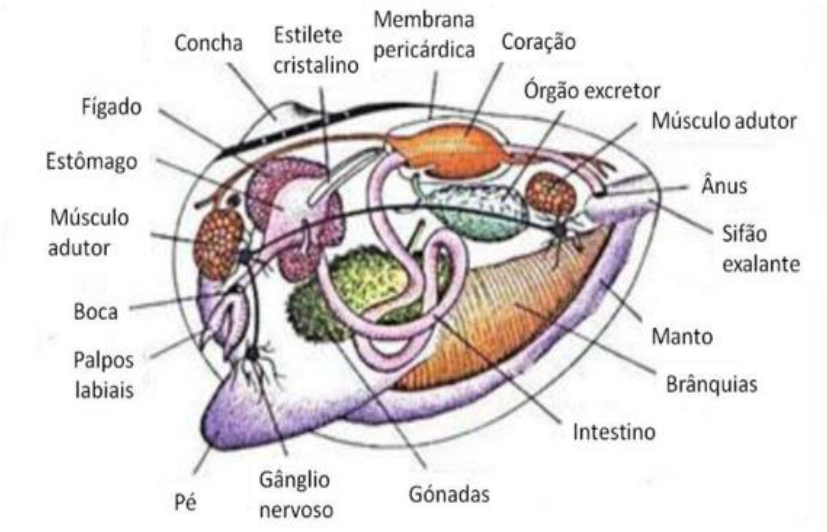


*Figura 1: Morfologia interna dos moluscos bivalves (Cabral, 2015).*

A reprodução de moluscos bivalves é controlada por fatores exógenos, tais como temperatura, salinidade, disponibilidade de alimento, profundidade e por fatores endógenos relacionados à genética dos indivíduos (MACKIE, 1984). O ciclo de vida de moluscos bivalves envolve os estágios de produção e liberação de gametas, fertilização externa na coluna da água, desenvolvimento embrionário e larval, assentamento das larvas, metamorfose em sementes e crescimento até o estágio de produção de gametas. Em moluscos bivalves, o ciclo reprodutivo é anual e envolve um período de gametogênese seguido de uma desova única, estendida, ou mesmo

vários eventos de desova (GOSLING, 2003). Entre os moluscos bivalves, um dos que mais se destaca pela seu consumo como alimento são as ostras.

### 1.2 Ostras (*Crassostrea gasar*)

As ostras são pertencentes à ordem Ostreoida e à família Ostreidae. O gênero *Crassostrea* apresenta cerca de 106 espécies com distribuição global em ambientes tropicais e temperados (DAME, 2011). No Brasil ocorrem pelo menos duas espécies nativas, *Crassostrea gasar* e *Crassostrea rhizophorae* e comumente são denominadas “ostras-do-mangue”. A *Crassostrea gasar* foi observada inicialmente na Costa Oeste da África, onde ocorre em manguezais entre o Senegal e Angola (AFINOWI, 1984). No Brasil, esta espécie é distribuída do Pará até Santa Catarina, principalmente costões rochosos e no fundo de rios associados a estuários ou em mangues (MELO *et al.,* 2010a).

Ostras do gênero *Crassostrea* são ovíparas ou não-incubatórias (MACKIE, 1984). São organismos dioicos sem dimorfismo sexual, sendo necessárias análises microscópicas e histológicas para diferenciar machos e fêmeas (CHRISTO e ABSHER, 2006). São também hermafroditas assíncronos, podendo ocorrer a mudança de sexo após a desova em resposta a condições ambientais específicas (MELO et al., 2010; GOSLING, 2003). A reprodução ocorre como na maioria dos moluscos bivalves, onde os gametas são formados através de células germinativas na parede epitelial dos folículos, sendo produzidos oócitos e espermatozoides por meio de divisões mitóticas e meióticas (GOSLING, 2003). Os oócitos e espermatozoides são liberados pelos poros genitais diretamente na coluna d’água onde ocorre a fertilização e o desenvolvimento larval (QUAYLE e NEWKIRK, 1989).

A reprodução das Ostras também possui relação com condições ambientais. Foi observada a relação entre o desenvolvimento gonadal dos indivíduos e o período de chuva e a salinidade, onde nos meses de seca ocorreu predominância de indivíduos imaturos e com folículos esvaziados e durante a transição entre os períodos seco e chuvoso (PAIXÃO *et al*. 2013). O desenvolvimento larval de moluscos bivalves, em geral, é planctônico. A duração do ciclo larval, crescimento e sobrevivência das larvas também são determinados por fatores ambientais como temperatura, salinidade e disponibilidade de alimento (GOSLING, 2003; DAME, 2011).

A base alimentar das ostras é de microalgas, bactérias, fitoplânctons, microzooplânctons e matéria orgânica dissolvida. A utilização dessas partículas em suspensão como alimento está associada ao mecanismo de seleção e capacidade de retenção nas brânquias. Alguns bivalves podem ingerir partículas te até 950 µm, em contrapartida possuem baixíssima capacidade de retenção de partículas menores que 5 µm (GOSLING, 2003; DAME, 2011).

### 1.3 Importância Ecológica e Econômica das Ostras

A maricultura representa um dos setores que mais crescem no cenário global da produção industrial de alimentos. Atualmente, a China é líder em produção aquícola, detentora de 83% do total de ostras produzidas no mundo. No Brasil, o cultivo de ostras é uma atividade caracterizada por uma produção baseada na unidade familiar, que oferece alternativas de opção de renda e de dinamização econômica para as comunidades pesqueiras, com fixação nas áreas de origem, graças à geração de empregos (BRANDINI *et al*., 2000).

A s ostras são mundialmente consumidas, algumas são cultivadas e outras são coletadas nas áreas entre marés para atividade de subsistência ou comercial (EFIUVWEVWERE E AMADI, 2015). Cerca de 5,1 milhões de toneladas de ostras cultivadas mundialmente são produzidas anualmente e espera-se que esse consumo aumente ainda mais até 2025 (FAO, 2018). Dente os moluscos, o gênero *Crassostrea* é o mais importante na pescaria artesanal *in situ* nos manguezais brasileiros (SANTOS *et al*., 2015). O estado de Santa Catarina é o segundo maior produtor de moluscos bivalves da América Latina. No ano de 2008, a produção total de moluscos (mexilhões, ostras e vieiras) foi de 13.107,92 toneladas.

No Estado do Pará a atividade do Cultivo de Ostras (Ostreicultura) é desenvolvida desde 2006 em cinco municípios: Associação dos Agricultores e Aquicultores de Nova Olinda (AGROMAR) no município de Augusto Corrêa (Figura 2) ; Associação dos Aquicultores, Produtores Rurais e Pescadores de Nazaré do Seco (AAPPNS) no município de Maracanã; Associação de Aquicultores da Vila de Lauro Sodré (AQUAVILA) e Associação Agropesqueira de Nazaré de Mocajuba (AGRONAM) no município de Curuçá; Associação dos Agricultores e Aquicultores de Santo Antônio de Urindeua (ASAPAQ) no município de Salinópolis; Associação de

Mulheres na Pesca e Agricultura de Pererú (AMPAP) e a Associação dos Produtores de Ostras de Pererú de Fátima (ASSOPEF) no município de São Caetano de Odivelas, Pará (SAMPAIO, 2007; SAMPAIO *et al.,* 2019; MACEDO *et al*., 2021).

*Figura 2: Representação de ostras cultivadas na comunidade de Augusto Corrêa. Retirado de https://dol.com.br/noticias/para/675376/augusto-correa-potencial-ainda-maior-para-cultivar-ostras.*

O único cultivo de ostras que está fora de uma Reserva Extrativista Marinha (RESEX) é no município de Salinópolis. As Reservas Extrativistas são espaços territoriais protegidos cujo objetivo é a proteção dos meios de vida e a cultura de populações tradicionais, bem como assegurar o uso sustentável dos recursos naturais da área. O sustento destas populações se baseia no extrativismo e, de modo complementar, na agricultura de subsistência e na criação de animais de pequeno porte.

Atualmente, a ostreicultura paraense vem se tornando uma alternativa de geração de renda para aproximadamente 84 famílias, com o aumento da produção nos últimos anos. Apesar do significativo tempo em que a atividade é exercida nessas comunidades litorâneas, a produção de ostras do Estado do Pará foi contabilizada nas estatísticas oficiais pelo Instituto Brasileiro de Geografia e Estatística (IBGE) apenas

em 2013, quando totalizou 8.250kg nos municípios de Curuçá e São Caetano de Odivelas. Em 2018, a cadeia produtiva da ostreicultura através dos municípios de Augusto Corrêa; Salinópolis; Curuçá e São Caetano de Odivelas produziu 39.850kg (IBGE, 2019). Com essa produção o Estado do Pará ocupou a 6ª. posição no Brasil no cultivo de ostras.

As ostras, por serem animais filtradores, utilizam as guelras para obter alimentos em suspensão. As bactérias correspondem a 4% do total de carbono necessário para o metabolismo dos bivalves e funcionam para o aporte de nitrogênio pelo animal (DAME, 2011). Dessa forma, tendem a acumular um grande número de microorganismos presentes na água, os quais podem compor a microbiota intestinal do animal.

A microbiota das ostras pode ser classificada como transiente, que são micro-organismos que sobrevivem e se proliferam ao passar pelo intestino, ou residente, que ocupam o intestino permanentemente por apresentarem mecanismos de adesão à parede intestinal. A microbiota é extremamente importante para a saúde e crescimento do molusco. E é importante ressaltar que assim como ocorre na reprodução das ostras, a biodiversidade do ambiente de cultivo também influencia diretamente na composição desta microbiota e, consequentemente, na qualidade da ostra e produtividade das associações de produtores do molusco.

### 1.4 Biodiversidade em ambientes aquáticos

Os ambientes aquáticos como oceanos, rios, lagos, estuários são sistemas ecológicos que possuem uma vasta diversidade de microorganismos. Estas comunidades microbianas estão envolvidas em diversos processos ambientais e sua diversidade é diretamente ligada a influencias em condições ecológicas e pressões seletivas (GLOCKNER *et al.*, 2012). Os animais marinhos ligados a este ambiente estão expostos a toda essa diversidade microbiana, podendo colonizar a superfície externa destes animais e também no trato digestivo. No caso das ostras, isto ocorre principalmente por serem organismos filtradores apartir da ingestão de alimentos e água (GLOCKNER *et al.*, 2012).

A composição da microbiota do trato digestivo em animais marinhos é considerada estável ao longo da fase adulta. Contudo, existem alguns fatores que

podem alterar a composição das diversidades microbianas do organismo hospedeiro, dependendo interamente das interações ecológicas deste como o estresse biótico, abiótico e mudanças na dieta do animal (NICHOLSON *et al*, 2012).

Em organismos filtradores como as ostras, os microorganismos hospedeiros podem atuar de três formas em interação ao hospedeiro: eles podem ser residentes, podendo ficar intimamente associados e colonizar a superfície externa, assim como podem através da ingestão passar a colonizar o trato digestivo; ou podem ser transientes, onde ao entrar em contato com o organismo, podem ser inibidos pela microbiota residente ou por compostos inibitórios naturais presentes no organismo (AUSTIN *et al*., 2006).

Por tanto, a eficiência e sustentabilidade nos sistemas de cultivo de animais aquáticos pode ser significativamente influenciado pela composição microbiana da espécie (FERREIRA *et al.,* 2018). Portanto, as características da microbiota estão dependentes das condições do trato gastrointestinal e de parâmetros específicos da espécie como anatomia, secreções digestivas, pH, osmolaridade, potencial redox, tamanho e estrutura, taxa de passagem e tempo de residência (RINGO *et al*., 2016). Portanto, esse consórcio entre os animais marinhos e as comunidades microbianas são sistemas-modelo para compreender a interação complexa entre os microrganismos e as células hospedeiras, e podem fornecer informações relevantes sobre o desenvolvimento de doenças humanas e identificação de novos alvos de drogas (FERREIRA *et al*., 2015; KING *et al*., 2021; MACEDO *et al.,* 2021).

Entre as suas funções, o conjunto da microbiota é envolvida na digestão e absorção dos nutrientes, manutenção e funcionalidade efetiva da mucosa intestinal e da proteção contra a colonização de microrganismos patogênicos, fornecendo nichos para aderência, colonização e proliferação de espécies microbianas mutualísticas e comensais (GIATSIS, 2016).

Os estuários são importantes ecossistemas que fornecem alimento e habitat, além de promover processos biogeoquímicos para outros ambientes por meio de sua conexão entre bacias hidrográficas e águas costeiras (BARLETTA *et al*., 2010). No entanto, há uma crescente interferência antrópica nesses ambientes, muitas vezes causada por atividades como agricultura, desenvolvimento industrial e usos múltiplos da água, levando ao descarte inadequado de resíduos, descargas de esgoto e controle de fluxo (BARLETTA *et al*., 2010). Os ambientes estuarinos são, portanto,

considerados importantes reservatórios de metais pesados e outros contaminantes (IP *et al*., 2004) e têm atraído muita atenção dos pesquisadores.

Os bivalves, como as ostras, são bons organismos sentinela frequentemente utilizados para avaliar a qualidade do ambiente marinho (SOLÉ *et al*., 1994). Além disso, destacam-se pela ampla distribuição no Brasil (espécies nativas) e no mundo, nichos ecológicos vitais, suscetibilidade à absorção de poluentes e estreita ligação com predadores marinhos e saúde humana, ao mesmo tempo, a exposição de organismos aquáticos a contaminantes tóxicos pode ser um risco para a saúde humana se os contaminantes forem incorporados na cadeia alimentar (SULLIVAN *et al*., 2005).

A capacidade de um organismo de sobreviver e se adaptar a mudanças ambientais repentinas depende da variação genética disponível e da plasticidade fenotípica. A plasticidade fenotípica pode amortecer mudanças ambientais repentinas e fornecer tempo para que a adaptação ocorra (KENKEL *et al*., 2016). A plasticidade fenotípica é importante para organismos sésseis que habitam zonas estuarinas que não podem usar a evasão para lidar com grandes flutuações nas condições ambientais (KENKEL *et al.,* 2016), o mesmo ocorre com ostras que habitam ambientes de estuario, que possuem uma diversidade genética significativamente menor, provavelmente devido ao impacto da glaciação passada em seu estilo de vida estuarino único.

As ostras estão entre o grupo socioeconômico mais valioso de espécies de bivalves nos desembarques pesqueiros globais e também fornecem inúmeros serviços ecossistêmicos. Portanto, é de suma importância entender os microorganismos presentes nas espécies de ostras nativas, permitindo entender de que forma os microbiomas de invertebrados podem afetados por essa remodelação metabólica e quais consequências um microbioma alterado pode ter para sua saúde e sobrevivência.

### 1.5 Importância do biomonitoramento de ostras.

Além das ostras bioconcentrarem microrganismos, elas também podem concentrar contaminantes existentes na água em que são cultivadas. Os contaminantes podem envolver substâncias tóxicas, toxinas produzidas por

microalgas e microrganismos patogênicos. No caso específico dos contaminantes microbianos, o risco é aumentado pelo hábito de se consumir os bivalves crus (BUTT *et al*., 2006). A contaminação marinha por patógenos de origem humana é comumente associada à inexistência ou a ineficiência de sistemas de coleta e tratamento de esgotos em áreas urbanas localizadas em bacias hidrográficas que drenam para áreas costeiras (GARBOSSA *et al*., 2017).

Um estudo que se estendeu por dois anos realizado no Reino Unido mostrou que 76,2% das amostras de ostras coletadas em 39 áreas de produção estavam contaminadas com Norovírus (CHENG *et al*., 2011). Em alguns países, como a Itália, a hepatite A é um problema importante. O consumo de moluscos tem sido associado a 62% dos casos dessa doença (CROMEANS *et al*., 1997).

Os filos Tenericutes, Chlamydiae, Proteobacteria e Firmicutes são os mais abundantes da microbiota intestinal de ostras da região de Puget Sound, no estado de Washington, EUA (LI *et al*., 2017). A presença de bactérias do filo Tenericutes pode ser explicada pela alimentação das ostras, que também é composta de algas, visto que foi relatado que a associação de bactérias deste filo à microbiota intestinal de moluscos (DAVIS *et al.*, 2013). Ademais, existem dados sobre bactérias pertencentes a esse filo como simbiontes intracelulares em animais, plantas e humanos, realizando diversas atividades simbiônticas nestes hospedeiros, além de serem consideradas bactérias residentes do microbioma intestinal de outra classe de molusco (*Gastropoda*) (SHTYKOVA *et al.*, 2018). A presença de bactérias do filo *Chlamydiae* já havia sido reportado anteriormente em moluscos bivalves na Cheapskate Bay, nos EUA, em 1977. Porém, a presença destas bactérias nestes animais, que são consumidos crus, é de extrema relevância a saúde pública por ocasionarem doenças em animais e humanos.

No Brasil, estudos identificaram a presença de diversos patógenos em moluscos e nas águas utilizadas para a malacocultura nas baías da Ilha de Santa Catarina (COELHO *et al*. 2003). A presença de bactérias do gênero *Vibrio* em ostras é de interesse sanitário devido algumas espécies serem causadoras de gastroenterites e septicemia fatal em humanos. Ademais, sabe-se que diversas espécies de bactérias deste gênero estão presentes em ambientes estuarinos e são naturalmente concentrados em bivalves durante do processo de filtração (COELHO et al., 2018).

Adicionalmente, estudos em ostras, mostraram que entre as bactérias isoladas

não apresentavam perfil de resistência a múltiplas drogas antimicrobianas e eram suscetíveis aos antibióticos de último recurso, como Cefotaxima e Imipenem (OLIVEIRA *et al.*, 2020).

Outra importante caracteristica de organismos filtradores também se dá no biomonitoramento de presença elementos tóxicos, como metais, uma vez que nos ambientes estuarinos as condições físicas e químicas (tais como pH, temperatura, salinidade, potencial redox) determinam a mobilidade, especiação, disponibilidade e toxicidade dos metais (SANY *et al*., 2013; MACHADO *et al*., 2016). Os elementos metálicos, após passarem por inúmeros gradientes físicos e químicos conjuntamente com a circulação estuarina, podem distribuir-se sob inúmeras formas nos compartimentos bióticos e abióticos (MACHADO *et al*., 2016).

Assim, para que possa se obter uma aquicultura sustentável é preciso manter uma interação harmônica com os ecossistemas e as comunidades locais. Sabe-se que o microbioma da ostra nativa altera de acordo com a estação (CONCEIÇÃO *et al*., 2021). Com isso para que sejam seguidos os princípios da sustentabilidade, na preservação ambiental e no desenvolvimento social, associado com o biomonitoramento e analise dos microorganismos presentes no animal é definidor para o desenvolvimento da aquicultura sustentável.

A microbiota do animal, bem como do seu ambiente natural de cultivo, pode ser avaliada por técnicas modernas de biologia molecular, independentes de cultivo. Este método consiste na extração do material genético da microbiota ambiental ou animal seguido de um sequenciamento massivo destas amostras.

## 1.6 A ascenção da era da metagenômica

Historicamente, a metagenômica como campo de estudo é precedida pela genômica bacteriana, que por sua vez é precedido pela microbiologia clássica. Ao contrário dos dois campos da ômica, a microbiologia foi a principal responsável por estudos microbianos por um grande período de tempo, começando com bacteriologistas do século 19 que seguiram de microscopistas como Antonie van Leeuwenhoek (WINSLOW, 1950). No final do século XX, microbiologistas, como Robert Koch, foram motivados a empregar um protocolo de cultura de células bacterianas para traçar uma conexão causal clara entre bactérias e doenças

(JACKSON *et al*., 1996). No entanto, diversos desafios começaram a surgir em decorrência a prevalência de técnicas relacionadas a cultura bacteriana, como a observação de uma grande discrepância entre o número de células em uma amostra e o número de colônias que elas produzem (STALEY & KONOPKA, 1985). Como resultado dessas descobertas altamente impactantes, o interesse em comunidades microbianas não cultivadas começaram a aumentar em meados da década de 1980, impulsionados também pelo advento da Reação em Cadeia da Polimerase (PCR) (BALLARD *et al*., 2000), um processo que permitiu a geração de inúmeras cópias de uma sequência de DNA produzida a partir de um único ou baixo número de sequências de origem.

Desta forma, as últimas décadas do século passado trouxeram consigo o surgimento da genômica, um campo que fornece uma perspectiva de todo o material genético de um determinado organismo (MEDINI *et al*., 2008). O sequenciamento do primeiro genoma microbiano, *Haemophilus influenzae* (FLEISCHMANN *et al*., 1995), foi seguido por constantes diminuições no custo total de sequenciamento. Isso resultou em um aumento exponencial no número de genomas sequenciados (HANDELMAN, 2004). Embora estes fossem principalmente genomas microbianos, o Genoma Humano (VENTER *et al*., 2001) foi uma inclusão altamente notável. A genômica aprimorou muito o campo da microbiologia, particularmente no que diz respeito ao esclarecimento da relação entre características fenotípicas e suas sequências de DNA e evolução dos microrganismos (ACHTMAN & WAGNER, 2008).

