UNIVERSIDAD JUÁREZ AUTÓNOMA DE TABASCO

DIVISIÓN ACADÉMICA DE CIENCIAS BIOLÓGICAS

MICROBIOTA Y FISIOLOGÍA DIGESTIVA DE LARVAS Y JUVENILES DE PEJELAGARTO (*Atractosteus tropicus*) DURANTE LA ADMINISTRACIÓN DE PROBIÓTICOS

TESIS PARA OBTENER EL TITULO DE:

DOCTORA EN CIENCIAS EN ECOLOGIA

Y MANEJO DE SISTEMAS TROPICALES

PRESENTA:

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EN CODIRECCIÓN DE:

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RESUMEN

El uso de probióticos en la acuicultura representa una herramienta eficaz, ya que estos contribuyen a mejorar el crecimiento, supervivencia y la salud de los organismos mediante la estimulación de la actividad digestiva, respuesta inmunológica y la modulación de la microbiota intestinal, lo que deriva en un mayor rendimiento del cultivo. En este contexto, el pejelagarto (*Atractosteus tropicus*), una especie de alta relevancia económica en el sureste de México, se presenta como modelo para evaluar el potencial de los probióticos en su cultivo. Aunque, se han logrado avances significativos en la acuicultura de esta especie a través del uso de aditivos alimenticios como los prebióticos, aún es escasa la evidencia sobre los beneficios que los probióticos podrían aportar para mejorar el bienestar y optimizar la rentabilidad del cultivo de esta especie.

En la presente investigación se evaluó el efecto de la administración de alimento enriquecido con la bacteria autóctona *Lactococcus lactis* (10^4, 10^6, y 10^8 UFC/g), así como con la levadura autóctona *Candida tropicalis* (10^4, 10^5, y 10^6 UFC/g) y la levadura probiótica *Debaryomyces hansenii* (10^3, 10^5, y 10^7 UFC/g), sobre los indicadores de productividad acuícola, la actividad enzimática digestiva, morfología digestiva, expresión de genes asociados a la función de barrera intestinal y el sistema inmunológico, y composición de la microbiota intestinal de larvas y juveniles de pejelagarto.

Los resultados en larvas, mostraron que las concentraciones más altas de *L. lactis* (10^6 y 10^8 UFC/g) influyeron positivamente en el crecimiento y en la morfología digestiva, ya que promovieron un aumento en el área de los hepatocitos y en la altura de los enterocitos. Específicamente, la inclusión de 10^6 UFC/g mejoró la supervivencia (46%) y la actividad de las enzimas digestivas, y la inclusión de 10^8 UFC/g promovió un incremento en la expresión de los genes *muc-2* e *il-10*, lo que sugiere un refuerzo en la función de barrera intestinal y en la respuesta antiinflamatoria. Aunque *L. lactis* no modificó de manera significativa la diversidad de la microbiota intestinal, se observó un aumento en la abundancia de *Lactobacillus* en las larvas alimentados con 10^6 UFC/g.

Además, las levaduras como la bacteria *L. lactis* exhibieron actividad antagónica *in vitro* contra patógenos de peces. En juveniles, la inclusión de las dosis más bajas administradas de *C. tropicalis*

(10^4 UFC/g) y de *D. hansenii* (10^3 UFC/g) promovieron el crecimiento, la supervivencia y la actividad enzimática digestiva. Sin embargo, *C. tropicalis* afectó negativamente la expresión de genes asociados a la integridad de la barrera intestinal, al reducir la expresión del gen *zo-1* y aumentar la expresión del gen *il-8*; también, alteró la composición de la microbiota intestinal favoreciendo a la familia Desulfovibrionaceae. Por el contrario, *D. hansenii* favoreció el incremento en la expresión de los genes asociados a la integridad de la barrera intestinal de *muc-2*, *zo-1* e *il-10*, al mismo tiempo que disminuyó la expresión del gen *il-8*, sin embargo, *D. hansenii* no indujo cambios en la microbiota.

En conclusión, los resultados de este estudio destacan que la inclusión de *C. tropicalis,* en la dieta de *A. tropicus* no aporta beneficios en su fisiología intestinal. En contraste, se recomienda la inclusión de 10^3 UFC/g de *D. hansenii* en la dieta de juveniles y 10^6 UFC/g de *L. lactis* en larvas.

Estos hallazgos establecen a la bacteria autóctona, *L. lactis*, como un candidato probiótico prometedor para su uso en la acuicultura larvaria de esta especie; y refuerzan la importancia del empleo de estrategias nutricionales basadas en probióticos y cepas autóctonas con potencial probiótico para su contribución a una acuicultura más sostenible del pejelagarto.

Palabras claves*:* pejelagarto; probióticos; *Lactococcus lactis; Debaryomyces hansenii;* microbiota intestinal

ABSTRACT

The use of probiotics in aquaculture represents an effective tool, as it contributes to improving the growth, survival, and health of organisms by stimulating digestive activity, immune response, and modulating the intestinal microbiota, resulting in increased culture yield. In this context, the alligator gar (*Atractosteus tropicus*), a species of high economic importance in southeastern Mexico, is presented as a model to evaluate the potential of probiotics in its culture. Although significant advances have been made in the aquaculture of this species through the use of feed additives such as Prebiotics, evidence on the benefits that probiotics could provide to improve welfare and optimize the profitability of farming this species is still scarce.