O dinamismo das comunidades microbianas e de suas estruturas genômicas trouxe consigo evoluções a metodologia das ômicas, com o surgimento de abordagens dinâmicas da composição genômica microbiana, como pan-genomas (MEDINI *et al*., 2008). Embora as contribuições da genômica tenham sido notáveis, trouxe consigo um problema que também era pertecente a microbiologia tradicional: a maioria sequências são determinadas a partir de culturas puras para evitar ambiguidades durante a montagem da sequência (SCHLOSS & HANDELSMAN, 2005).

No entanto, estima-se que mais de 99% dos microrganismos não podem ser cultivados (HANDELSMAN *et al.,* 2004; SCHLOSS & HANDELSMAN, 2005). A metagenômica surgiu como um método para contornar essa limitação, sequenciando amostras de DNA amplificadas diretamente do ambiente e, portanto, contendo uma

variedade de fontes genômicas, em vez de um organismo de uma única fonte (HANDELSMAN, 2004). O benefício da metagenômica é a obtenção de informação genéticas de microorganismos sem a necessidade de cultiva-los. Por exemplo, organismos simbiontes e patógenos obrigatórios que não podem sobrevivem fora de seus hospedeiros e os micróbios ambientais são muitas vezes incapazes de crescer em estado puro cultura. No entanto, o DNA pode ser extraído diretamente de tais organismos enquanto eles estão em seus habitats naturais, produzindo assim uma mistura heterogênea de DNA que podem ser separados em bibliotecas de sequências de dados (TRINGE & RUBIN, 2005).

A ideia de clonar o DNA diretamente do meio ambiente foi proposta em 1986 (PACE, 1986) após isolar com sucesso sequências de rRNA 16S diretamente de populações microbianas naturais para fins de caracterização filogenética. Este conceito foi implementado vários anos depois, em 1994 (SCHMIDT *et al*., 1991) utilizando clonagem em um vetor de fago, progredindo assim para a construção de uma biblioteca metagenômica completa. Estas iniciativas foram posteriormente seguida por mais elaborados esforços de construção de bibliotecas metagenômicas (STEIN *et al*., 1996). Dessa forma, a metagenômica como campo distinto de pesquisa começou a tomar forma no final do século 20. O termo metagenoma foi cunhado pela primeira vez por Handelsman *et al*. (1998) com respeito ao conceito de meta-análise sendo aplicada a conjuntos de dados semelhantes, mas não idênticos. O interesse na metagenômica floresceu no novo milênio, desencadeando vários projetos marcantes. O estudo do Mar dos Sargaços (VENTER *et al*., 2004) representou um esforço para entender melhor populações microbianas.

Análises metagenômicas são mais frequentemente realizadas por sequenciamento do RNA ribossomal (rRNA) 16S ou sequenciamento total (shotgun). O estudo conhecido como shotgun ou metagenômica de DNA total, busca estudar todo o material genético encontrado na amostra. Esse material é sequenciado em pequenos fragmentos que, após disso são juntados em fragmentos maiores utilizando sobreposição de sequências em uma técnica denominada assembly (WANG, 2014). Após isso, esses fragmentos maiores, são classificados taxonomicamente (binning) baseados em bancos de dados de referências ou através da metodologia de novo (CARR, 2013). Este estudo permite uma abordagem mais ampla, onde se pode ser realizado estudos relacionados a genes de interesse e enzimas, proteínas e demais

produtos de origem transcricional.

As abordagens independentes de cultivo tem contribuído para o estudo de comunidades microbianas possibilitando o acesso a ampla diversidade de microrganismos que não são cultiváveis com as técnicas e meios comumente empregados. O emprego do sequenciamento de gene do rRNA16S em trabalhos de diversidade de comunidades microbianas utilizando-se como fonte de estudo o DNA extraído de amostras ambientais foi proposto na década de 1980 (PACE *et al*., 1985). Após isso, o surgimento das tecnologias de sequenciamento de alto desempenho a um relativo menor custo financeiro, menor tempo e menor exigência de infraestrutura laboratorial permitiu o rápido aumento do número de trabalhos descrevendo comunidades ambientais e associadas a organismos hospedeiros (VENTER *et al*., 2004).

A utilização do gene rRNA 16S apresenta características importantes como marcador filogenético, entre elas a baixa taxa de transferência horizontal, baixa taxa de recombinação gênica (SCHLOSS *et al*., 2011; YARZA *et al*., 2014). Esta abordagem permite o acesso direto a esta microbiota sem a interferência do genoma do hospedeiro, e com um número menor de sequências a serem analisadas computacionalmente, além de permitir com que sejam realizadas comparações entre diferentes comunidades (RAJAN *et al*, 2015; SHARPTON, 2014), sendo assim referido como amplicon (Figura 3).

Através desta abordagem são identificadas as OTUs (Unidades Taxonômicas Operacionais) presentes na comunidade microbiana. As OTUs representam um grupo de sequências muito próximas (>97% de identidade) pela aplicação de técnicas de agrupamento hierárquico utilizando limites de identidade de sequência independentemente de inferências filogenéticas, na caracterização de uma comunidade microbiana, além da descrição da sua composição taxonômica, normalmente são realizadas análises de riqueza e de diversidade, as quais permitem omparar ambientes distintos e oferecem uma medida da complexidade da comunidade (YARZA *et al*., 2014).

Nas últimas décadas as pesquisas que utilizam abordagens de metagenômica foram de extrema importância e mostraram que entender propriedades como riqueza, estrutura e a dinâmica de comunidades microbianas assim como o ambiente em que estas estão inseridas é essencial para desvendar os processos implícitos que regulam

a organização desses sistemas. Portanto, a metagenômica é capaz de revelar um universo de organismos não cultiváveis.

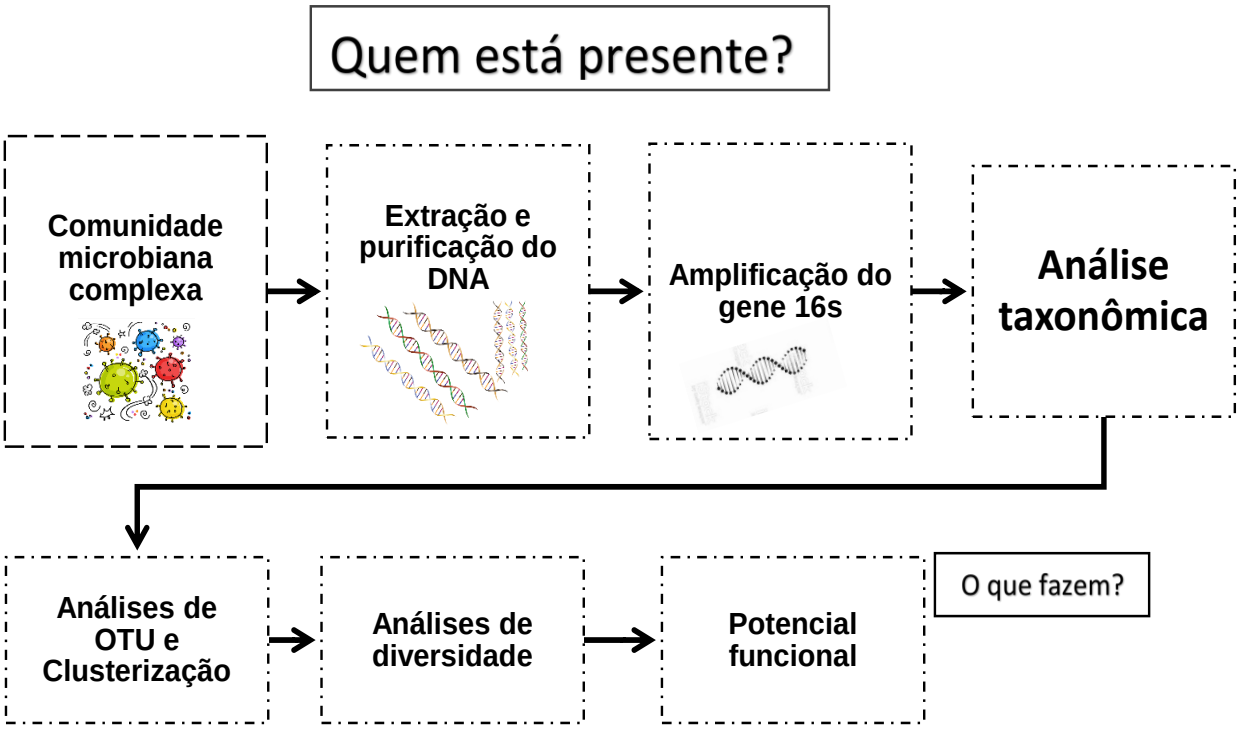


*Figura 3: Estrutura de analise de metagenomica 16s.*

.

## 2 JUSTIFICATIVA

A ostra-do-mangue (*Crassostrea gasar*) é uma espécie de bivalve filtrador nativa da costa atlântica do Brasil e tem um papel fundamental nos ecossistemas de manguezais, particularmente na região amazônica. Esses organismos alimentam-se filtrando partículas orgânicas suspensas, como microalgas, fitoplâncton e bactérias, que são fontes essenciais de nutrientes para seu crescimento e sobrevivência. A colonização bacteriana no trato digestivo das ostras é um processo ecológico complexo, influenciado diretamente pelo microbioma presente no ambiente aquático. Fatores como salinidade, pH e disponibilidade de nutrientes, que variam ao longo das estações, afetam diretamente essa dinâmica microbiana. Dessa forma, compreender a interação entre o microbioma das ostras e o ambiente é de extrema importância tanto para a saúde das ostras quanto para a segurança alimentar das comunidades que dependem dessa prática. As ostras são de grande relevância econômica e ecológica, especialmente no contexto amazônico, onde a aquicultura de ostras-do-mangue é uma atividade tradicional que sustenta cerca de 100 famílias no nordeste do Pará. Essa prática é majoritariamente familiar, desempenhando um papel vital na subsistência das comunidades pesqueiras locais. No entanto, o sucesso e a sustentabilidade dessa atividade dependem diretamente da manutenção da qualidade ambiental dos estuários e manguezais. O uso de tecnologias avançadas, como o sequenciamento de DNA de alta resolução pela plataforma Nanopore, permite uma investigação mais detalhada e precisa da diversidade microbiana associada ao trato digestivo das ostras e ao ambiente em que estão inseridas. Anteriormente, as técnicas de sequenciamento eram limitadas a pequenos fragmentos de genes, o que dificultava a obtenção de uma visão completa do microbioma. Com a nova tecnologia, é possível sequenciar o gene 16S rRNA completo, proporcionando uma maior resolução filogenética e permitindo uma análise aprofundada da composição microbiana até o nível de gênero. Este é um dos primeiros estudos a aplicar essa tecnologia no microbioma de ostras, o que representa uma inovação significativa na pesquisa sobre ecossistemas costeiros.

# 3 OBJETIVOS

## 3.1 Objetivo Geral

Avaliar a dinâmica do pan-bacterioma das ostras-do-mangue (*Crassostrea gasar*) e dos seus habitats amazônicos, utilizando uma abordagem de metabarcoding com alta resolução filogenética. O estudo busca entender como os fatores abióticos, como salinidade e pH, influenciam a composição microbiana do trato digestivo das ostras e do ambiente em que elas estão inseridas.

## 3.2 Objetivos específicos

- Determinar como os fatores abióticos, como pH, salinidade e sólidos dissolvidos totais, afetam a composição microbiana nas ostras e na água em diferentes períodos sazonais;
- Investigar as mudanças sazonais na diversidade microbiana (alfa e beta) das ostras e dos habitats aquáticos nos estuários do nordeste do estado do Pará;
- Identificar o pan-bacterioma associado às ostras e determinar as diferenças entre o bacterioma das ostras cultivadas e das naturais;
- Comparar a diversidade e a abundância de filos e gêneros bacterianos nas ostras e na água em regiões com diferentes influências de maré e condições ambientais;
- Verificar se a presença de táxons específicos tem um impacto positivo ou negativo na fisiologia das ostras.

# 4 CAPITULO 1

**The pan-bacteriome dynamics of mangrove oysters (*Crassostrea gasar*) and their Amazonian habitats revealed by a high phylogenetic resolution metabarcoding approach**

Sávio de Souza Costa[1*], Diego Assis das Graças[1], Artur Silva[1], Adonney Allan de Oliveira Veras[1], Maria Paula Cruz Schneider[2], Dioniso de Souza Sampaio[3], Rafael Azevedo Baraúna[1]

[1] Laboratório de Engenharia Biológica, Espaço Inovação, Parque de Ciência e Tecnologia Guamá, 66075-750, Belém, PA, Brazil.
[2] Centro de Genômica e Biologia de Sistemas, Instituto de Ciências Biológicas, Universidade Federal do Pará, Guamá, 66075-110, Belém, PA, Brazil.
[2] Instituto de Estudos Costeiros (IECOS), Universidade Federal do Pará, Campus de Bragança, 68600-000, PA, Brazil.

* Corresponding author: Sávio S. Costa. Laboratório de Engenharia Biológica, Espaço Inovação, Parque de Ciência e Tecnologia Guamá, Av. Perimetral, km 01, 66075-750, Belém, PA, Brazil. E-mail: savscosta@gmail.com

**Abstract**

The mangrove oyster (*Crassostrea gasar*) feeds by filtering suspended organic particles, microalgae, phytoplankton and bacteria, which provide essential nutrients for the mollusk's growth and survival. The process of bacterial colonization in the oyster gut is complex and directly influenced by the microbiome of the aquatic habitat. The *C. gasar* species is indigenous to the Atlantic coast of Brazil and is prevalent in the mangrove ecosystems of the Amazon region, where it is farmed. Oysters are a globally consumed seafood, and = farming this species in the Amazon contributes to the economy and livelihoods of traditional communities. In this study, we employed a high phylogenetic resolution metabarcoding approach to describe the richness, structure and dynamics of the bacterial communities associated with the oyster gut and the aquatic habitat in which they reside. Four farming sites were analyzed during the Amazon summer and winter: Santo Antônio de Urindeua (SAU); Nova Olinda (NO); Pereru de Fátima (PF); and Lauro Sodré (LS). Total DNA was extracted from the water and oyster gut samples, and the full-length 16S rRNA gene was amplified and sequenced on the PromethION P2 Solo platform (Oxford Nanopore Technologies). Water samples were analyzed for the following parameters: salinity, pH, total dissolved solids, resistivity and conductivity. The bacteriome of oysters and rivers exhibited a pronounced response to abiotic factors, such as salinity and pH. The alpha diversity was higher in water samples than in the oyster gut. Significant seasonal differences in beta diversity were observed between the oyster samples from all locations. The most abundant phyla in the mollusks were Actinomycetota and Pseudomonadota, particularly in the SAU and NO regions. The bacteriome included genera such as *Sphaerochaeta* and *Crinalium*, which are involved in crucial ecological functions such as nitrogen fixation and the digestion of complex organic matter, thereby providing nutritional benefits to the host. The abundance of *Salmonella* in periods of low rainfall highlights the necessity of continuous monitoring throughout the production chain. Our data contributed to the description of the bacteriome associated with *C. gasar*, demonstrating that the microbiota of these mollusks is unique and exhibits a composition that is significantly influenced by the environmental conditions of the Amazon.

**Keywords:** 16S rRNA, mangrove, oyster**,** microbiome, metabarcoding.

## Introduction

Mangrove oysters (*Crassostrea gasar*, Phylum: Mollusca, Class: Bivalvia) are animals that feed by filtering suspended organic particles, microalgae, phytoplankton, and bacteria. These organic materials provide essential nutrients such as proteins, lipids, and carbohydrates, that are necessary for oyster growth and survival [1]. Thus, the bacterial colonization of the oyster gut is a complex ecological process that is directly influenced by the microbiome of the aquatic habitat [2]. Coastal environments harbor a vast diversity of microorganisms essential for several biogeochemical processes [3,4] and for maintaining biodiversity [5]. These environments are also the habitats for several species of mollusks.

*C. gasar* is native to the Atlantic coast of Brazil and is particularly abundant in the mangrove ecosystems of the Amazon region [6]. Along the Amazon coast, these mollusks benefit from the unique ecological conditions of mangroves, which provide a rich habitat for their growth and reproduction [7,8]. The bacterial diversity of the oyster's gut changes according to the season in Amazonian rivers with less tidal influence [9]. This compositional change may affect the life cycle, immunity, and other physiological characteristics of oysters [5, 10].

In 1988, Whipps and colleagues proposed the term microbiome to describe the set of microorganisms living in a well-defined habitat with unique physicochemical properties. Later, metabarcoding approaches were used to describe the taxa that comprise a particular group of microorganisms. For example, the 16S rRNA gene is commonly used to describe the bacterial diversity of a sample, referred to as a bacteriome. For years, taxonomic assignment was done using short sequences that encompassed only a portion of the gene, such as the V2-V3 or V3-V4 regions. This approach was characterized by a strong methodological bias [11]. However, with the advent and chemical improvement of nanopore sequencers, a high phylogenetic resolution metabarcoding approach is now possible, as the entire gene can be massively sequenced and analyzed [12,13].

The gut bacteriome composition of other species such as *Crassostrea virginica* [14], *Crassostrea gigas*, *Crassostrea corteziensis*, and *Crassostrea sikamea* was previously described [15]. In *C. virginica*, the predominant phyla were Proteobacteria, Tenericutes, and Verrucomicrobia [16], being the phyla Chloroflexi, Firmicutes, Proteobacteria, and Verrucomicrobia especially found in the core bacteriome [14]. In *C. corteziensis*, Proteobacteria, Bacteroidetes, and Actinobacteria were the most abundant phyla [15], while in *C. gigas*, Tenericutes, Spirochaetes, and Proteobacteria were dominant [17]. Therefore, the bacterial composition of oyster's gut varies according to the species and location.

Oysters are widely consumed as food in many regions of the world. The global oyster market has grown significantly over the past two decades, driven by the rising demand for seafood, particularly in Asia, which accounted for 45.1% of the global revenue in 2022 [18].

The market is expected to continue expanding, with a global value estimated at USD 111.64 billion in 2023, and a projected growth to USD 143.28 billion by 2030 [18]. In Brazil, about 9.978.480 kg were produced in 2022, being the North region responsible for 62.192 kg of this production [19].

Oyster farming in the Amazon region is a family practice, that contributes to the income of about 100 families living in traditional communities [7]. Previous studies showed that farming sites have optimal sanitary conditions [9, 20]. These results were important because the entire production chain depends on the ecosystem services provided by the forest. Producers manually collect oyster seeds from natural banks and sell them to other producers who fatten the mollusks in saline rivers before selling them to the public. Therefore, understanding the ecological dynamics of the interaction between the oyster bacteriome and its natural environment is of utmost importance. In this work, we performed a high phylogenetic resolution metabarcoding approach using the Nanopore sequencing platform to answer several questions: How do abiotic factors influence the composition of the oyster gut bacteriome? Does this bacteriome change with the Amazonian seasons? If so, does this change reflect a change in the bacteriome of the rivers? What is the core bacteriome of the mangrove oyster? Is there a difference in bacterial diversity between rivers with different tidal influences? Our data reveal complex ecological processes that shape the oyster gut bacteriome.

## Material and Methods

### *Ethical issues*

Sampling of oysters in Marine Extractive Reserves was approved by the Instituto Chico Mendes de Conservação da Biodiversidade (SisBio/ICMBio) under the authorization Nº 80264-1.

### *Sampling and physicochemical analysis*

A total of two expeditions for oyster and water sampling were performed. One of them during the Amazonian dry season in October/November 2022, and the other one during the rainy season on March/April 2023. In each expedition, samples from four municipalities were collected: Pereru de Fátima river in Pereru de Fátima – PF – P1 (0º43’2.823’’W 48º1’2.5608’’S); Mocajuba river in Lauro Sodré – LS – P2 (0º51’16.9344’’W 47º54’38.6706’’S; Urindeua river in Santo Antônio de Urindeua – SAU – P3 (0º39’20.5956’’W 47º24’10.926’’S) and Emboraí Velho river in Nova Olinda – NO – P4 (1º2’28.8414’’W 46º26’35.9376’’S) (Figure 1).

The river water was collected from the photic zone (about 0.5 m deep) above the oyster’s farming structures. Three liters of water were collected in triplicate using van Dorn

bottles and maintained at 4ºC in sterile bottles. In the same day, 2 L of the collected water were vacuum filtered through 0.22 µm nitrocellulose membranes using a sterile Millipore system. Membranes were maintained in 50 mL polypropylene tubes with RNAlater (Invitrogen) at -20ºC until total DNA extraction. One liter of water was used for physicochemical analysis. Resistivity, salinity, conductivity, and total dissolved solids were analyzed using a portable multiparametric probe AT 215 (Alfakit). pH was measured using a portable pHmeter AT 315 (Alfakit). All analyzes were performed in triplicate. The sediment was collected with a van Veen dragger next to the oyster's farming structures.

Farmed and natural oysters were hand-collected during high-tide. The mollusks were kept in a container with ice until being processed on the same day. They were opened with sterile knives and the gut content was dissected using sterile scalpel and forceps inside a biological safety cabinet. The gut content of three oysters (about 10 g of biological material) was mixed and maintained in 50 mL polypropylene tubes with RNAlater (Invitrogen) 1:1 (m/v) at -20ºC. Three replicates of 10 g were processed for each sampling point.

*Full-length 16S rRNA gene sequencing*

To understand the complex ecological dynamics of the oyster and water bacteriome, we used a high phylogenetic resolution metabarcoding approach based on the sequencing of the whole 16S rRNA gene in the PromethION P2 Solo platform (Oxford Nanopore Technologies). Total DNA was extracted from the water, sediment and oyster's gut samples using the FastDNA Spin kit for Soil (MP Biomedicals) according to the manufacture's protocol. The DNA integrity was evaluated using 1% agarose gel electrophoresis and nucleic acid quantification was performed on NanoDrop spectrophotometer (Thermo Fisher). The 16S rRNA gene was amplified using the primer pairs Bact16S-8F (5'-AGAGTTTGATCATGGCTCAG-3') and Uni16S-1492R (5'-CGGTTACCTGTTACGACTT-3') in the thermocycler GeneAmp 9700 (Thermo Fisher). The cycling condition was an initial denaturation at 95ºC for 2 minutes, followed by 35 cycles of 95ºC for 30 seconds, 55ºC for 30 seconds, 72ºC for 90 seconds, and a final extension step at 72ºC for 5 minutes. Sequencing was performed on PromethION P2 Solo (Oxford Nanopore Technologies) using a FLO-PRO114M flow cell in a 24 h run.

*Bioinformatics*

Base calling was performed using Guppy v.6.3.7 integrated with EPI2ME v.5.2.13 software. The FAST5 files were converted to FASTQ where data with quality >7 were filtered out for analysis. The processed files were loaded into the EPI2ME-16S workflow using Kraken2 [21] with a minimum accuracy level set at 77%. Data analysis was performed in the cloud and included demultiplexing, quality control, and taxonomic assignment using the

BLAST algorithm against the GenBank Reference Sequence (RefSeq) database [22]. The abundance data were then used to calculate alpha and beta diversities and taxa composition using the Vegan [23] and Phyloseq [24] packages in RStudio. Correlation between the bacteriome composition and the physicochemical parameters was performed using Redundancy Analysis (RDA) using microbiome [25] package in RStudio.

## Results

*Salinity, pH, and TDS vary according to season and geographic location*

The physicochemical parameters pH, total dissolved solids (TDS), conductivity, and salinity were evaluated, and the results are shown in Table 1. Salinity and pH are the main parameters for evaluating oyster farming because they directly influence oyster growth (Sampaio et al., 2020). SAU and NO are near the sea and therefore have a greater influence from the tide, unlike PF and LS. During both seasons, SAU and NO had higher salinity than PF and LS, remarkably during the rainy season (Table 1). The pH of the four locations was very similar, with a slight difference in PF and LS during the dry season, with a value closer to neutrality. Another parameter with a marked difference between the regions was TDS. During the rainy season, TDS increased at the sampling points nearest to the sea and decreased at the points with less tidal influence (Table 1).

The pH and salinity data were organized into discrete values to determine which intervals had the greatest influence on the alpha diversity of the samples. The data was grouped as follows: samples with pH between 6 and 7 were grouped in value 1; 7 to 8 in value 2; 8 to 9 in value 3 and > 9 in value 4. The analysis indicated that bacterial diversity is greater in samples with pH values of 6 to 7 or > 9 (Figure S1). Salinity was also grouped into discrete values: samples with salinity < 2 were grouped in value 1; 20 to 30 in value 2; 30 to 40 in value 3; and > 30 in value 4. In this case, the higher the salinity, the lower the bacterial diversity (Figure S1).

*Oyster gut bacteriome changes with Amazonian seasons*

The Chao1 index was used to estimate alpha diversity. We observed a higher bacterial diversity in oysters collected during the rainy season than in the dry season (Figure 2A). The same result was observed for other alpha diversity indices such as Shannon and Simpson (Table S1). In addition, the bacterial diversity of the river water was significantly higher than that of oyster samples, irrespective of season and geographic location, except for samples collected in PF during the dry season (Figure 2A). The beta-diversity analysis revealed a significant difference in the bacterial composition of the water and oyster samples (Figure 2B). In addition, a comparison of oyster samples from different locations revealed that

the bacterial composition varied according to the season (Figures 2D and 2C).

The three most abundant phyla found were Pseudomonadota, Actinomycetota, and Bacteroidota (Figure 3). During the dry season, the bacterial composition was more uniform. In addition, the bacteriome of oysters was directly affected by the bacterial composition of the water. For example, oysters from NO and SAU, which are sampling sites near the ocean, have a higher abundance of Actinomycetota than oysters from PF and LS, where the most abundant phylum was Pseudomonadota (Figure 3). The same pattern of bacterial composition was observed in the water samples. Significant differences (Table S2) among samples included the increased abundance of Bacteroidota, Actinomycetota and Spirochaetota in oysters collected at NO and SAU during the rainy season. The prevalence of Bacteroidota only in water samples, suggesting that oysters do not retain these taxa in their gut; and the higher abundance of Cyanobacteriota during the dry season. This last result highlights the need for increased environmental monitoring during the dry season to avoid cyanotoxin poisoning due to the consumption of oysters *in natura*.

Analysis of the bacterial composition at the genus level revealed an abundance of *Corynebacterium*, *Salmonella*, *Enterobacter*, Candidatus *Pelagibacter*, *Mycoplasmopsis*, and *Crinalium* (Figure 4). The presence of these taxa in water and oyster samples is a warning of the anthropization of these environments. Since the production chain depends on the natural oyster seed banks and the maintenance of the river quality for their farming processes, it is imperative to establish an environmental monitoring routine in these farming sites.

We used redundancy analysis (RDA) to determine the relationship between environmental parameters and bacterial community composition. We observed that an increase in pH and salinity favored the phylum Cyanobacteriota and had a negative effect on the abundance of the phyla Pseudomonadota, Mycoplasmatota and Spirochaetota (Figure 5). At the genus level, the filamentous cyanobacterium *Crinalium* was favored by the increase in salinity and pH (Figure 5). Other finding included a positive relationship between the increase in resistivity and the abundance of *Enterobacter* (Figure 5).

*Mangrove oysters have a large core bacteriome and many unique taxa associated with specimens from areas with natural seed banks*

The Venn diagram in Figure 6A shows the comparison of all predicted taxa in the 30 oyster samples using a detection threshold of 0.010 (Figure 6B). The pan-bacteriome consists of 5,409 taxa and 2,330 core taxa (Figure 6A). Interestingly, the site with the highest number of unique taxa was LS which is the sampling region with the largest natural oyster seed banks. Pseudomonadota and Actinomycetota were the most abundant phyla of the pan-bacteriome followed by Mycoplasmatota and Bacillota. Figure 6C shows the percentage of bacterial genera considering the 2,330 taxa detected in the core bacteriome. *Corynebacterium* and

*Enterobacter* corresponded to 48% and 10% of the core bacteriome, respectively, and a relevant proportion (27%) was composed of less abundant taxa. Among the 730 unique taxa found in LS oysters, 34% were affiliated to *Streptomyces*, 30.8% to *Pseudomonas*, in addition to a relevant abundance of *Paenibacillus* and *Sphaerochaeta* (Figure 7).

*The tides influence the bacterial composition of the oyster, which is highly diverse regardless of geographic location*

As presented before, several differences in bacteriome composition were observed in oysters collected at PF and LS compared to NO and SAU. PF and LS are regions of brackish water with less tidal influence, while NO and SAU are regions of saline water with high tidal influence. Figure 2A shows that, regardless of location, the rainy season increases the diversity of bacteria associated with the oyster gut. Despite the similar alpha diversity, the histograms of Figures 3 and 4 show that oysters from PF and LS have a different bacterial abundance and composition from that observed in SAU and NO, both during the dry and rainy seasons. For example, the abundance of *Mycoplasmopsis* (mainly *M. phocirhinis*) was higher in PF and LS oysters than in SAU and NO. *Enterobacter* species such as *E. asburiae* were prevalent only in oyster samples collected from PF and LS in both seasons. They were abundant in both natural and farmed oysters, suggesting an important role in the mollusk physiology. Interestingly, these taxa were not detected or were in very low abundance in water samples (Figure 4). *Corynebacterium* species such as *C. canis* were abundant in oysters collected at NO and SAU, but unlike *Enterobacter*, *Corynebacterium* was also detected in water samples (Figure 4).

*The farming process does not affect the bacterial composition of the oysters but makes them more susceptible to environmental changes*

Samples of farmed oysters, natural oysters (those attached to the submerged rocks or tree roots), water, and sediment were grouped and analyzed using beta-diversity parameters. This analysis was conducted only with samples collected at LS in both seasons. The data highlighted significant differences between the bacterial composition of natural oysters and environmental samples (water and sediment) (Figure 8A). The distance to the environmental samples suggests that their natural growth condition selects groups of bacteria that are rare in the river water. On the other hand, farmed oysters were more related to the taxa found in environmental samples, suggesting that this growth condition might be more sensitive to environmental impacts, such as those caused by human activities or climate changes (Figure 8A). Finally, no significant difference was observed when comparing only the oyster samples suggesting that the farming process does not affect the bacterial composition of the oysters but makes them more susceptible to environmental changes (Figure 8B and

8C).

**Discussion**

Microbiomes are influenced by biotic and abiotic factors and are involved in regulating the physiology and health of animals, plants and humans [26,27]. Abiotic factors such as pH, temperature, salinity, and nutrient availability, determine the composition and function of microbial communities, including dynamic estuarine environments. Previous studies have shown that these parameters influence the microbiome of both oysters and their aquatic habitat [27,28,29]. During the rainy season, intense rainfall leads to a significant decrease in salinity in regions such as PF and LS, due to the high freshwater inflow (Table 1). This phenomenon is common in tropical estuarine regions, where precipitation is a dominant factor in salinity regulation [30,31]. In addition, the lower pH observed during this period is also related to the freshwater inflow, which acidifies the water due to the decomposition of organic matter that is carried by rain into the rivers [32].

The NO and SAU regions exhibited elevated salinity levels during the rainy season (Table 1) due to the geomorphological characteristics and water flow dynamics of these areas. For example, these regions can act as aquifer recharge zones, where rainfall is absorbed by the permeable soils of the Pirabas and Barreiras geological formations, thereby limiting surface runoff and salinity dilution [32,33]. These data illustrate the intricate nature of hydrological processes in estuaries, where local factors can cause significant variations in physicochemical parameters, even during periods of high rainfall.

The alpha diversity of water samples was significantly higher than that of the oyster bacteriome (Figure 1A). Environmental samples are more heterogeneous and dynamic, providing a variety of microhabitats that support a rich microbial communities compared to host-associated microhabitats [34,35]. Furthermore, although the richness of bacterial taxa in the oyster gut is higher during the rainy season, there is no significant difference in alpha diversity between the brackish water (PF and LS) and saline water (SAU and NO) regions (Figure 1A). However, the histograms demonstrated a clear difference in the bacterial composition of the two groups, which can be attributed to the different abiotic conditions, primarily pH and salinity [36,37]. The predominance of Actinomycetota, Pseudomonadota, and Cyanobacteriota in SAU and NO indicates a microbial profile typical of environments with higher salinity and basic pH. Acidification has been identified as an important factor contributing to shifts in the microbiome composition in oysters and other mollusks [38,39]. The analysis of beta diversity among the farmed oyster samples (Figure 2C and 2D) revealed significant differences between the dry and rainy periods for all locations. This result corroborates previous research conducted exclusively in LS [9].

The use of nanopore sequencing enabled the taxonomic affiliation to be determined

down to the genus level, thereby making this one of the first studies to employ this technology to describe the bacteriome of mollusks. The significant difference between samples indicates the presence of a sophisticated biological mechanism governing bacteriome selection during the filtration process (Figures 3 and 4) [40,41]. Regarding bacteriome composition at the phylum level, Actinomycetota was predominant across all oyster samples, with particular abundance in the SAU and NO regions (Figure 3). It has been demonstrated that members of the Actinomycetota have important symbiotic functions, contributing to the defense mechanisms and degradation of organic compounds [42]. Members of this phylum are also known to produce bioactive compounds, including antibiotics [43,44]. Previous studies have confirmed the presence of Actinomycetota in mollusks cultivated in a range of coastal regions, where these bacteria are crucial for maintaining oyster health. These bacteria can protect the host against pathogens through the production of antimicrobial substances [45]. The taxon has already been described in other oyster species including *C. gasar, C. sikamea, C. gigas e C. corteziensis* [17,9,15]. Furthermore, *C. gasar* gut-associated taxa previously described in other studies, such as Pseudomonadota and Bacteroidota, were also identified in our analysis [9,46,47]. The Pseudomonadota have already been described in the oyster microbiome, from the larval phase up to the adult phase of the mollusk, reinforcing its importance and adaptability [17,9,48].

We expected less drastic changes in some abiotic parameters during the dry period due to the low rainfall. Consequently, we also expected low species richness in the bacteriomes during this period. Conversely, the higher species richness and abundance of Cyanobacteriota and Mycoplasmatota during the rainy season reflect the environmental changes promoted by the intense rainfall, which modified nutrient availability and water turbidity (Figure 3) [49]. It is worth noting that the higher abundance of Cyanobacteriota has a negative impact on oyster growth and survival rate [50]. However, most taxa belonging to the phylum Cyanobacteriota were affiliated to the genera *Crinalium* (Figure 4). *Crinalium* is a poorly studied cyanobacterium that is often associated with both aquatic and terrestrial environments. It plays an important role in nitrogen fixation and provides essential nutrients to the oyster microbiota [51]. The abundance of this genus suggests a positive ecological interaction between oysters and their natural habitats. However, regular monitoring is essential to identify or predict blooms of other groups of cyanobacteria that are harmful to animals and humans. RDA has identified a significant correlation between alkaline and saline waters and the occurrence of cyanobacteria (Figure 5). This finding is consistent with other studies [48,52].

A stable and diverse gut microbiota is important for oyster health [50]. Based on the abundance and stability between seasons, our data suggest that Actinomycetota and Spirochaetota are part of the resident microbiota, while Bacteroidota are likely part of the transient microbiota [53]. In addition, the higher abundance of Spirochaetota and

Mycoplasmatota in the oyster gut compared to the water samples suggests that these taxa have effective mechanisms of adaptation to the host tissue. The genus *Sphaerochaeta* was the major taxon found in the phylum Spirochaetota. The genus consists of anaerobic bacteria commonly found in freshwater sediments, animal guts, and in methanogenic consortia [54,55]. This taxon probably play an important role in the digestion of complex organic matter through fermentative processes, providing nutritional benefits to the host. This genus has not yet been reported in the gut of other oyster species.

Other genera found in the oyster gut include *Salmonella*, *Corynebacterium*, *Enterobacter, Mycoplasmopsis*, and Candidatus *Pelagibacter* (Figure 4). It is well established that *Salmonella* is frequently associated with the contamination of water bodies by fecal matter. Periods of low rainfall led to the accumulation of organic matter and other nutrients from human sources that favor the proliferation of these pathogens [56]. Interestingly, all Amazonian communities involved in the oyster production chain are small, with less than 10,000 inhabitants. *Pelagibacter* is a genus adapted to oligotrophic zones and plays a crucial role in the carbon cycle in environments with low nutrient availability [57]. Its abundance during dry periods suggests an adaptation of the oyster bacteriome to conditions of nutrient stress due to the low rainfall. In addition, during the dry season, there is less freshwater inflow to the oceans, which contributes to an increase in estuarine salinity, allowing seasonal colonization of the oyster gut by oligotrophic species adapted to the pelagic zone.

*Corynebacterium* is commonly found in animal and aquatic microbiomes and is associated with protection against pathogens and maintenance of immune homeostasis [58]. *Enterobacter* is frequently detected in nutrient-rich environments, including mangroves [59,60]. It is noteworthy that the abundance of *Enterobacter* was higher in PF and LS, which are regions characterized by smaller riverine systems and greater contact with the mangrove.

Detection of *Mycoplasmopsis* raises concerns, as this bacterium is associated with diseases in aquatic organisms [61,62]. Some members of the Mycoplasmataceae family were identified as the etiological agents of the Pacific Oyster Mortality Syndrome (POMS) [63]. However, this genus has also been observed to perform beneficial functions in oysters and mussels through symbiotic interactions [40,64]. They confer benefits to the host by reducing parasitic infections through competitive sequestration of arginine [64]. Nevertheless, given the scarcity of studies involving this genus in mollusks, further research is needed to better understand they role in the microbiome of mangrove oysters.

In our study, we sought to identify the bacterial taxa that constitute the pan-bacteriome of mangrove oysters, which can be defined as the collection of bacteria found in a specific environment or host (Figure 6A, 6B and 6C). To this end, we conducted a comparative analysis of all the samples, regardless of location and season. Pseudomonadota and Actinomycetota were identified in all samples, even at low levels of abundance (≥1%). These phyla are widely

recognized for playing essential roles in biogeochemical cycles, including the degradation of organic compounds and other metabolic processes, such as ammonia oxidation [65,66]. The presence of Actinomycetota may be associated with the degradation of complex organic matter, as many members of this phylum possess the ability to degrade refractory compounds. This is particularly pertinent in detritus-rich environments such as mangroves [53,60].

There are few studies describing the pan-bacteriome of oysters. In *C. gigas*, the families *Rhodobacteraceae*, *Nitrosomonadaceae*, *Flavobacteriaceae*, *Pirellulaceae*, and *Saprospiraceae* were identified [47]. In *C. virginica*, members of the Firmicutes and Spirochaetota phyla were identified, as well as the *Mycoplasmataceae* and *Spirochaetaceae* families [67]. Despite the difference in the oyster species and the environmental conditions analyzed, a substantial number of taxa were identified as constituents of the bacteriome of oysters and other marine animals. Moreover, the detection of Bacillota and Mycoplasmatota in the bacteriome of mangrove oysters suggests that these groups play a crucial role in maintaining the health of the host, even under environmental fluctuations in pH and salinity [68].

Finally, our study also aimed to assess whether the farming method employed affects the bacteriome of oysters. To this end, we compared farmed oysters with native oysters, which were sampled directly from rocks and plant roots. This analysis was conducted only in LS region. Although the natural oyster exhibited a slightly higher species richness during the rainy period, this difference did not reach statistical significance. This indicates that the type of cultivation does not exert a significant influence on the diversity of the bacteriome. Both types of oysters share similar distribution of bacterial phyla, including Pseudomonadota, Spirochaetota, Actinomycetota, and Mycoplasmota. However, farmed oysters exhibit a bacterial composition more closely related with that of the water and sediment samples. Therefore, they require more attention as they can easily affected by environmental changes.

## Conclusion

The present study employed an innovative metabarcoding approach with high phylogenetic resolution to investigate the dynamics of the pan-bacteriome of mangrove oysters and their aquatic habitats. The study demonstrated that the bacteriome of oysters and their surrounding aquatic environment is influenced by abiotic factors, primarily salinity and pH. Microbial diversity was higher in water samples, and no significant seasonal variations were observed in the beta diversity of oyster gut samples. The microbial composition differs between various sample types, including water, sediment and oysters. Actinomycetota was the predominant phylum in oyster samples. This phylum usually plays an important role in host defense and the degradation of complex organic compounds. Furthermore, other phyla, including Pseudomonadota, Bacteroidota, and Cyanobacteriota, demonstrated seasonal

fluctuations, reflecting environmental shifts induced by rainfall. The abundance of Salmonella during periods of low rainfall highlights the necessity for constant monitoring throughout the production chain. In addition, the pan-bacteriome of mangrove oysters farmed in the Amazon encompasses 5,409 taxa and 2,330 core taxa, with the phyla Pseudomonadota and Actinomycetota being the most abundant in the pan-bacteriome. The farming method did not exert an influence on the microbial diversity of the oysters' gut. However, the bacterial composition of the mollusks was found to be more like that of the water and sediment samples, indicating that the farming process may render mollusks more susceptible to environmental changes compared to their native counterparts.

**Acknowledgments**

Authors would like to thank for the valuable support of all associations of mollusk farmers involved in this research and Pró-Reitoria de Pesquisa e Pós-Graduação (PROPESP) from Universidade Federal do Pará (UFPA) for the payment of the Article Processing Charge (APC) through the PAPQ 2024 Program.

**Funding Declaration**

Authors would like to thank Fundação Amazônia de Amparo a Estudos e Pesquisas (FAPESPA) for the financial support under the research grant Nº 013/2022.

**Conflict of Interest Statement**

The authors have no conflicts of interest to declare.

**References**

1. **Gosling, E.** (2003). *Bivalve Molluscs: Biology, Ecology and Culture*. Oxford: Foshing News Books. https://doi.org/10.1002/9780470995532.

2. **Asha, K. K., Anandan, R., Mathew, S., Lakshmanan, P. T.** (2014). Biochemical profile of oyster *Crassostrea madrasensis* and its nutritional attributes. *The Egyptian Journal of Aquatic Research*, **40**(1), 35–41. https://doi.org/10.1016/j.ejar.2014.02.00.

3. **King, N. G., Moore, P. J., Thorpe, J. M., et al.** (2023). Consistency and variation in the kelp microbiota: Patterns of bacterial community structure across spatial

scales. *Microbial Ecology*, **85**, 1265–1275. https://doi.org/10.1007/s00248-022-02038-0.

4. **Nguyen, V. K., King, W. L., Siboni, N., Mahbub, K. R., Dove, M., O'Connor, W., Seymour, J. R., & Labbate, M.** (2020). The Sydney rock oyster microbiota is influenced by location, season and genetics. *Aquaculture*, **527**, 735472. https://doi.org/10.1016/j.aquaculture.2020.735472.

5. **Khan, B., Clinton, S. M., Hamp, T. J., Oliver, J. D., & Ringwood, A. H.** (2018). Potential impacts of hypoxia and a warming ocean on oyster microbiomes. *Marine Environmental Research*, **139**, 27–34. https://doi.org/10.1016/j.marenvres.2018.04.018.

6. **Ferreira, J. P. R., Legat, A. P., Lazoski, C., Freire, T. B., Gomes, C. H. A. M., & Melo, C. R. M.** (2023). A historical and integrative taxonomic account of mangrove oyster species native to the Atlantic American coast: A re-evaluation of Brazilian *Crassostrea* species. *Zoologischer Anzeiger – A Journal of Comparative Zoology*, **305**, 52–81. https://doi.org/10.1016/j.jcz.2023.06.004.

7. **Sampaio, D. S., Tagliaro, C. H., Schneider, H., & Beasley, C. R.** (2017). Oyster culture on the Amazon mangrove coast: Asymmetries and advances in an emerging sector. *Reviews in Aquaculture*, **11**, 88–104. https://doi.org/10.1111/raq.12227.

8. **Mendes, D. C. S., Rodrigues, D. T. A., Gomes, H. M., Lenz, T. M., Silva, C. M., & Antonio, I. G.** (2023). Pathogens and microorganisms in the mangrove oyster *Crassostrea gasar* cultivated in an estuarine environment in Northeast Brazil. *Brazilian Journal of Biology*, **83**, e272789. https://doi.org/10.1590/1519-6984.272789.

9. **Conceição, M. V. R., Costa, S. S., Schaan, A. P., Ribeiro-dos-Santos, A. K. C., Silva, A., Graças, D. A., Schneider, M. P. C., & Baraúna, R. A.** (2021). Amazonia seasons have an influence in the composition of bacterial gut microbiota of mangrove oysters (*Crassostrea gasar*). *Frontiers in Genetics*, **11**, 602608. https://doi.org/10.3389/fgene.2020.602608.

10. **Froelich, B., & Oliver, J.** (2013). Increases in the amounts of *Vibrio* spp. in oysters upon addition of exogenous bacteria. *Applied and Environmental Microbiology*, **79**(17), 5208–5213. https://doi.org/10.1128/AEM.01110-13.

11. **Bukin, Y., Galachyants, Y., Morozov, I., et al.** (2019). The effect of 16S rRNA region choice on bacterial community metabarcoding results. *Scientific Data*, **6**, 190007. https://doi.org/10.1038/sdata.2019.7.

12. **Matsuo, Y., Komiya, S., Yasumizu, Y., et al.** (2021). Full-length 16S rRNA gene amplicon analysis of human gut microbiota using MinION™ nanopore sequencing confers species-level resolution. *BMC Microbiology*, **21**, 35. https://doi.org/10.1186/s12866-021-02094-5.

13. **Rubiola, S., Macori, G., Civera, T., Fanning, S., Mitchell, M., & Chiesa, F.** (2022). Comparison between full-length 16S rRNA metabarcoding and whole metagenome sequencing suggests the use of either is suitable for large-scale microbiome studies. *Foodborne Pathogens and Disease*, **19**(7), 495-504. https://doi.org/10.1089/fpd.2022.0027.

14. **King, G. M., Judd, C., Kuske, C. R., & Smith, C.** (2012). Analysis of stomach and gut microbiomes of the Eastern oyster (*Crassostrea virginica*) from coastal Louisiana, USA. *PLoS ONE*, **7**(12), e51475. https://doi.org/10.1371/journal.pone.0051475.

15. **Trabal Fernández, N., Mazón-Suástegui, J. M., Vázquez-Juárez, R., Ascencio-Valle, F., & Romero, J.** (2014). Changes in the composition and diversity of the bacterial microbiota associated with oysters (*Crassostrea corteziensis*, *Crassostrea gigas* and *Crassostrea sikamea*) during commercial production. *FEMS Microbiology Ecology*, **88**(1), 69-83. https://doi.org/10.1111/1574-6941.12270.

16. **Pierce, M. L., & Ward, J. E.** (2019). Gut microbiomes of the Eastern oyster (*Crassostrea virginica*) and the blue mussel (*Mytilus edulis*): Temporal variation and the influence of marine aggregate-associated microbial communities. *mSphere*, **4**(6), e00730-19. https://doi.org/10.1128/mSphere.00730-19.

17. **Horodesky, A., Castilho-Westphal, G. G., Pont, G. D., Faoro, H., Balsanelli, E., Tadra-Sfeir, M. Z., Cozer, N., Pie, M. R., & Ostrensky, A.** (2020). Metagenomic analysis of the bacterial microbiota associated with cultured oysters

(*Crassostrea* sp.) in estuarine environments. *Anais da Academia Brasileira de Ciências*, **92**(supl.1), 1-15. https://doi.org/10.1590/0001-3765202020180432.

18. **Iitembu, J. A., Fitzgerald, D., Altintzoglou, T., Boudry, P., Britz, P., Byron, C. J., Delago, D., Girard, S., Hannon, C., Kafensztok, M., et al.** (2023). Comparative description and analysis of oyster aquaculture in selected Atlantic regions: Production, market dynamics, and consumption patterns. *Fishes*, **8**, 584. https://doi.org/10.3390/fishes8120584.

19. **IBGE (Instituto Brasileiro de Geografia e Estatística).** (2022)

20. **Oliveira, A. M. S., Baraúna, R. A., Marcon, D. J., Lago, L. A. B., Silva, A., Lusio, J., Tavares, R. D. S., Tacão, M., Henriques, I., & Schneider, M. P. C.** (2020). Occurrence, antibiotic-resistance and virulence of *E. coli* strains isolated from mangrove oysters (*Crassostrea gasar*) farmed in estuaries of Amazonia. *Marine Pollution Bulletin*, **157**, 111302. https://doi.org/10.1016/j.marpolbul.2020.111302.

21. **Wood, D. E., Lu, J., & Langmead, B.** (2019). Improved metagenomic analysis with Kraken 2. *Genome Biology*, **20**, 257. https://doi.org/10.1186/s13059-019-1891-0.

22. **Pruitt, K. D., Tatusova, T., & Maglott, D. R.** (2007). NCBI reference sequences (RefSeq): A curated non-redundant sequence database of genomes, transcripts

and proteins. *Nucleic Acids Research*, **35**(Database issue), D61–D65. https://doi.org/10.1093/nar/gkl842.

23. **Oksanen, J., Simpson, G. L., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Solymos, P., Stevens, M. H. H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., Evangelista, H. B. A., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M. O., Lahti, L., McGlinn, D., Ouellette, M. H., Ribeiro Cunha, E., Smith, T., Stier, A., Ter Braak, C. J. F., & Weedon, J.** (2024). *vegan: Community Ecology Package* (R package version 2.7-0). https://github.com/vegandevs/vegan.

24. **McMurdie, P. J., & Holmes, S.** (2013). phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS ONE*, **8**(4), e61217. https://doi.org/10.1371/journal.pone.0061217.

25. **Lahti, L., & Shetty, S.** (2012–2019). *microbiome R package* (Bioconductor version: Release 3.19). https://doi.org/10.18129/B9.bioc.microbiome.

26. **Le Chatelier, E., Nielsen, T., Qin, J., et al.** (2013). Richness of human gut microbiome correlates with metabolic markers. *Nature*, **500**, 541–546. https://doi.org/10.1038/nature12506.

27. **Liu, M., Li, Q., Tan, L., Wang, L., Wu, F., Li, L., & Zhang, G.** (2023). Host-microbiota interactions play a crucial role in oyster adaptation to rising

seawater temperature in summer. *Environmental Research*, **216**(Part 2), 114585. https://doi.org/10.1016/j.envres.2022.114585.

28. **Zhao, J., Zhao, B., Kong, N., Li, F., Liu, J., Wang, L., & Song, L.** (2023). Increased abundances of potential pathogenic bacteria and expressions of inflammatory cytokines in the intestine of oyster *Crassostrea gigas* after high temperature stress. *Developmental & Comparative Immunology*, **141**, 104630. https://doi.org/10.1016/j.dci.2023.104630.

29. **Stevick, R. J., Post, A. F., & Gómez-Chiarri, M.** (2021). Functional plasticity in oyster gut microbiomes along a eutrophication gradient in an urbanized estuary. *Animal Microbiome*, **3**, 5. https://doi.org/10.1186/s42523-020-00066-0.

30. **Madeira, D., Narciso, L., Cabral, H. N., & Vinagre, C.** (2012). Thermal influence and potential impacts of climate change on coastal and estuarine organisms. *Journal of Sea Research*, **70**, 32–41.

31. **Whitfield, A. K.** (2021). Estuaries – how challenging are these constantly changing aquatic environments for associated fish species? *Environmental Biology of Fishes*, **104**(4), 517–528. https://doi.org/10.1007/s10641-021-01085-9.

32. **Sampaio, D. S., Tagliaro, C. H., Schneider, H., & Beasley, C. R.** (2017). Oyster culture on the Amazon mangrove coast: Asymmetries and advances in an

emerging sector. *Reviews in Aquaculture*, **11**(1), 88–104. https://doi.org/10.1111/raq.12227.

33. **Consentine, P. J., Pedreno, G. Y. de O., Moreira, J. P. P. C., Bruce Neta, I. B., Lino, P. R. de M., Azevedo, R. L., de Melo, M. da G. G., & Alves, N. de S.** (2023). Estimativa de recarga do aquífero Alter do Chão na região do município de Óbidos–PA utilizando o método WTF. *Revista Foco*, **16**(10), e3098. https://doi.org/10.54751/revistafoco.v16n10-154.

34. **Robidart, J. C., Magasin, J. D., Shilova, I. N., Turk-Kubo, K. A., Wilson, S. T., Karl, D. M., Scholin, C. A., & Zehr, J. P.** (2019). Effects of nutrient enrichment on surface microbial community gene expression in the oligotrophic North Pacific Subtropical Gyre. *ISME Journal*, **13**(2), 374–387. https://doi.org/10.1038/s41396-018-0280-0.

35. **Al-Tarshi, M., Dobretsov, S., & Al-Belushi, M.** (2024). Bacterial communities across multiple ecological niches (water, sediment, plastic, and snail gut) in mangrove habitats. *Microorganisms*, **12**, 1561. https://doi.org/10.3390/microorganisms12081561.

36. **Nocker, A., Lepo, J. E., & Snyder, R. A.** (2004). Influence of an oyster reef on development of the microbial heterotrophic community of an estuarine biofilm. *Applied and Environmental Microbiology*, **70**(11), 6834–6845. https://doi.org/10.1128/AEM.70.11.6834-6845.2004.

37. **Sampaio, D. S., Santos, M. L. S., Tagliaro, C. H., & Beasley, C. R.** (2020). Variation in environmental characteristics of waters among Amazon coast oyster culture units. *Acta Amazonica*, **50**, 295-304.

38. **Unzueta-Martínez, A., Downey-Wall, A. M., Cameron, L. P., Ries, J. B., Lotterhos, K. E., & Bowen, J. L.** (2021). Ocean acidification alters the diversity and structure of oyster associated microbial communities. *Limnology and Oceanography Letters*, **6**, 2021. https://doi.org/10.1002/lol2.10214.

39. **Scanes, E., Parker, L. M., Seymour, J. R., Siboni, N., King, W. L., Danckert, N. P., Wegner, K. M., Dove, M. C., O'Connor, W. A., & Ross, P. M.** (2021). Climate change alters the haemolymph microbiome of oysters. *Marine Pollution Bulletin*, **164**, 111991. https://doi.org/10.1016/j.marpolbul.2021.111991.

40. **Offret, C., Paulino, S., Gauthier, O., et al.** (2020). The marine intertidal zone shapes oyster and clam digestive bacterial microbiota. *FEMS Microbiology Ecology*, **96**, fiaa078. https://doi.org/10.1093/femsec/fiaa078.

41. **Kachor, A., Tistechok, S., Rebets, Y., et al.** (2024). Bacterial community and culturable actinomycetes of *Phyllostachys viridiglaucescens* rhizosphere. *Antonie van Leeuwenhoek*, **117**, 9. https://doi.org/10.1007/s10482-023-01906-0.

42. **Rath, S., Rud, T., Karch, A., et al.** (2018). Pathogenic functions of host microbiota. *Microbiome*, **6**, 174. https://doi.org/10.1186/s40168-018-0542-0.

43. **Ward, A. C., & Bora, N.** (2006). Diversity and biogeography of marine actinobacteria. *Current Opinion in Microbiology*, **9**(3), 279–286. https://doi.org/10.1016/j.mib.2006.04.004.

44. **Jagannathan, S. V., et al.** (2021). Marine actinomycetes, new sources of biotechnological products. *Marine Drugs*, **19**(7), 365. https://doi.org/10.3390/md19070365.

45. **Tian, L., Lin, X., Tian, J., Ji, L., Chen, Y., & Tran, L. P., & Tian, C.** (2020). Research advances of beneficial microbiota associated with crop plants. *International Journal of Molecular Sciences*, **21**(5), 1792. https://doi.org/10.3390/ijms21051792.

46. **Peng, Z., Wang, P., Luo, X., et al.** (2024). Community structure and carbon metabolism functions of bacterioplankton in the Guangdong coastal zone. *Marine Life Science & Technology*, **6**, 547–561. https://doi.org/10.1007/s42995-024-00245-x.

47. **Cho, A., Finke, J. F., Zhong, K. X., Chan, A. M., Saunders, R., Schulze, A., Warne, S., Miller, K. M., & Suttle, C. A.** (2024). The core microbiome of cultured Pacific oyster spat is affected by age but not mortality. *Host-Microbial Interactions*. https://doi.org/10.1128/spectrum.00031-24.

48. **Cyphert, E. L., Nand, S., Franco, G., Hajkowski, M., Soto, L., Lee, D. M., Ferner, M., Zabin, C., Blumenthal, J., Deck, A., Boyer, K., Burrus, K., Hernandez, C. J., &**

**Anand, A.** (2024). Combinatorial characterization of bacterial taxa-driven differences in the microbiome of oyster reefs. *bioRxiv* [Preprint]. https://doi.org/10.1101/2024.05.15.594453.

49. **Ishaq, S. E., Ahmad, T., Liang, L., et al.** (2024). Cultivation of diverse novel marine bacteria from deep ocean sediment using spent culture supernatant of *Ca. Bathyarchaeia* enrichment. *Journal of Microbiology*, **62**, 611-625. https://doi.org/10.1007/s12275-024-00145-w.

50. **Liu, M., Li, Q., Xu, W., Wang, L., Wu, F., Tan, L., Li, L., & Zhang, G.** (2024). Characterization of water microbiota and their relationship with resident oysters during an oyster mortality event. *Microbiology Spectrum*, **e02881-23**. https://doi.org/10.1128/spectrum.02881-23.

51. **Mikhailyuk, T., Vinogradova, O., Holzinger, A., Glaser, K., Samolov, E., & Karsten, U.** (2019). New record of the rare genus *Crinalium* Crow (Oscillatoriales, Cyanobacteria) from sand dunes of the Baltic Sea, Germany: Epitypification and emendation of *Crinalium magnum* Fritsch et John based on an integrative approach. *Phytotaxa*, **400**(3), 165-179. https://doi.org/10.11646/phytotaxa.400.3.4.

52. **Mehdizadeh Allaf, M., & Peerhossaini, H.** (2022). Cyanobacteria: Model microorganisms and beyond. *Microorganisms*, **10**(4), 696. https://doi.org/10.3390/microorganisms10040696.

53. **Zurel, D., Benayahu, Y., Or, A., Kovacs, A., & Gophna, U.** (2011). Composition and dynamics of the gill microbiota of an invasive Indo-Pacific oyster in the eastern Mediterranean Sea. *Environmental Microbiology*, **13**(6), 1462-2920. https://doi.org/10.1111/j.1462-2920.2011.02448.x.

54. **Bidzhieva, S. Kh., Sokolova, D. Sh., Grouzdev, D. S., Kostrikina, N. A., Poltaraus, A. B., Tourova, T. P., Shcherbakova, V. A., Troshina, O. Yu., & Nazina, T. N.** (2020). *Sphaerochaeta halotolerans* sp. nov., a novel spherical halotolerant spirochete from a Russian heavy oil reservoir, emended description of the genus *Sphaerochaeta*, reclassification of *Sphaerochaeta coccoides* to a new genus *Parasphaerochaeta* gen. nov. as *Parasphaerochaeta coccoides* comb. nov. and proposal of *Sphaerochaetaceae* fam. nov. *International Journal of Systematic and Evolutionary Microbiology*, **70**(8), 004340. https://doi.org/10.1099/ijsem.0.004340.

55. **Zakem, E. J., Polz, M. F., & Follows, M. J.** (2020). Redox-informed models of global biogeochemical cycles. *Nature Communications*, **11**, 5680. https://doi.org/10.1038/s41467-020-19454-w.

56. **Billah, M. M., & Rahman, M. S.** (2024). *Salmonella* in the environment: A review on ecology, antimicrobial resistance, seafood contaminations, and human health implications. *Journal of Hazardous Materials Advances*, **2024**, 100407. https://doi.org/10.1016/j.hazadv.2024.100407.

57. **Fuhrman, J.** (2009). Microbial community structure and its functional implications. *Nature*, **459**, 193-199. https://doi.org/10.1038/nature08058.

58. **Wilkins, L. G. E., Leray, M., O'Dea, A., Yuen, B., Peixoto, R. S., Pereira, T. J., et al.** (2019). Host-associated microbiomes drive structure and function of marine ecosystems. *PLoS Biology*, **17**(11), e3000533. https://doi.org/10.1371/journal.pbio.3000533.

59. **Ngugi, C. C., et al.** (2017). Fingerprints of upstream catchment land use in suspended particulate organic matter (SPOM) at the river discharge sites in Lake Victoria (Kenya): Insights from element, stable isotope and lipid biomarker analysis. *Aquatic Sciences*, **79**, 73–87.

60. **Fernandes, S. O., Kirchman, D. L., Michotey, V. D., Bonin, P. C., & LokaBharathi, P. A.** (2014). Bacterial diversity in relatively pristine and anthropogenically-influenced mangrove ecosystems (Goa, India). *Brazilian Journal of Microbiology*, **45**(4), 1161–1171. https://doi.org/10.1590/S1517-83822014000400006.

61. **Rostama, B., & May, M.** (2021). *Mycoplasma* and related organisms. In R. N. H. Smith & G. G. Parker (Eds.), *Diagnostic Microbiology* (pp. 497–512). CRC Press. https://doi.org/10.1201/9781003099277-36.

62. **Galosi, L., Ridolfi, N., Fellini, C., Pelizzone, I., Cusaro, S., Marchetti, G., Canonico, M., Ghelfi, E., Di Girolamo, N., & Preziuso, S.** (2023). Detection and identification of *Mycoplasmopsis agassizii* in captive tortoises with different clinical signs in Italy. *Animals*, **13**, 588. https://doi.org/10.3390/ani13040588.

63. **Clerissi, C., de Lorgeril, J., Petton, B., Lucasson, A., Escoubas, J.-M., Gueguen, Y., Dégremont, L., Mitta, G., & Toulza, E.** (2020). Microbiota composition and evenness predict survival rate of oysters confronted to Pacific oyster mortality syndrome. *Frontiers in Microbiology*, **11**, 311. https://doi.org/10.3389/fmicb.2020.00311.

64. **Pimentel, Z. T., Dufault-Thompson, K., Russo, K. T., et al.** (2021). Microbiome analysis reveals diversity and function of mollicutes associated with the Eastern oyster, *Crassostrea virginica*. *mSphere*, **6**, e00227-21. https://doi.org/10.1128/mSphere.00227-21.

65. **Hernandez-Agreda, A., Leggat, W., Bongaerts, P., & Ainsworth, T. D.** (2016). The microbial signature provides insight into the mechanistic basis of coral success across reef habitats. *mBio*, **7**, 1-10. https://doi.org/10.1128/mBio.00001-16

66. **Li, Z., Nicolae, V., Akileh, R., & Liu, T.** (2017). A brief review of oyster-associated microbiota. *Microbiology Research Journal International*, **20**, 1-14.

67. **Hines, I. S., Madanick, J. M., Smith, S. A., Kuhn, D. D., & Stevens, A. M.** (2023). Analysis of the core bacterial community associated with consumer-ready Eastern oysters (*Crassostrea virginica*). *PLoS ONE*, **18**(2), e0281747. https://doi.org/10.1371/journal.pone.0281747.

68. **Trabal Fernández, N., Mazón-Suástegui, J. M., Vázquez-Juárez, R., Ascencio-Valle, F., & Romero, J.** (2014). Changes in the composition and diversity of the bacterial microbiota associated with oysters (*Crassostrea corteziensis*, *Crassostrea gigas* and *Crassostrea sikamea*) during commercial production. *FEMS Microbiology Ecology*, **88**(1), 69–83. https://doi.org/10.1111/1574-6941.12270.

## Tables

**Table 1.** Results of the physicochemical analyses conducted with multiparametric probes. The results are divided by sampling point and season. The following abbreviations are used throughout the table: SAU: Santo Antônio de Urindeua; NO: Nova Olinda; PF: Pereru de Fátia; LS: Lauro Sodré. SAU and NO are closer to the ocean and therefore have a greater influence from the tides.

| Parameter | Dry season | | | | Rainy season | | | |
|---|---|---|---|---|---|---|---|---|
| | SAU | NO | PF | LS | SAU | NO | PF | LS |
| pH | 9.41 ± 0.02 | 9.11 ± 0.04 | 8.61 ± 0.07 | 8.38 ± 0.12 | 7.87 ± 0.25 | 8.36 ± 0.16 | 7.63 ± 0.04 | 7.11 ± 0.50 |
| TDS (ppm) | 30,484.33 ± 245.23 | 31,605.00 ± 127.80 | 21,556.67 ± 56.55 | 21,526.00 ± 108.58 | 35,866.00 ± 939.42 | 39,241.33 ± 956.42 | 1,387.00 ± 39.68 | 1,281.00 ± 45.60 |
| Resistivity (ohm) | 20.00 ± 0.00 | 20.00 ± 0.00 | 29.00 ± 0.00 | 29.00 ± 0.00 | 17.00 ± 0.00 | 19.67 ± 3.67 | 29.00 ± 0.00 | 27.67 ± 1.15 |
| Salinity (ppt) | 31.23 ± 0.48 | 32.47 ± 0.48 | 22.50 ± 0.14 | 22.53 ± 0.09 | 36.27 ± 2.33 | 47.17 ± 4.25 | 1.20 ± 0.04 | 1.07 ± 0.08 |
| Conductivity (ms) | 48.53 ± 0.19 | 50.17 ± 0.29 | 35.23 ± 0.17 | 35.10 ± 0.14 | 57.93 ± 3.03 | 61.20 ± 1.16 | 2.49 ± 0.09 | 2.31 ± 0.08 |

## Table S1.

| Samples | Shannon | Simpson | Observed |
|---|---|---|---|
| LS_Rainy_Water1 | 4,67514E+14 | 9,67393E+14 | 2702 |
| LS_Rainy_Water2 | 4,5336E+14 | 9,64819E+14 | 2262 |
| LS_Rainy_Water3 | 4,54379E+14 | 9,62835E+14 | 2386 |
| LS_Rainy_Oyster1 | 2,4962E+14 | 8,06904E+14 | 975 |
| LS_Rainy_Oyster2 | 2,94716E+13 | 8,5582E+14 | 455 |
| LS_Rainy_Oyster3 | 3,04688E+14 | 8,61798E+13 | 910 |
| LS_Dry_Water1 | 4,16841E+14 | 9,66255E+14 | 141 |
| LS_Dry_Water2 | 4,5313E+14 | 9,6809E+14 | 831 |
| LS_Dry_Water3 | 4,57061E+13 | 9,70703E+14 | 665 |
| LS_Dry_Oyster1 | 2,93342E+14 | 8,82312E+14 | 235 |
| LS_Dry_Oyster2 | 2,93601E+14 | 8,03226E+14 | 181 |
| LS_Dry_Oyster3 | 3,41826E+14 | 9,15053E+14 | 252 |
| NO_Rainy_Water1 | 3,79975E+14 | 8,5994E+14 | 1928 |
| NO_Rainy_Water2 | 3,99769E+14 | 8,89427E+13 | 2163 |
| NO_Rainy_Water3 | 4,04085E+14 | 8,92455E+14 | 2256 |
| NO_Rainy_Oyster1 | 1,92919E+14 | 5,00375E+14 | 1181 |
| NO_Rainy_Oyster2 | 1,67388E+14 | 4,22228E+13 | 1621 |
| NO_Rainy_Oyster3 | 1,51996E+13 | 3,86665E+14 | 1230 |
| NO_Dry_Water1 | 3,85823E+14 | 8,97945E+14 | 802 |

| | | | |
|---|---|---|---|
| NO_Dry_Water2 | 4,08343E+14 | 9,19922E+14 | 848 |
| NO_Dry_Water3 | 4,11421E+14 | 9,23518E+14 | 756 |
| NO_Dry_Oyster1 | 2,12765E+14 | 6,02103E+14 | 272 |
| NO_Dry_Oyster2 | 2,36869E+14 | 7,05994E+14 | 358 |
| NO_Dry_Oyster3 | 3,14916E+14 | 8,91142E+14 | 80 |
| PF_Rainy_Water1 | 4,67869E+14 | 9,67658E+14 | 1806 |
| PF_Rainy_Water2 | 4,69107E+13 | 9,64845E+14 | 2295 |
| PF_Rainy_Water3 | 4,76763E+13 | 9,69465E+14 | 2286 |
| PF_Rainy_Oyster1 | 3,38051E+14 | 8,44164E+13 | 1473 |
| PF_Rainy_Oyster2 | 3,73653E+14 | 9,24501E+14 | 898 |
| PF_Rainy_Oyster3 | 2,45525E+14 | 7,14547E+14 | 1033 |
| PF_Dry_Water1 | 4,67895E+14 | 9,70484E+12 | 544 |
| PF_Dry_Water2 | 4,44287E+14 | 9,57718E+14 | 636 |
| PF_Dry_Water3 | 4,84822E+14 | 9,75164E+14 | 953 |
| PF_Dry_Oyster1 | 3,42909E+14 | 9,08497E+14 | 329 |
| PF_Dry_Oyster2 | 3,2978E+14 | 9,18133E+14 | 174 |
| PF_Dry_Oystert3 | 3,02584E+14 | 8,75402E+14 | 206 |
| SAU_Rainy_Water1 | 3,24618E+14 | 8,08604E+14 | 2365 |
| SAU_Rainy_Water2 | 3,02995E+14 | 8,01635E+14 | 543 |
| SAU_Rainy_Water3 | 4,57833E+14 | 9,44705E+14 | 2583 |
| SAU_Rainy_Oyster1 | 2,4503E+13 | 6,15846E+14 | 1303 |
| SAU_Rainy_Oyster2 | 2,47382E+14 | 6,03245E+14 | 1283 |
| SAU_Rainy_Oyster3 | 2,10739E+14 | 5,8651E+14 | 974 |
| SAU_Dry_Water1 | 4,09537E+13 | 9,19178E+14 | 905 |
| SAU_Dry_Water2 | 3,91879E+14 | 9,09156E+14 | 684 |
| SAU_Dry_Water3 | 4,02961E+14 | 9,17573E+14 | 865 |
| SAU_Dry_Oyster1 | 2,99985E+14 | 8,12848E+14 | 506 |
| SAU_Dry_Oyster2 | 2,92755E+14 | 7,8083E+14 | 506 |
| SAU_Dry_Oyster3 | 2,45851E+14 | 7,66802E+14 | 352 |

**Table S2.**

| Phylum | log2FC | Pvalues, | FDR |
|---|---|---|---|
| Bacteroidota | 26.297 | 2,65E-03 | 5,47E-02 |
| Actinomycetota | 30.272 | 3,49E-03 | 5,47E-02 |
| Spirochaetota | 42.949 | 3,96E-02 | 4,66E-01 |
| Pseudomonadota | 22.715 | 5,78E-03 | 5,43E-01 |
| Mycoplasmatota | 28.544 | 1,21E-02 | 9,47E-01 |
| Planctomycetota | 30.153 | 1,63E-01 | 1,04E+00 |
| Thermodesulfobacteria | 29.922 | 1,78E-01 | 1,04E+00 |
| Bacillota | 1.826 | 1,73E+00 | 9,03E+00 |
| Verrucomicrobiota | 2.228 | 3,58E+00 | 0.0016808 |
| Kiritimatiellaeota | 21.921 | 4,08E+00 | 0.0017432 |

**Figure Legend**

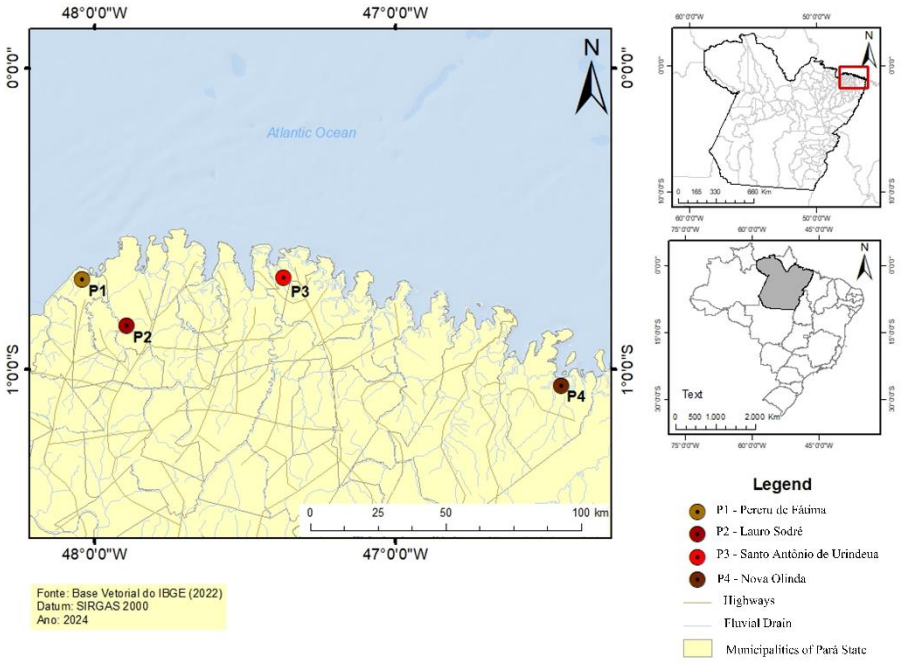


**Figure 1.** The map shows the geographical location of the four municipalities that were the subject of the research, situated in the northeastern region of the state of Pará, in the Brazilian Amazon: Pereru de Fátima (PF) (P1), Lauro Sodré (LS) (P2), Santo Antônio do Urindeua (SAU) (P3), and Nova Olinda (NO) (P4). Furthermore, the figure includes two sub-maps on the right: one illustrating the location of the study areas taking into account the entire map of the state of Pará and the other indicating the location of Pará on the map of Brazil. The data source employed was the IBGE Vector Base (2022).

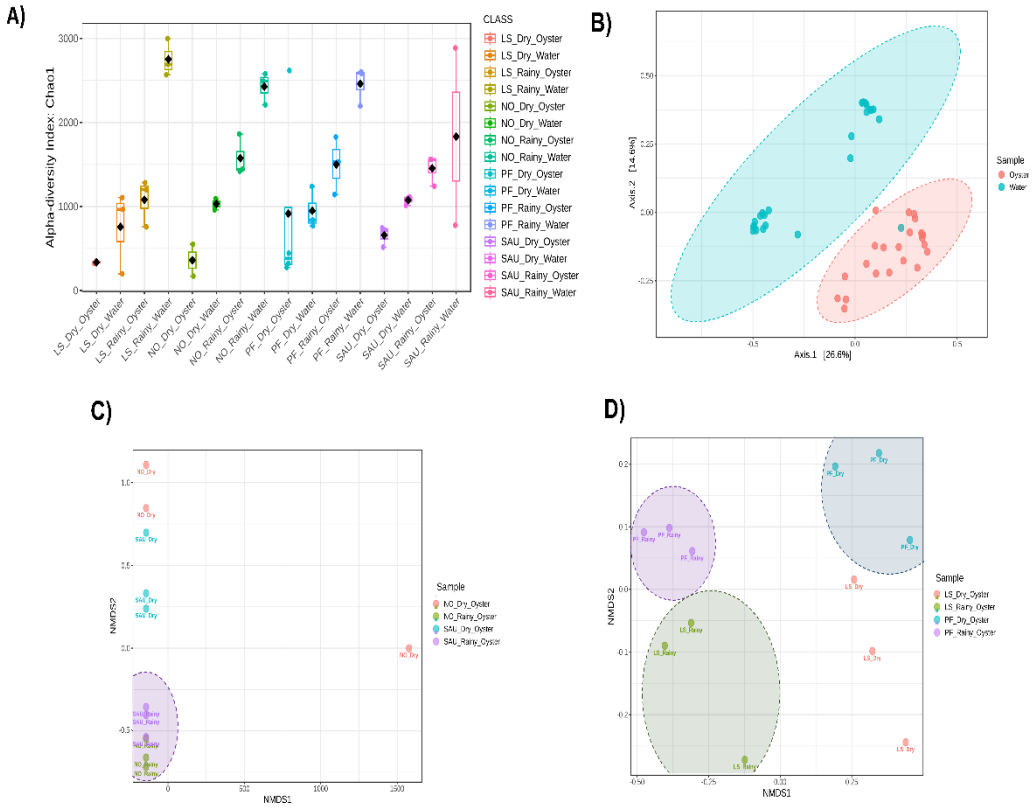


**Figure 2.** Alpha and beta-diversity analyses of farmed oyster and water samples collected in the four municipalities. (A) Alpha-diversity index (Chao1) for oyster and water samples collected at different locations and seasonal periods (dry and rainy). It is evident that the water samples exhibit higher alpha-diversity values than the oyster samples, except for the oyster and water samples collected in PF during the dry period. (B) Beta-diversity analysis by principal component analysis (PCA), which compares the microbial composition between oyster and water samples. The colored ellipses serve to highlight the separation between the two types of samples, with oysters represented in red and water in blue. In Figures C and D, the beta-diversity analysis is presented, comparing only the farmed oyster samples collected in PF and LS (C) and SAU and NO (D), during the different seasons. It is evident that there is a clear differentiation between the samples collected during the dry and rainy periods across all locations.

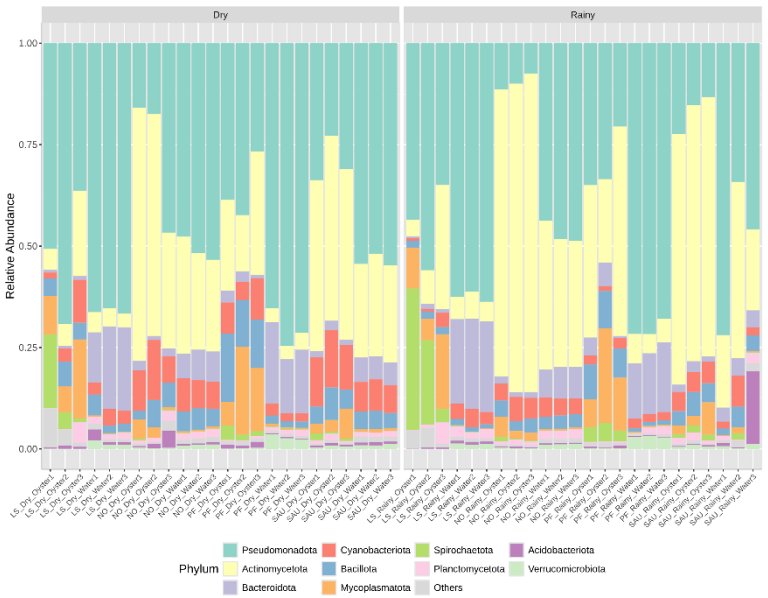


**Figure 3.** The histogram depicts the relative abundance of different bacterial phyla in the oyster and water samples, grouped by season (dry and rainy). Each bar represents a specific sample, and the color within the bars indicates the proportion of each bacterial phylum. Among the predominant phyla are Pseudomonadota, Actinomycetota, Bacillota, Cyanobacteriota and Bacteroidota. It is evident that variations in microbial composition can be observed when taking into account the seasons and geographical location.

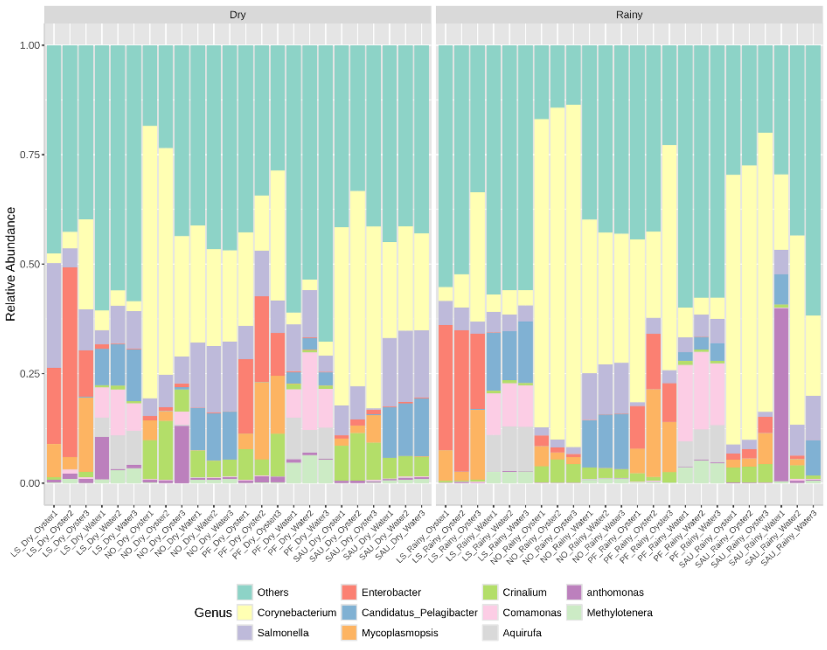


**Figure 4.** The histogram depicts the relative abundance of different bacterial genera present

in oyster and water samples, grouped by season (dry and rainy). Each bar represents a specific sample, and the color within the bars indicates the proportion of each bacterial genus. Among the predominant genera are *Corynebacterium*, *Enterobacter, Crinalium, Candidatus Pelagibacter, Comamonas, Methylotenera, Salmonella*, and other less frequent bacterial genera. It is evident that variations in microbial composition can be observed when taking into account the seasons and geographical location.

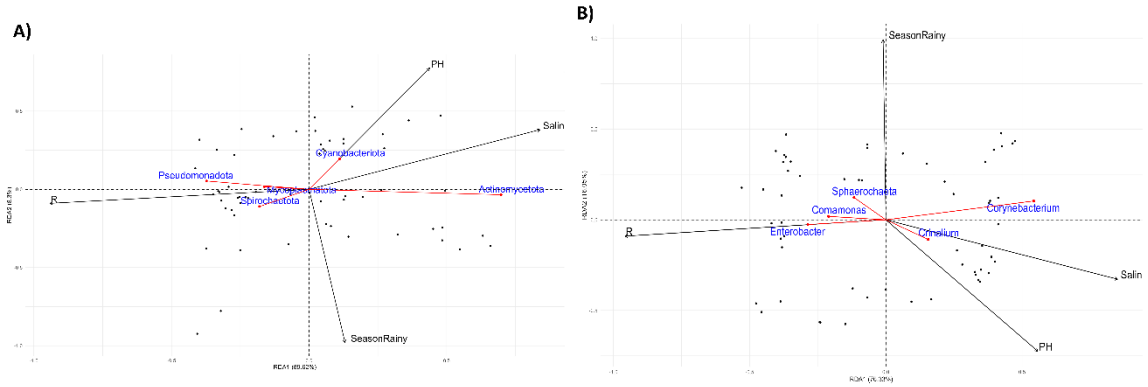


**Figure 5.** The graphs show the relationship between different taxa (phylum on the left and genus on the right) and the abiotic factors under investigation, employing redundancy analysis (RDA). The red dots represent a bacterial taxon, while the black arrows indicate the magnitude and direction of the correlation between the taxa and the principal components of the analysis. The phyla analyzed include Pseudomonadota, Mycoplasmatota, Spirochaetota, Cyanobacteriota and Actinomycetota (A). The genera analyzed include Enterobacter, Comamonas, Sphaerochaeta, Crinalium and Corynebacterium (B). The X-axis and Y-axis represent the principal components of the analysis, and the distribution of the taxa along these axes reflects their associations with the environmental variables.

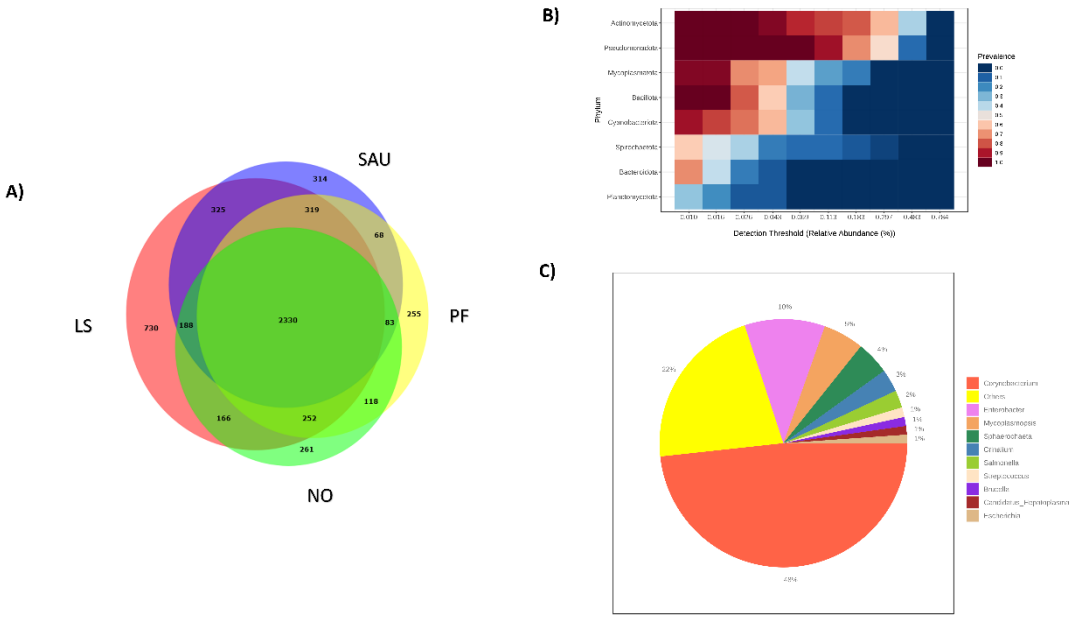


**Figure 6.** A comprehensive analysis of the pan-bacteriome of mangrove oysters was conducted. (A) Venn diagram illustrating the number of taxa identified in each sample. The coloured circles represent the collection sites of the oyster samples. A total of 2,330 taxa were identified as being present in all samples. It is noteworthy that the oyster samples collected in LS exhibit the highest number of unique taxa. (B) A heat map is provided, which demonstrates the shared bacteriome and the prevalence of each phylum as a function of different relative abundance detection thresholds (%). The color scale indicates the prevalence of the phyla at each threshold, with red indicating the highest prevalence and blue the lowest. The most prevalent bacterial phyla at the different levels of relative abundance were Actinomycetota and Pseudomonadota. (C) A pie chart illustrating the relative composition of the predominant bacterial genera within a specific sample. The genera represented include *Corynebacterium* (48%), Others (22%), *Enterobacter* (10%), and other genera such as *Mycoplasmopsis*, *Sphaerochaeta*, *Crinalium* and *Escherichia* in smaller proportions.

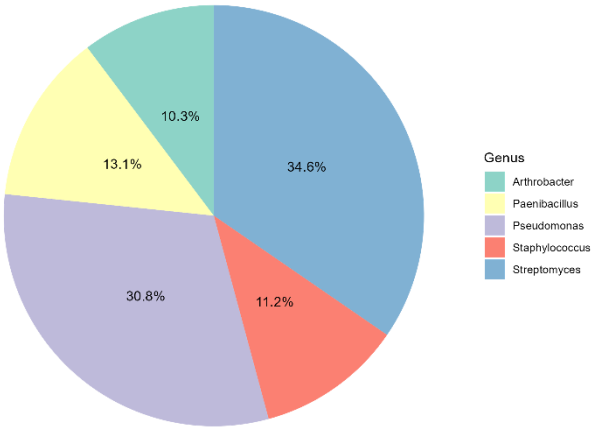


**Figure 7.** Pie chart showing the percentage of taxa within a sample. The main taxa found were Streptomyces (34.6%), Pseudomonas (30.8%), Paenibacillus (13.1%), Staphylococcus (11.2%), and Arthobacter (10.3%).

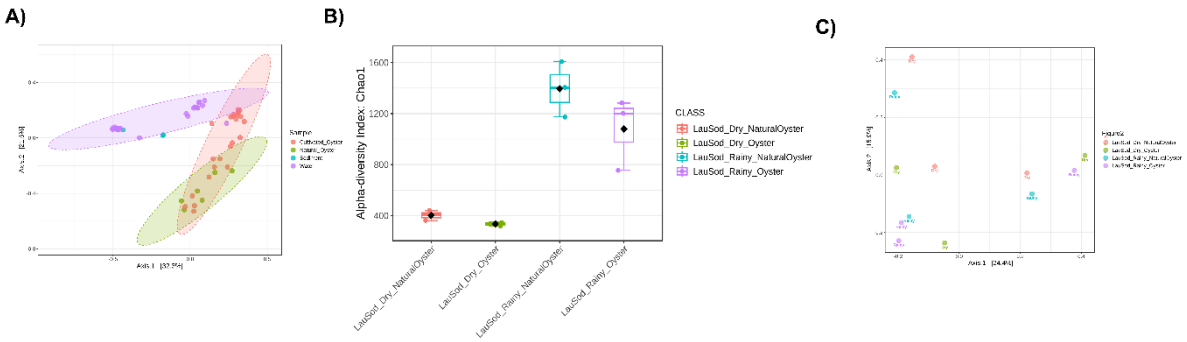


**Figure 8.** A comparative analysis of the bacterial diversity present in samples of farmed and native oysters, collected during both the dry and rainy seasons. (A) Beta-diversity by principal coordinates (PCoA), evaluating the similarity between samples of water (purple dots), sediment (blue dots), native oyster (green dots), and farmed oyster (red dots). A distinct separation between the samples is evident, particularly between the group of natural oysters and the other environmental samples, including water and sediment. (B) Alpha-diversity of oyster samples collected in Lauro Sodré, in the dry and rainy seasons. The Chao1 index provides an estimate of the species richness present in each sample. The rainy season exhibited greater richness for both samples, with the natural oyster samples demonstrating the greatest diversity. (C) Beta-diversity analysis by principal coordinates (PCoA) for the native and farmed oyster samples in both periods. No distinction could be discerned between the samples, indicating that the farming method does not exert a significant influence on the bacterial diversity of the oyster gut.

# 5 CONCLUSÃO

Este estudo demonstrou que o microbioma das ostras de mangue (*Crassostrea gasar*) e de seus habitats aquáticos amazônicos é fortemente influenciado por fatores abióticos, como salinidade, pH e disponibilidade de nutrientes, com variações sazonais particularmente acentuadas durante o período chuvoso. A diversidade microbiana foi consistentemente maior nas amostras de água, o que reflete a maior complexidade do ambiente aquático em comparação com o microbioma intestinal das ostras. No entanto, a diversidade beta das ostras mostrou uma relativa estabilidade sazonal, sugerindo que o microbioma destes moluscos mantém uma composição microbiana adaptada às condições ambientais flutuantes da Amazônia.

Os resultados apontam para a predominância do filo Actinomycetota nas ostras, especialmente nas áreas de maior influência de maré, destacando o papel simbiótico desses microrganismos na proteção contra patógenos e na degradação de compostos orgânicos. A identificação de táxons como Pseudomonadota, Bacteroidota e Cyanobacteriota reflete as mudanças sazonais na composição microbiana, com a abundância de Cyanobacteriota sendo mais proeminente durante o período seco, o que sugere a necessidade de monitoramento ambiental para prevenir intoxicações por cianotoxinas. Embora o método de cultivo não tenha influenciado significativamente a diversidade do microbioma das ostras, observou-se que as ostras cultivadas estão mais suscetíveis a mudanças nas condições ambientais, o que pode impactar sua saúde e a segurança alimentar das populações que dependem desse recurso.

Em síntese, este estudo fornece insights valiosos sobre a ecologia microbiana de ostras de mangue na Amazônia, contribuindo para o entendimento das interações complexas entre o microbioma dos moluscos e seu ambiente aquático. Esses achados têm implicações diretas para a gestão sustentável dos recursos naturais e para a segurança alimentar das comunidades que dependem da produção de ostras. A continuidade do monitoramento ambiental e microbiológico é crucial para garantir a saúde desses ecossistemas e a qualidade do produto final, com vistas à sustentabilidade econômica e ecológica da atividade de cultivo de ostras na Amazônia.

## 6 REFERÊNCIAS BIBLIOGRÁFICAS


ACHTMAN M, WAGNER M. Microbial diversity and the genetic nature of microbial species. **Nat Rev Microbiol**. 6(6):431-40, 2008.

AFINOWI, M.A. The mangrove oyster, *Crassostrea gasar* cultivation and potential in the Niger Delta (Nigeria). Lagos: **Nigerian Institute for Oceanography and Marine Research**, (Technical Paper: 14), 14p, 1984.

AMADI L. O, EFIUVWEVWERE B. Seasonal variations in physico-chemical and bacteriological parameters of Ogodogbo creek, Niger Delta, Nigeria. **International J. Environment and Bioenergy** 10(2):94-106. 2005.

ANDREWS, S. FASTQC. A quality control tool for high throughput sequence data, 2010.

ASHA, K et al. “Biochemical profile of oyster *Crassostrea* madrasensis and its nutritional attributes.” **The Egyptian Journal of Aquatic Research** 40: 35-41. 2014.

AUSTIN B, ZHANG XH. Vibrio harveyi: a significant pathogen of marine vertebrates and invertebrates. **Lett Appl Microbiol**. Aug;43(2):119-24, 2006.

BALLARD, A. L. et al. Detection of Legionella pneumophila using a real-time PCR hybridization assay. **Journal of clinical microbiology**, v. 38, n. 11, p. 4215-4218, 2000.

BARLETTA, M., BARLETTA-BERGAN, A., SAINT-PAUL, U. & HUBOLD, G. 2005. The role of salinity in structuring the fish assemblages in a tropical estuary. **J. Fish. Biol.** 66:45-72, 2005.

BARLETTA, M., et al., Fish and aquatic habitat conservation in South America: a continental overview with emphasis on neotropical systems. **J. Fish. Biol.** 76(9):2118-2176, 2010.

BRANDINI, F.P.; SILVA, A.S.; PROENÇA, L.A.O. Oceanografia e maricultura. In: VALENTI, W.C.; POLI C.R.; PEREIRA, JA; BORGHETTI, J.R. (ed.) **Aquicultura no Brasil: bases para um desenvolvimento sustentável**. Brasília: Ministério Ciência e Tecnologia, p.107-141. 2000.

BUTT, D., SHADDICK, K., & RAFTOS, D. (The effect of low salinity on phenoloxidase activity in the Sydney rock oyster, Saccostrea glomerata. **Aquaculture**, 251(2-4), 159–166, 2006.

CALLAHAN BJ, MCMURDIE PJ, ROSEN MJ, HAN AW, JOHNSON AJ,

HOLMES SP. DADA2: High-resolution sample inference from Illumina amplicon data. **Nat Methods**. Jul;13(7):581-3. 2016

CAPORASO JG, LAUBER CL, WALTERS WA, BERG-LYONS D, LOZUPONE CA, TURNBAUGH PJ, FIERER N, KNIGHT R. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample**. Proc Natl Acad Sci U S A**.;108 Suppl 1(Suppl 1):4516-22, 2011.

CARR R, SHEN-ORR SS, BORENSTEIN E. Reconstructing the genomic content of microbiome taxa through shotgun metagenomic deconvolution. **PLoS Comput Biol**. 2013.

CHENG, P. K. C., WONG, D. K. K., CHUNG, T. W. H., & LIM, W. W. L.. Norovirus contamination found in oysters worldwide. **Journal of Medical Virology**, 76(4), 593–597, 2005.

CHRISTO, S. W. & ABSHER, T. M. Reproductive period of *Crassostrea* rhizophorae (GUILDING, 1828) and *Crassostrea* brasiliana (Lamark, 1819) (Bivalvia: ostreidae) in Guaratuba bay, Paraná, Brazil. **Journal of Coastal Research**, SI 39, 1215 - 1218. Itajaí, SC, Brazil. 2006.

CLARK MS, THORNE MA, AMARAL A, VIEIRA F, BATISTA FM, REIS J, POWER DM. Identification of molecular and physiological responses to chronic environmental challenge in an invasive species: the Pacific oyster, *Crassostrea* gigas. **Ecol Evol.** Sep;3(10):3283-97. 2013.

COELHO C, HEINERT AP, SIMÕES CM, BARARDI CR. HEPATITIS A virus detection in oysters (*Crassostrea* gigas) in Santa Catarina State, Brazil, by reverse transcription-polymerase chain reaction**. J Food Prot**. Mar;66(3):507-11., 2003.

COELHO, PAULA NUNEs et al. Updated distribution and range expansion of the gastropod invader Melanoides tuberculata (Müller, 1774) in Brazilian waters. **BioInvasions Records,** v. 7, n. 4, p. 405–409, 2018.

CONCEIÇÃO, Marcos Vinícius Reis et al. Amazonia Seasons Have an Influence in the Composition of Bacterial Gut Microbiota of Mangrove Oysters (*Crassostrea gasar*). **Frontiers in genetics**, v. 11, p. 602608, 2021.

CROMEANS TL, NAINAN OV, MARGOLIS HS. Detection of hepatitis A virus RNA in oyster meat. Appl **Environ Microbiol**. Jun;63(6):2460-3, 1997.

DAME, Richard. Ecology of Marine Bivalves. 2. ed. **Boca Raton**. CRC Press. 2011.

DAVIS, A. R., COLEMAN, D., BROAD, A., BYRNE, M., DWORJANYN, S. A., &

PRZESLAWSKI, R. Complex Responses of Intertidal Molluscan Embryos to a Warming and Acidifying Ocean in the Presence of UV Radiation. **PLoS ONE**, 8(2), e55939, 2013.

do extrativismo para o cultivo. **Histórias de Sucesso - Agronegócio - Aquicultura e Pesca (Sebrae)**, Brasília, p.18, 2007.

DUMBAULD, B. R.; RUESINK, J. L.; RUMRILL, S. S. The ecological role of bivalve shellfish aquaculture in the estuarine environment: A review with application to oyster and clam culture in West Coast (USA) estuaries. **Aquaculture,** v. 290, n. 3–4, p. 196–223, 2009.

EFIUVWEVWERE, B.J.O. AND AMADI, L.O. Effects of Preservatives on the Bacteriological, chemical and sensory qualities of mangrove oyster (*Crassostrea gasar*) harvested from the Niger Delta Region, Nigeria. **British Journal of Applied Science and Technology**; 5(1):76-84, 2015.

FAO - Food and Agriculture Organization of the United Nations. The State of World Fisheries and Aquaculture - **Meeting the sustainable development goals**. Rome, v. 35, 2018.

FERREIRA, et al. Ecological Carrying Capacity for Shellfish Aquaculture—Sustainability of Naturally Occurring Filter-Feeders and Cultivated Bivalves. **Journal of Shellfish Research** 37(4), 709-726, (1 October 2018.

FLEISCHMANN RD, ADAMS MD, WHITE O, CLAYTON RA, KIRKNESS EF, KERLAVAGE AR, BULT CJ, TOMB JF, DOUGHERTY BA, MERRICK JM, et al. Whole-genome random sequencing and assembly of Haemophilus influenzae, **Rd. Science.;**269(5223):496-512. 1995

GARBOSSA, L. H. P. et al. Thermotolerant coliform loadings to coastal areas of SantaCatarina (Brazil) evidence the effect of growing urbanisation and insufficient provision of sewerageinfrastructure. **Environmental Monitoring and Assessment**, v. 189, n. 1, 12 p., 2017.

GIATSIS C, SIPKEMA D, RAMIRO-GARCIA J, BACANU GM, ABERNATHY J, VERRETH J, SMIDT H, VERDEGEM M. Probiotic legacy effects on gut microbial assembly in tilapia larvae. **Sci Rep.** 2016.

GLÖCKNER F.O., STAL L.J., SANDAA R.-A., GASOL J.M., O'GARA F., HERNANDEZ F., LABRENZ M., STOICA E., VARELA M.M., BORDALO A., PITTA P. Marine Microbial Diversity and its role in Ecosystem Functioning and Environmental Change. **Marine Board Position Paper** 17, 2012.

GOMES, L.A.O. **Cultivo de crustáceos e moluscos.** São Paulo: Nobel, 244p 1986.

GOSLING, Elizabeth. Buvalve Molluscs: Biology, ecology and culture. 1 ed. **Oxford: Foshing News Books.** p.455. 2003.

HANDELSMAN J. Metagenomics: application of genomics to uncultured microorganisms. Microbiol Mol **Biol Rev**.Dec;68(4):669-85, 2004.

IBGE Instituto Brasileiro de Geografia e Estatística. **Prod. da Pecuária Municipal**.
Vol.46, 2019.

IBGE. Instituto Brasileiro de Geografia e Estatística. **Prod. da Pecuária Municipal**. Vol. 41, 2014.

IP, C. C. M., LI, X., ZHANG, G., FARMER, J. G., WAI, W. H. O., & LI, Y. S. (2004). Over one hundred years of trace metal fluxes in the sediments of the Pearl River Estuary, South China. **Environmental Pollution,** 132(1), 157-172,2004.

JACKSON, M. PAULINE M. H. MAZUMDAR, Species and Specificity: An Interpretation of the History of Immunology. Cambridge: Cambridge University Press,. Pp. xiii 457. ISBN 0-521-43172-7. **The British Journal for the History of Science**, 29. 1996.

KENKEL, C., MATZ, M. Gene expression plasticity as a mechanism of coral adaptation to a variable environment. **Nat Ecol Evol 1,** 0014, 2017.

KIM BR, SHIN J, GUEVARRA R, LEE JH, KIM DW, SEOL KH, LEE JH, KIM HB, ISAACSON R. Deciphering Diversity Indices for a Better Understanding of Microbial Communities. **J Microbiol Biotechnol.** Dec 28;27(12):2089-2093, 2017.

KING W.L. et al., Regional and oyster microenvironmental scale heterogeneity in the Pacifig oyster bacterial Community. FEMS Microbiology Ecology. V. 96, n. 5, 2020.

LI, C., M. HAWS, H. WANG & X. GUO. Taxonomic classificationof three oyster (Ostreidae) species from Myanmar. **J. Shellfish Res**.36:365–371, 2017b.

MACEDO, S. SÜHNEL AND C.A.M. CORDEIRO et al. Growth and survival of the native oyster *Crassostrea gasar* cultured under different stocking densities in two grow-out systems in tropical climate. **Arq. Bras. Med. Vet. Zootec**.. Vol. 73(4):893-901, 2021

MACHADO AA, SPENCER K, KLOAS W, TOFFOLON M, ZARFL C. Metal fate and effects in estuaries: A review and conceptual model for better understanding of

toxicity. **Sci Total Environ.** 541:268-281 .09.045, 2016.

Mackie, G. L. Bivalves. Pages 305–418 in K. M. Wilburn**. The Mollusca**. vol. 7. Reproduction, S. A. Tompa, N. H. Verdonk and J. A. M van den Biggelaar, eds. Academic Press, Orlando, Florida. 1984.

MEDINI D, SERRUTO D, PARKHILL J, RELMAN DA, DONATI C, MOXON R, FALKOW S, RAPPUOLI R. Microbiology in the post-genomic era. **Nat Rev Microbiol.** 2008.

MELO, A.G.C., VARELA, E.S., BEASLEY, C.R., SCHNEIDER, H., SAMPAIO, I., GAFFNEY, P.M., REECE, K.S., TAGLIARO, C.H.. Molecular identification, phylogeny and geographic distribution 55 of Brazilian mangrove oysters (*Crassostrea*). **Genetics and Molecular Biology** 33( 3), 564-572. 2010b.

MELO, C.M.R., SILVA, F.C., GOMES, C.H.A.M., SOLE´-CAVA, A.M., LAZOSKI, C. *Crassostrea* gigas in natural oyster banks in southern Brazil. **Biological Invasions** 12, 441–449. 2010a .

NATHAN G KING, SOPHIE B WILMES, DAVID SMYTH, JONATHAN TINKER, PETER E ROBINS, JAMIE THORPE, LAURENCE JONES, SHELAGH K MALHAM, Climate change accelerates range expansion of the invasive non-native species, the Pacific oyster, *Crassostrea* gigas, **ICES Journal of Marine Science,** Volume 78, Issue 1, January-February 2021, Pages 70–81. 2021.

NICHOLSON JK, HOLMES E, KINROSS J, BURCELIN R, GIBSON G, JIA W, PETTERSSON S. Host-gut microbiota metabolic interactions. **Science.** Jun 8;336(6086):1262-7. doi: 10.1126/science.1223813. Epub 2012 Jun 6. PMID: 22674330, 2012.

OLIVEIRA AMS, BARAÚNA RA, MARCON DJ, LAGO LAB, SILVA A, LUSIO J, TAVARES RDS, TACÃO M, HENRIQUES I, SCHNEIDER MPC. Occurrence, antibiotic-resistance and virulence of E. coli strains isolated from mangrove oysters (*Crassostrea gasar*) farmed in estuaries of Amazonia. **Mar Pollut Bull.** 2020.

PACE, R. et al. The Analysis of Natural Microbial Populations by Ribosomal RNA Sequences. **Advances in Microbial Ecology: Collected Works.** New South Whales. v. 9, p, 394. 1985.

PAIXÃO, L. , FERREIRA, M. A., NUNES, Z. FONSECA-SIZO, F., ROCHA, R. Effects of salinity and rainfall on the reproductive biology of the mangrove oyster (*Crassostrea gasar*): Implications for the collection of broodstock oysters. **Aquaculture** 380-383: 6-

12, 2013.

PARKS, D. H., TYSON, G. W., HUGENHOLTZ, P., & BEIKO, R. G. STAMP: statistical analysis of taxonomic and functional profiles. **Bioinformatics (Oxford, England),** 30(21), 3123–3124, 2014.

PHA, AWWA, WEF. Standard Methods for exa- mination of water and wastewater. 22nd ed. Washington: **American Public Health Association**; 1360 pp , 2012.

QUAYLE, D. B.; NEWKIRK, G. F. Farming bivalve molluscs: Methods for study and development. **Advances in World Aquaculture**, v. 1, 294 p., 1989.

R CORE TEAM; R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, 2015.

RANJAN, Ravi; RANI, Asha; KUMAR, Rajesh. Exploration of microbial cells: The storehouse of bio-wealth through metagenomics and metatranscriptomics. **Microbial Factories**, p. 7-27, 2015

SAMPAIO, D., TAGLIARO, C.H., SCHNEIDER, H. AND BEASLEY, C.R. Oyster culture on the Amazon mangrove coast: asymmetries and advances in an emerging sector. **Rev Aquacult**, 11: 88-104, 2019.

SAMPAIO, DIONISO de S. & BOULHOSA, R. L. DE M. Energia que vem da ostra:

SANTOS AA, COSTA SW. **Síntese informativa da maricultura**. Santa Catarina: EPAGRI; 2015

SANY SBT, HASHIM R, REZAYI M, SALLEH A, SAFARI O. A review of strategies to monitor waterand sediment quality for a sustainability assessment of marine environment. **Environ Sci PollutRes**. 2013.

SCHETTINO T, CARICATO R, CALISI A, GIORDANO M, LIONETTO M. Biomarker approach in marinemonitoring and assessment: new insights and perspectives. **Open Environ Sci** 6:20–27, 2012.

SCHLOSS PD, GEVERS D, WESTCOTT SL. Reducing the Effects of PCR Amplification and Sequencing Artifacts on 16S rRNA-Based Studies. **PLoS ONE** 6(12), 2011.

SCHLOSS PD, HANDELSMAN J. Metagenomics for studying unculturable microorganisms: cutting the Gordian knot. **Genome Biol.;**6(8):229, 2005.

SCHMIDT, Thomas M.; DELONG, Edward F.; PACE, Norman R. Analysis of a

marine picoplankton community by 16S rRNA gene cloning and sequencing. **Journal of bacteriology**, v. 173, n. 14, p. 4371-4378, 1991.

SHARPTON, Thomas J. An introduction to the analysis of shotgun metagenomic data. **Frontiers in plant science**, v. 5, p. 209, 2014.

SHEELA A, LETHA J, JOSEPH S, CHACKO M, THOMAS J. Water quality assessment of a tropicalcoastal lake system using multivariate cluster, principal component and factor analysis. **Lakes Reserv Res Manag** 17(2):143–159, 2012.

Sherman LS, Blum JD, Franzblau A, Basu N (2013) New insight into biomarkers of human mer-cury exposure using naturally occurring mercury stable isotopes. Environ Sci Technol47:3403–3409

Shokrollahi A, Ghaedi M, Niband MS, Rajabi HR (2008) Selective and sensitive spectrophotomet-ric method for determination of sub-micro-molar amounts of aluminium ion. J Hazard Mat151(2–3):642–648

SHTYKOVA, Y.R., SITNIKOVA, T.Y., KULAKOVA, N.V. et al. First Reports on the Bacterial Community of the Digestive System of Endemic Baikal Gastropods Benedictia baicalensis. **Microbiology 87,** 825–832 2018.

SMITH BA, GREENBERG B, STEPHANSON GL. Comparison of biological and chemical measuresof metal bioavailability in field soils: test of a novel simulated earthworm gut extraction.**Chemosphere** 81:755–766, 2010.

SOLÉ, M., PORTE, C., & ALBAIGÉS, J. Mixed-function oxygenase system components and antioxidant enzymes in different marine bivalves: Its relation with contaminant body burdens. **Aquatic Toxicology**, 30(3), 271–283, 1994.

STALEY JT, KONOPKA A. Measurement of in situ activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. **Annu Rev Microbiol.** 1985.

SULLIVAN, J. M., TWARDOWSKI, M. S., DONAGHAY, P. L., & FREEMAN, S. AUse of optical scattering to discriminate particle types in coastal waters. **Applied Optics**, 44(9), 1667, 2005.

SUN J, WANG MH, HO YS. A historical review and bibliometric analysis of research onestuary pollution. **Mar Pollut Bull** 64:13–21, 2012.

TABINDA AB, BASHIR S, YASAR A, HUSSAIN M. Metals concentrations in the riverine water,sediments and fishes from river Ravi at Bolloki headworks. **J Anim Plant Sci** 21(1):76–84, 2013.

THOMPSON, P.A., GUO, M. & HARRISON, P.J. The influence of irradiance on

the biochemical composition of three phytoplankton species and their nutritional value for larvae of the Pacific Oyster (*Crassostrea* gigas). **Marine Biology 117,** 259–268. 1993.

TONG Y, ZHANG W, HU X, QU L, HU D, YANG T, WEI W, WANG X. Model description of tropho-dynamic behaviour of methylmercury in a marine aquatic system. **Environ Pollut** 166:89–97. 2012.

TRINGE SG, RUBIN EM. Metagenomics: DNA sequencing of environmental samples. **Nat Rev Genet**. ;6(11):805-14, 2005.

TURNER A, MILLWARD GE. Suspended particles: their role in estuarine biogeochemical cycles.**Estuar Coast Shelf Sci** 55(6):857–883, 2002.

VALENTI, W.C.; POLI C.R.; PEREIRA, JA; BORGHETTI, J.R. (ed.) **Aquicultura no Brasil: bases para um desenvolvimento sustentável.** Brasília: Ministério Ciência e Tecnologia. p.107-141. 2000.

VALTER AMARAL, HENRIQUE N. CABRAL, MELANIE J. BISHOP. Resistance among wild invertebrate populations to recurrent estuarine acidification, Estuarine, **Coastal and Shelf Science**, Volume 93, Issue 4, Pages 460-467. 2011.

VENTER J.C. et al., The sequence of the human genome. **Science**. 291(5507):1304-51, 2001.

VENTER JC, REMINGTON K, HEIDELBERG JF, HALPERN AL, RUSCH D, EISEN JA, WU D, PAULSEN I, NELSON KE, NELSON W, FOUTS DE, LEVY S, KNAP AH, LOMAS MW, NEALSON K, WHITE O, PETERSON J, HOFFMAN J, PARSONS R, BADEN-TILLSON H, PFANNKOCH C, ROGERS YH, SMITH HO. Environmental genome shotgun sequencing of the Sargasso Sea. **Science**. 304(5667):66-74. 2004.

VERMEULEN LA, WEPENER V. Spatial and temporal variations of metals in Richards BayHabour (RHB), South Africa. **Mar Poll Bull** 39(1):304–307A.Z. Aris and L.J. Looi Page 25, 1999.

WANG, JUN et al. Dietary history contributes to enterotype-like clustering and functional metagenomic content in the intestinal microbiome of wild mice. **Proceedings of the National Academy of Sciences**, v. 111, n. 26, p. E2703-E2710, 2014.

WINSLOW CE. Some leaders and landmarks in the history of microbiology. **Bacteriol Rev**. Jun;14(2):99-114, 1950.

YARZA, P., YILMAZ, P., PRUESSE, E. et al. Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. **Nat**

**Rev Microbiol 12,** 635–645, 2014.

# 7 ARTIGOS PUBLICADOS NO PERÍODO

frontiers in Genetics

ORIGINAL RESEARCH
published: 12 February 2021
doi: 10.3389/fgene.2020.602608

# Amazonia Seasons Have an Influence in the Composition of Bacterial Gut Microbiota of Mangrove Oysters (*Crassostrea gasar*)

*Marcos Vinicius Reis Conceição*[1,2], *Sávio Souza Costa*[1,2], *Ana Paula Schaan*[3], *Ândrea Kely Campos Ribeiro-dos-Santos*[3], *Artur Silva*[1,2], *Diego Assis das Graças*[1,2], *Maria Paula Cruz Schneider*[1,2] *and Rafael Azevedo Baraúna*[1,2]*

[1] Laboratory of Genomics and Bioinformatics, Center of Genomics and Systems Biology, Institute of Biological Sciences, Federal University of Pará, Belém, Brazil, [2] Laboratory of Biological Engineering, Guamá Science and Technology Park, Belém, Brazil, [3] Laboratory of Medical and Human Genetics, Institute of Biological Sciences, Federal University of Pará, Belém, Brazil

OPEN ACCESS

**Edited by:**
*András Moya,*
*University of Valencia, Spain*

**Reviewed by:**
*Daniel Martinez,*
*Imperial College London, United Kingdom*
*Daniel A. Medina,*
*San Sebastián University, Chile*

***Correspondence:**
*Rafael Azevedo Baraúna*
*rabarauna@ufpa.br*

**Specialty section:**
*This article was submitted to Evolutionary and Genomic Microbiology, a section of the journal Frontiers in Genetics*

**Received:** *03 September 2020*
**Accepted:** *17 December 2020*
**Published:** *12 February 2021*

**Citation:**
*Conceição MVR, Costa SS, Schaan AP, Ribeiro-dos-Santos ÂKC, Silva A, das Graças DA, Schneider MPC and Baraúna RA (2021) Amazonia Seasons Have an Influence in the Composition of Bacterial Gut Microbiota of Mangrove Oysters (Crassostrea gasar). Front. Genet. 11:602608. doi: 10.3389/fgene.2020.602608*

The mangrove oysters (*Crassostrea gasar*) are molluscs native to the Amazonia region and their exploration and farming has increased considerably in recent years. These animals are farmed on beds built in the rivers of the Amazonia estuaries and, therefore, the composition of their microbiome should be directly influenced by environmental conditions. Our work aimed to evaluate the changes in bacterial composition of oyster's microbiota at two different seasons (rainy and dry). For this purpose, we amplified and sequenced the V3-V4 regions of the 16S rRNA gene. Sequencing was performed on the Illumina MiSeq platform. According to the rarefaction curve, the sampling effort was sufficient to describe the bacterial diversity in the samples. Alpha-diversity indexes showed that the bacterial microbiota of oysters is richer during the rainy season. This richness is possibly associated with the diversity at lower taxonomic levels, since the relative abundance of bacterial phyla in the two seasons remained relatively constant. The main phyla found include Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria. Similar results were found for the species *Crassostrea gigas*, *Crassostrea sikamea*, and *Crassostrea corteziensis*. Beta-diversity analysis showed that the bacterial composition of oyster's gut microbiota was quite different in the two seasons. Our data demonstrate the close relationship between the environment and the microbiome of these molluscs, reinforcing the need for conservation and sustainable management of estuaries in the Amazonia.

**Keywords: mangrove oyster, oyster, oyster microbiota, Amazonia, *Crassostrea gasar***

## INTRODUCTION

The phylum Mollusca is one of the largest and most important in the animal kingdom. From the six classes that make up the phylum, we can highlight the Bivalvia class, composed of about 7,500 species of soft-bodied animals protected by a shell, which acts as a skeleton for the connection of muscles and protects against predators (Gosling, 2003; Dame, 2011). Oysters are molluscs belonging to the *Ostreidae* family found in various marine and estuarine environments around the globe (Dame, 2011).

# First Steps in the Analysis of Prokaryotic Pan-Genomes

Bioinformatics and Biology Insights
Volume 14: 1–9
© The Author(s) 2020
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/1177932220938064
SAGE

Sávio Souza Costa[1,2], Luís Carlos Guimarães[1], Artur Silva[1,2], Siomar Castro Soares[3] and Rafael Azevedo Baraúna[1,2]

[1]Centro de Genômica e Biologia de Sistemas, Universidade Federal do Pará, Belém, Brazil. [2]Laboratório de Engenharia Biológica, Espaço Inovação, Parque de Ciência e Tecnologia Guamá, Belém, Brazil. [3]Instituto de Ciências Biológicas e Naturais, Universidade Federal do Triângulo Mineiro, Uberaba, Brazil.

**ABSTRACT:** Pan-genome is defined as the set of orthologous and unique genes of a specific group of organisms. The pan-genome is composed by the core genome, accessory genome, and species- or strain-specific genes. The pan-genome is considered open or closed based on the alpha value of the Heap law. In an open pan-genome, the number of gene families will continuously increase with the addition of new genomes to the analysis, while in a closed pan-genome, the number of gene families will not increase considerably. The first step of a pan-genome analysis is the homogenization of genome annotation. The same software should be used to annotate genomes, such as GeneMark or RAST. Subsequently, several software are used to calculate the pan-genome such as BPGA, GET_HOMOLOGUES, PGAP, among others. This review presents all these initial steps for those who want to perform a pan-genome analysis, explaining key concepts of the area. Furthermore, we present the pan-genomic analysis of 9 bacterial species. These are the species with the highest number of genomes deposited in GenBank. We also show the influence of the identity and coverage parameters on the prediction of orthologous and paralogous genes. Finally, we cite the perspectives of several research areas where pan-genome analysis can be used to answer important issues.

**KEYWORDS:** Pan-genome, core genome, accessory genome

**RECEIVED:** May 13, 2020. **ACCEPTED:** May 26, 2020.

**TYPE:** Review

**FUNDING:** The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: R.A.B. would like to thank Fundação Amazônia Paraense de Amparo a Estudos e Pesquisas (FAPESPA) for supporting the research (grant number 2155/2017), and Pró-Reitoria de Pesquisa e Pós-Graduação of Universidade Federal do Pará (Programa de Apoio à Publicação Qualificada – PAPQ 2020).

**DECLARATION OF CONFLICTING INTERESTS:** The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**CORRESPONDING AUTHOR:** Rafael Azevedo Baraúna, Laboratório de Engenharia Biológica, Parque de Ciência e Tecnologia Guamá, Avenida Perimetral da Ciência, km 01, Guamá, 66075-750 Belém, Brasil. Email: rabarauna@ufpa.br

## Introduction

Pan-genome was a term coined by Tettelin et al[1] to describe the gene content of several strains of *Streptococcus agalactiae*. The pan-genome is divided into core genome, dispensable or accessory genome, and singleton genes (ie, species-specific genes). Fifteen years after the publication of Tettelin's article, the number of genomes sequenced and available in databases have grown exponentially surpassing 30 000 complete and draft genomes in 2020 (https://gold.jgi.doe.gov/statistics). The evolution of sequencing technologies from classic chain termination method to fourth-generation sequencing, based on a massively parallel analysis, has been facilitating cost reduction over the years.[2] However, in many countries, the sequencing value still exceeds the prediction of USD 1000 per genome.[3] Despite criticisms about the use of draft genomes in pan-genome analysis, several new software have been developed to improve the assembly of these draft genomes.[4] For example, *Escherichia coli* has 15 275 genomes in scaffold or contigs available in GenBank (https://www.ncbi.nlm.nih.gov/genome/genomes/167) and their use for pan-genome studies is considered limited.

A search in PubMed database using the words "pan genome" or "pan-genome" returns a total of 494 works published in the last 5 years (2015 to date). This number tends to increase because the results of pan-genome analyses are becoming more accurate. Zeng et al[5] used a new pan-genome reverse vaccinology approach and found 121 cell surface-exposed proteins belonging to the core genome of *Leptospira interrogans*. These proteins proved to be highly antigenic and widely distributed in the species. Thus, these proteins are potential candidates for vaccine development. Pan-genome analysis was also applied to the discovery of antiphage defense systems,[6] in RNAseq analysis,[7] and evolutionary studies of adaptation to different hosts.[8]

In this review, we present the main concepts and software used for the analysis of prokaryotic pan-genomes. First, introduction to basic concepts is presented followed by an up-to-date description of the most recent software for pan-genome analysis. We also present a pan-genome analysis of the 9 bacterial species with the highest number of genomes deposited in GenBank.

## Basic Concepts

### *Pan-genome structure*

The sequence of a single genome does not reflect the entire genetic variability of a bacterial species. Complex analysis such as evolutionary genomics and molecular pathogenesis require a large number of sequenced genomes.[1,9] Fortunately, the constant evolution of sequencing technologies has been allowing the reduction of sequencing time and cost. Consequently, an exponential increase in the number of genomes available in the databases has been observed. New research fields have emerged such as comparative genomics, whose principle is to compare the genetic content of several taxonomically related microorganisms.[10] For example, in the pre-genomic era, 2 strains were classified in the same species

*Genetics and Molecular Biology*, 45, 1, e20210204 (2022)

DOI: https://doi.org/10.1590/1678-4685-GMB-2021-0204

Research Article
Genetic of Microorganisms

# Diversity of bacteriocins in the microbiome of the Tucuruí Hydroelectric Power Plant water reservoir and three-dimensional structure prediction of a zoocin

Sávio S. Costa[1], Leticia, A. B. Lago[1], Artur Silva[1], Diego A. das Graças[1], Jerônimo Lameira[2] and Rafael A. Baraúna[1]

[1]*Parque de Ciência e Tecnologia Guamá, Laboratório de Engenharia Biológica, Belém, PA, Brazil.*
[2]*Universidade Federal do Pará, Instituto de Ciências Exatas e Naturais, Laboratório de Planejamento e Desenvolvimento de Fármacos, Belém, PA, Brazil.*

## Abstract

Bacteriocins are antimicrobial peptides expressed by bacteria through ribosomal activity. In this study, we analyzed the diversity of bacteriocin-like genes in the Tucuruí-HPP using a whole-metagenome shotgun sequencing approach. Three layers of the water column were analyzed (photic, aphotic and sediment). Detection of bacteriocin-like genes was performed with blastx using the BAGEL4 database as subject sequences. In order to calculate the abundance of bacteriocin-like genes we also determined the number of 16S rRNA genes using blastn. Taxonomic analysis was performed using RAST server and the metagenome was assembled using IDBA-UD in order to recover the full sequence of a zoocin which had its three-dimensional structure determined. The photic zone presented the highest number of reads affiliated to bacteriocins. The most abundant bacteriocins were sonorensin, Klebicin D , pyocin and colicin. The zoocin model was composed of eight anti-parallel β-sheets and two α-helices with a $Zn^{2+}$ ion in the active site. This model was considerably stable during 10 ns of molecular dynamics simulation. We observed a high diversity of bacteriocins in the Tucuruí-HPP, demonstrating that the environment is an inexhaustible source for prospecting these molecules. Finally, the zoocin model can be used for further studies of substrate binding and molecular mechanisms involving peptidoglycan degradation.

*Keywords*: Bacteriocin, whole metagenome sequencing, Zoocin, Tucuruí-HPP.

Received: July 15, 2021; Accepted: November 05, 2021.

## Introduction

In the environment, several species of free-living microorganisms coexist and their adaptive success depends, in part, on the molecular mechanisms of defense and competition (Nes *et al.*, 1996; Quereda *et al.*, 2016). Among these mechanisms there are the so-called antimicrobial peptides (AMPs). They are synthesized in ribosomes and the gene clusters that encode AMPs are widely distributed in nature (Nissen-Meyer and Nes, 1997). AMP expression has already been reported in mammals, plants, insects, and bacteria (Hancock and Chapple, 1999). The AMPs produced by bacteria are narrow-spectrum anti-bacterial agents called bacteriocins. These peptides have activity against bacteria that are taxonomically related to the producing species. However, some broad-spectrum AMPs have already been described (Cleveland *et al.*, 2001). Some studies suggest that approximately 99% of bacterial species produce bacteriocins (Riley and Wertz, 2002).

The first bacteriocins characterized were produced by the model species *Escherichia coli* and were called colicins (Rehm and Lazdunski, 1988). Colicin acts by forming a voltage-dependent channel into the inner membrane of bacteria causing an imbalance of electrochemical gradient and, consequently, cell death. Colicin also acts as an endonuclease on DNA, rRNA, or tRNA of the target cells (Riley and Wertz, 2002). Several other bacteriocins have been described and studied since then as enterocin K1 (Ovchinnikov *et al.*, 2017), listeriolysin S (Quereda *et al.*, 2016), nisin O (Hatziioanou *et al.*, 2017), among others.

Different methods for the classification of bacteriocins were proposed (Klaenhammer 1993; Franz *et al.*, 2007; Zouhir *et al.*, 2010). Klaenhammer (1993) proposed the classification of bacteriocins produced by lactic acid bacteria (LAB) into four classes according to molecular weight, mechanism of action, and biochemical characteristics. This classification is one of the most used today.

Bacteriocins have a wide range of application. The most successful applications are related to the food industry and agriculture (Snyder and Worobo, 2014). AMPs are a promising alternative to the use of chemical preservatives in food production (Chopra *et al.*, 2015). Nisin, a bacteriocin of class Ia according to the Klaenhammer classification, was one of the first AMPs to be commercialized as a natural preservative for foods under the name Nisaplin™. This product is currently commercialized in several countries around the world. The use of bacteriocins as an alternative to antibiotics is also widely discussed today (Cotter *et al.*, 2013). For example lacticin 3147 acts in synergy with polymyxin to inhibit Gram-negative bacteria such as *Cronobacter* and *E. coli* (Draper *et al.*, 2013). Additionally, several studies have analyzed the ability of bacteriocins to inhibit the formation of biofilms in order to assist in the clinical treatment of pathogenic biofilm-forming

Send correspondence to Sávio S. Costa. Parque de Ciência e Tecnologia Guamá, Laboratório de Engenharia Biológica, Avenida Perimetral, 66075-750, Guamá, Belém, PA, Brazil. E-mail: savscosta@gmail.com.

Science of The Total Environment
Volume 812, 15 March 2022, 152324

# Pathogenicity of Shiga toxin-producing *Escherichia coli* (STEC) from wildlife: Should we care?

Diana Dias [a], Sávio Costa [b], Carlos Fonseca [a c], Rafael Baraúna [b], Tânia Caetano [a], Sónia Mendo [a]



https://doi.org/10.1016/j.scitotenv.2021.152324



## Highlights

- Cultivable STEC were isolated from 17% of the wild animals.
- All the isolates were non-O157, encoding *stx1* (4%) and/or *stx2* genes (98%).
- Serotypes O27:H30, O146:H28, O146:H21, O178:H19 and O103:H2 were identified.
- Besides Shiga toxin, all genomes encode, at least, 10 additional virulence factors.
- Some wild animals STEC have close evolutionary relationships with human-derived STEC.

Meeting Report | Open Access

# Shiga toxin-producing E. coli (STEC) isolated from wild mammals in Portugal

Diana Dias[1], Sávio Costa[2], Rafael Baraúna[2], Carlos Fonseca[3], Tânia Caetano[1] and Sónia Mendo[1]

View Affiliations

Published: 27 May 2022 | **https://doi.org/10.1099/acmi.ac2021.po0104**



**Background**

Zoonoses are diseases common to humans and animals (livestock, wildlife, and pets). In 2018 about 360 000 zoonoses were reported in European Union. Shiga toxin-producing Escherichia coli (STEC) infections were among the most reported causes of these zoonotic diseases.

**Methods**

Faecal samples of mammal species (n=286) with distinct phenology (wild boar, red deer, otter, and red fox) were collected in Portugal. After the initial processing, the presence of STEC was screened by PCR, and suspicious samples were platted on CHROMagar STEC. STEC positive isolates were tested for antibiotic susceptibility. Thephylogenetic relationship of STEC strains was evaluated by PFGE. Of these, 20 representative strains were selected for whole genome sequencing with the Illumina NovaSeq 6000 system. For the assembly, annotation and genome characterization, multiple web-based bioinformatic tools were employed.

**Results**

Cultivable STEC (n=52) were recovered from 17% (n=49) of the samples collected from the four mammals. All the isolates were non-O157:H7 STEC encoding stx1 (n=2; 4%) and/or stx2 genes (n=51; 98%). Only one strain (2%) of red fox was resistant to ceftazidime, aztreonam and nalidixic acid. The 20 strains that were sequenced belong mainly to serotype O27:H30 (n=15), followed by O146:H28 (n=2), O146:H21 (n=1), O178:H19 (n=1) and O103:H2 (n=1). In addition to stx, all strains encode several virulence factors, mainly toxins, adhesins, fimbrae, secretion systems, among others. Additionally, several pathogenicity islands have been predicted for these strains.

**Conclusions**

Our results show that wild animals are reservoirs of STEC, potentially pathogenic to humans.

Published Online: 27/05/2022

Costa et al. BMC Bioinformatics (2023) 24:24
https://doi.org/10.1186/s12859-022-05106-x

BMC Bioinformatics

SOFTWARE Open Access

# BADASS: BActeriocin-Diversity ASsessment Software

Sávio S. Costa[1*], Gislenne da Silva Moia[2], Artur Silva[1], Rafael A. Baraúna[1] and Adonney Allan de Oliveira Veras[3]

*Correspondence: savscosta@gmail.com

[1] Parque de Ciência e Tecnologia Guamá, Laboratório de Engenharia Biológica, Belém, Pará, Brazil
[2] Faculty of Computer Engineering, Federal University of Pará, Campus Tucuruí (CAMTUC-UFPA), Belém, Pará, Brazil
[3] Faculty of Computing, Federal University of Pará, Campus Castanhal (FACOMP/CCAST), Belém, Pará, Brazil

## Abstract

**Background:** Bacteriocins are defined as thermolabile peptides produced by bacteria with biological activity against taxonomically related species. These antimicrobial peptides have a wide application including disease treatment, food conservation, and probiotics. However, even with a large industrial and biotechnological application potential, these peptides are still poorly studied and explored. BADASS is software with a user-friendly graphical interface applied to the search and analysis of bacteriocin diversity in whole-metagenome shotgun sequencing data.

**Results:** The search for bacteriocin sequences is performed with tools such as BLAST or DIAMOND using the BAGEL4 database as a reference. The putative bacteriocin sequences identified are used to determine the abundance and richness of the three classes of bacteriocins. Abundance is calculated by comparing the reads identified as bacteriocins to the reads identified as 16S rRNA gene using SILVA database as a reference. BADASS has a complete pipeline that starts with the quality assessment of the raw data. At the end of the analysis, BADASS generates several plots of richness and abundance automatically as well as tabular files containing information about the main bacteriocins detected. The user is able to change the main parameters of the analysis in the graphical interface. To demonstrate how the software works, we used four datasets from WMS studies using default parameters. Lantibiotics were the most abundant bacteriocins in the four datasets. This class of bacteriocin is commonly produced by *Streptomyces* sp.

**Conclusions:** With a user-friendly graphical interface and a complete pipeline, BADASS proved to be a powerful tool for prospecting bacteriocin sequences in Whole-Metagenome Shotgun Sequencing (WMS) data. This tool is publicly available at https://sourceforge.net/projects/badass/.

**Keywords:** Antimicrobial peptides, Bacteriocin, Metagenome mining, Software development

## Background

Characterization of bioactive molecules produced by free-living microorganisms has been very important in recent years because of their biotechnological applications. It is well known that the overwhelming majority of free-living microorganisms are not

*microorganisms*

MDPI

*Article*

# Effects of Degradation on Microbial Communities of an Amazonian Mangrove

**Gleyciane Machado da Costa [1,2], Sávio Souza Costa [1,2], Rafael Azevedo Baraúna [1,2], Bruno Pureza Castilho [1], Izabel Cruz Pinheiro [1], Artur Silva [1,2], Ana Paula Schaan [3], Ândrea Ribeiro-dos-Santos [3] and Diego Assis das Graças [1,2,*]**

[1] Laboratory of Biological Engineering, Guamá Science and Technology Park, Belém 66075-750, Brazil; gleyci.ane2219@gmail.com (G.M.d.C.); savscosta@gmail.com (S.S.C.); rabarauna@ufpa.br (R.A.B.)
[2] Laboratory of Genomics and Bioinformatics, Center of Genomics and Systems Biology, Institute of Biological Sciences, Federal University of Pará, Belém 66075-110, Brazil
[3] Laboratory of Medical and Human Genetics, Institute of Biological Sciences, Federal University of Pará, Belém 66075-110, Brazil; apschaan@gmail.com (A.P.S.); akelyufpa@gmail.com (Â.R.-d.-S.)
* Correspondence: diegoassis@ufpa.br

**Abstract:** Mangroves provide a unique ecological environment for complex microbial communities, which play important roles in biogeochemical cycles, such as those for carbon, sulfur, and nitrogen. Microbial diversity analyses of these ecosystems help us understand the changes caused by external influences. Amazonian mangroves occupy an area of 9000 km$^2$, corresponding to 70% of the mangroves in Brazil, on which studies of microbial biodiversity are extremely scarce. The present study aimed to determine changes in microbial community structure along the PA-458 highway, which fragmented a mangrove zone. Mangrove samples were collected from three zones, (i) degraded, (ii) in the process of recovery, and (iii) preserved. Total DNA was extracted and submitted for 16S rDNA amplification and sequencing on an MiSeq platform. Subsequently, reads were processed for quality control and biodiversity analyses. The most abundant phyla were Proteobacteria, Firmicutes, and Bacteroidetes in all three mangrove locations, but in significantly different proportions. We observed a considerable reduction in diversity in the degraded zone. Important genera involved in sulfur, carbon, and nitrogen metabolism were absent or dramatically reduced in this zone. Our results show that human impact in the mangrove areas, caused by the construction of the PA-458 highway, has resulted in a loss of biodiversity.

**Keywords:** 16S rRNA; deforestation; anthropogenic impact; microbiome



**Citation:** Costa, G.M.d.; Costa, S.S.; Baraúna, R.A.; Castilho, B.P.; Pinheiro, I.C.; Silva, A.; Schaan, A.P.; Ribeiro-dos-Santos, Â.; Graças, D.A.d. Effects of Degradation on Microbial Communities of an Amazonian Mangrove. *Microorganisms* **2023**, *11*, 1389. https://doi.org/10.3390/microorganisms11061389

Academic Editors: Huixin Li, Li Xu and Hui Cao

Received: 10 April 2023
Revised: 4 May 2023
Accepted: 5 May 2023
Published: 25 May 2023



## 1. Introduction

Mangroves are one of the most dynamic and productive ecosystems and have great ecological importance. Located along tropical and subtropical regions, they constitute more than half of the terrestrial coastline forming a transitional environment between sea and land [1]. Mangroves are subjected to periodic tidal flooding, which results in variable salinity, redox potential, and anaerobic/aerobic conditions [2,3]. These characteristics make mangroves capable of harboring highly diverse microbial communities, which in turn are responsible for many essential features of biogeochemical cycles [4,5], thus microbial communities play a central role in maintaining the health and equilibrium of the mangrove environment.

The nitrogen cycle in mangroves is mediated by diverse groups of microorganisms and involves the transformation of nitrogen in various forms through key processes, such as nitrogen fixation, nitrification, denitrification, and ammonification [4]. Microbial communities in mangrove soils play a critical role in the phosphorus (P) cycle by mediating key processes involved in P cycling, such as (re)mineralization, immobilization, and adsorption, which influence P availability and cycling in mangrove ecosystems [5]. Microorganisms

*microorganisms*

MDPI

*Article*

# Soil Fertilization with Palm Oil Mill Effluent Has a Short-Term Effect on the Bacterial Diversity of an Amazonian Agricultural Land Area

**Johnes Pinto Sanches [1], Sávio Souza Costa [1], Diego Assis das Graças [1], Artur Silva [1], Guilherme Costa Baião [2], Rennan G. Moreira [3], Marcelo Murad Magalhães [4], Roberto Lisboa Cunha [4] and Rafael Azevedo Baraúna [1,*]**

[1] Biological Engineering Laboratory, Innovation Space, Guamá Science and Technology Park, Belém 66075-750, Brazil; johnes.sanches@icb.ufpa.br (J.P.S.); savscosta@gmail.com (S.S.C.); diego.a87@gmail.com (D.A.d.G.); arturluizdasilva@gmail.com (A.S.)
[2] Laboratory of Molecular and Computational Biology of Fungi, Department of Microbiology, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte 30120-140, Brazil; gcbaiao@gmail.com
[3] Multiuser Laboratories Center, Center of Genomics, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte 31270-901, Brazil; rennangm@gmail.com
[4] Brazilian Agricultural Research Corporation (EMBRAPA), Eastern Amazon, Belém 66095-100, Brazil; marcelo.magalhaes@embrapa.br (M.M.M.); roberto.cunha@embrapa.br (R.L.C.)
* Correspondence: rabaraúna@ufpa.br

**Abstract:** Palm oil derived from the fruits of *Elaeis guineensis* Jacq. has global economic importance and is largely produced in tropical regions. The palm oil production process leads to a highly polluting waste called palm oil mill effluent (POME). A strategy commonly used by producers to overcome environmental issues and to improve soil fertility is the reuse of POME as a fertilizer due to the chemical and biological characteristics of the effluent. In this research, three groups were analyzed: soil without POME application (control group) and soil samples after 4 and 9 days of POME application. An environmental DNA metabarcoding approach was used. eDNA was extracted, and the V4 region of the 16S rRNA gene was amplified and sequenced in the Illumina MiSeq platform. The abundance of Proteobacteria (48.1%) and Firmicutes (9.0%) was higher in fertilized soil, while Bacteroidetes (20.3%) and Verrucomicrobia (7.8%) were more abundant in control soil. Additionally, the effluent seemed to modify soil characteristics favoring taxa responsible for the mineralization of organic compounds and nitrogen fixation such as species of *Gammaproteobacteria* class. Our study highlights the influence of POME on soil biological components and contributes to the sustainable production of palm oil in the Amazon.

**Keywords:** 16S rRNA; bioinformatics; POME; microbiome; palm oil; metabarcoding



**Citation:** Sanches, J.P.; Costa, S.S.; das Graças, D.A.; Silva, A.; Baião, G.C.; Moreira, R.G.; Magalhães, M.M.; Cunha, R.L.; Baraúna, R.A. Soil Fertilization with Palm Oil Mill Effluent Has a Short-Term Effect on the Bacterial Diversity of an Amazonian Agricultural Land Area. *Microorganisms* **2024**, *12*, 507. https://doi.org/10.3390/microorganisms12030507

Academic Editors: Nikolay Vassilev and Stefan Shilev

Received: 8 December 2023
Revised: 22 January 2024
Accepted: 25 January 2024
Published: 1 March 2024



## 1. Introduction

*Elaeis guineensis* Jacq., the oil palm, is a perennial crop native to equatorial Africa that was introduced in other tropical regions of the world such as the Brazilian Amazon. The oil extracted from the palm fruits is used in several products including food, cosmetics, and biodiesel. Oil palm has been used as a semi-wild food by traditional communities for more than 7000 years [1]. In South America, the plant is also used in traditional medicine by indigenous people [2]. The industrial farming of oil palm remains predominantly concentrated in Southeast Asia. However, other tropical regions in the world have significantly expanded their production, including West Africa and South America [1].

*Elaeis guineensis* Jacq. offers an extracting yield significantly higher than other palm trees and presented a worldwide production of 77.2 million tons in the crop year 2022/2023 [3,4]. Brazil represents 15% of this worldwide production, with 11.4 million tons produced in the same period [4]. The state of Pará has the largest agricultural land area, producing 98% of the exported oil [5]. The Amazon region offers ideal ecological conditions

www.nature.com/scientificreports

scientific reports

Check for updates

OPEN

# BioPipeline Creator—a user-friendly Java-based GUI for managing and customizing biological data pipelines

Cléo Maia Cordeiro[1], Gislenne da Silva Moia[2], Mônica Silva de Oliveira[3], Lucas da Silva e Silva[4], Maria Paula da Cruz Schneider[4], Sávio S. Costa[5], Rafael Azevedo Baraúna[5], Diego Assis das Graças[5], Artur Silva[5] & Adonney Allan de Oliveira Veras[5]✉

**Bioinformatics tools are essential for performing analyses in the omics sciences. Given the numerous experimental opportunities arising from advances in the field of omics and easier access to high-throughput sequencing platforms, these tools play a fundamental role in research projects. Despite the considerable progress made possible by the development of bioinformatics tools, some tools are tailored to specific analytical goals, leading to challenges for non-bioinformaticians who need to integrate the results of these specific tools into a customized pipeline. To solve this problem, we have developed the BioPipeline Creator, a user-friendly Java-based GUI that allows different software tools to be integrated into the repertoire while ensuring easy user interaction via an accessible graphical interface. Consisting of client and server software components, BioPipeline Creator provides an intuitive graphical interface that simplifies the use of various bioinformatics tools for users without advanced computer skills. It can run on less sophisticated devices or workstations, allowing users to keep their operating system without having to switch to another compatible system. The server is responsible for the processing tasks and can perform the analysis in the user's local or remote network structure. Compatible with the most important operating systems, available at https://github.com/allanverasce/bpc.git.**

**Keywords** Research reproducibility, Customized pipeline, Bioinformatics

The development of computational tools in the field of bioinformatics has completely changed the possibilities of biological analysis and made bioinformatics an indispensable resource for numerous research projects in omics sciences. These software applications have evolved in recent years and have become critical components and, in many cases, integral parts of various biological workflows. They are able to process the large amounts of data that are produced and enable discoveries to be made. In the field of genomics, with next-generation sequencing platforms and the increased accessibility of these technologies, their application has expanded to various tasks such as gene expression analysis, gene prediction, genome assembly and annotation, genetic diversity analysis, and comparative genomic analysis[1].

Many tools used in omics research are designed to perform automated actions sequentially based on an input file containing biological information, typically using the result of one step as input for the next step, creating a so-called pipeline or workflow, such as the widely used Broad Institute's Genome Analysis Toolkit (GATK) bioinformatics pipeline[2]. In structural genomics, GATK is a comprehensive software package that includes genomics tools for variant detection, genotyping, and annotation. It provides a standardized pipeline for processing next-generation sequencing data, including read alignment, variant calling, and quality control. It has been used extensively in large genome projects, such as the 1000 Genomes Project and the Cancer Genome Atlas[2].

[1]Faculty of Computing, Federal University of Pará campus Castanhal, Pará, Brazil. [2]Postgraduate Program in Applied Computing, Federal University of Pará campus Tucuruí Pará, Tucuruí, Brazil. [3]Federal Institute of Pará campus Tucuruí, Pará, Brazil. [4]Center of Genomics and System Biologics, Federal University of Pará, Belém, Pará, Brazil. [5]Biological Engineering Laboratory, Guamá Science and Technology Park, Belém, Pará, Brazil. ✉email: allanveras@ufpa.br

# 8 PATENTES DEPOSITADAS NO PERÍODO

**REPÚBLICA FEDERATIVA DO BRASIL**
MINISTÉRIO DO DESENVOLVIMENTO, INDÚSTRIA, COMÉRCIO E SERVIÇOS
**INSTITUTO NACIONAL DA PROPRIEDADE INDUSTRIAL**
DIRETORIA DE PATENTES, PROGRAMAS DE COMPUTADOR E TOPOGRAFIAS DE CIRCUITOS

## Certificado de Registro de Programa de Computador

Processo Nº: **BR512023000694-7**

O Instituto Nacional da Propriedade Industrial expede o presente certificado de registro de programa de computador, válido por 50 anos a partir de 1° de janeiro subsequente à data de 20/01/2023, em conformidade com o §2°, art. 2° da Lei 9.609, de 19 de Fevereiro de 1998.

**Título:** BADASS: BActenocin-Diversity ASsessment Software

**Data de publicação:** 20/01/2023

**Data de criação:** 20/03/2022

**Titular(es):** ARTUR LUIZ DA COSTA DA SILVA; ADONNEY ALLAN DE OLIVEIRA VERAS; RAFAEL AZEVEDO BARAUNA

**Autor(es):** ARTUR LUIZ DA COSTA DA SILVA; ADONNEY ALLAN DE OLIVEIRA VERAS; GISLENNE DA SILVA MOIA; SÁVIO DE SOUZA COSTA; RAFAEL AZEVEDO BARAUNA

**Linguagem:** JAVA

**Campo de aplicação:** BL-02

**Tipo de programa:** IA-02

**Algoritmo hash:** SHA-256

**Resumo digital hash:** bdb00f247bbc674f14ec6bb336fb4a2aa48b3cad8ee1477e06abf70abd369a1b

**Expedido em:** 21/03/2023

**Aprovado por:**
Carlos Alexandre Fernandes Silva
Chefe da DIPTO

**Dados Internacionais de Catalogação na Publicação (CIP) de acordo com ISBD**
**Sistema de Bibliotecas da Universidade Federal do Pará**
**Gerada automaticamente pelo módulo Ficat, mediante os dados fornecidos pelo(a) autor(a)**

---

C837a Costa, Sávio.
ANÁLISE DA COMUNIDADE MICROBIANA INTESTINAL DE OSTRAS E DA ÁGUA PROVENIENTES DE RESERVAS EXTRATIVISTAS MARINHAS DO ESTADO DO PARÁ / Sávio Costa. — 2024.
83 f. : il. color.

Orientador(a): Prof. Dr. Rafael Baraúna
Tese (Doutorado) - Universidade Federal do Pará, Instituto de Ciências Biológicas, Programa de Pós-Graduação em Genética e Biologia Molecular, Belém, 2024.

1. Microbioma. 2. Ostras. 3. Nanopore. I. Título.

CDD 576

---