In this study, the effect of feeding feed enriched with the native bacterium *Lactococcus lactis* (10^4, 10^6, 10^8 CFU/g), as well as the native yeast *Candida tropicalis* (10^4, 10^5 y 10^6 CFU/g), and the probiotic yeast *Debaryomyces hansenii* (10^3, 10^5, y 10^7 CFU/g), on aquaculture productivity indicators, digestive enzyme activity, digestive morphology, expression of genes associated with intestinal barrier function and the immune system, and gut microbiota composition of alligator gar larvae and juveniles was evaluated.

The results showed in larvae, higher concentrations of *L. lactis* (10^6 and 10^8 CFU/g) positively influenced growth and digestive morphology, as they increased hepatocyte area and enterocyte height. Specifically, the inclusion of 10^6 CFU/g improved survival (46%) and digestive enzyme activity, and the inclusion of 10^8 CFU/g promoted an increase in the expression of *muc-2* and *il-10* genes, suggesting a reinforcement of intestinal barrier function and the anti-inflammatory response. Although *L. lactis* did not significantly modify the diversity of the intestinal microbiota, an increment in the abundance of *Lactobacillus* was observed in larvae fed 10^6 CFU/g.

Furthermore, the results showed the both the yeasts and the bacteria *L. lactis* exhibited antagonistic activity in vitro against fish pathogens. In juveniles, including of the lowest administered doses of *C. tropicalis* (10^4 CFU/g) and *D. hansenii* (10^3 CFU/g) promoted growth, survival, and digestive enzyme activity. However, *C. tropicalis* negatively affected the expression of genes associated with intestinal barrier integrity by reducing the expression of the *zo-1* gene and increasing the expression of the *il-8* gene. It also altered the intestinal microbiota composition, favoring the

Desulfovibrionaceae family. In contrast, *D. hansenii* favored an increase in the expression of the genes associated with intestinal barrier integrity: *muc-2*, *zo-1*, and *il-10*, while decreasing the expression of the *il-8* gene. However, *D. hansenii* did not induce changes in the microbiota.

In conclusion, the results of this study highlight that the inclusion of *C. tropicalis* in the diet of *A. tropicus* does not benefit its intestinal physiology. In contrast, including 10^3 CFU/g of *D. hansenii* in the diet of juveniles and 10^6 CFU/g of *L. lactis* in larvae is recommended.

These findings proposed the native bacterium *L. lactis* as a promising probiotic candidate for use in larval aquaculture of this species. Moreover, they reinforce the relevance of implementing nutritional strategies based on native strains with probiotic potential, thereby contributing to a more sustainable aquaculture of alligator gar.

Keywords: tropical gar; probiotics; *Lactococcus lactis; Debaryomyces hansenii*; gut microbiota

Capítulo I

PROTOCOLO DE TESIS

MICROBIOTA Y FISIOLOGIA DIGESTIVA DE LARVAS Y JUVENILES DE PEJELAGARTO (*Atractosteus tropicus*) DURANTE LA ADMINISTRACIÓN DE PROBIÓTICOS

1. INTRODUCCIÓN

La acuicultura es una actividad sostenible dirigida a la producción de alimentos de origen acuático, que ha experimentado un crecimiento exponencial para satisfacer la creciente demanda de la población mundial. Sin embargo, su intensificación se enfrenta a diversos desafíos, entre ellos, la aparición de enfermedades en los organismos cultivados, lo que puede provocar altas tasas de mortalidad y pérdidas económicas significativas para la industria (Carrias et al., 2012). Para mitigar estos efectos, se ha recurrido al uso de antibióticos, no obstante, su uso indiscriminado favorece la aparición y propagación de cepas bacterianas resistentes y, como consecuencia, altera el crecimiento, la fisiología digestiva, así como la composición y función de la microbiota intestinal de los organismos de cultivo (Wichmann et al., 2014; Meek et al., 2015). Ante esta problemática, la industria acuícola ha optado por uso de los probióticos como estrategia para minimizar el impacto económico asociado a las enfermedades y a la mortalidad (Martínez-Porchas & Martínez-Córdova, 2012). De acuerdo con la FAO/WHO (2001), los probióticos son microorganismos vivos que, cuando se administran en cantidades adecuadas, confieren un beneficio para la salud del hospedero. En la acuicultura, estos microorganismos son considerados aditivos alimenticios funcionales debido a los múltiples efectos positivos que ejercen en la salud y bienestar de los peces, así como en el medio ambiente (Ibrahem, 2015). Si bien los efectos de los probióticos son específicos de cada cepa y dependen de la duración, vía y modo de administración, se ha demostrado que pueden favorecer la digestión, la absorción de nutrientes, la respuesta inmunológica y el equilibrio de la microbiota intestinal (Nayak, 2010; Pérez-Sánchez et al., 2013).

La microbiota intestinal en los peces representa un ecosistema dinámico y complejo, influenciado por diversos factores ambientales, fisiológicos y dietéticos. En general, los filos bacterianos Actinobacteria, Bacteroidetes, Fusobacteria, Firmicutes y Proteobacteria predominan en la microbiota intestinal de peces marinos y dulceacuícolas (Ringo et al., 2006; Desai et al., 2012; Liu et al., 2016; Yukgehnaish et al., 2020). Estudios previos han demostrado, que las alteraciones en esta comunidad bacteriana pueden conducir a diversas patologías, como trastornos digestivos, intestinales e inmunológicos. En contraste, la comunidad microbiana intestinal equilibrada se asocia a un estado de salud y bienestar de los peces en cultivo, lo que resalta la importancia del uso de probióticos en la acuicultura.

Entre los probióticos bacterianos con mayor potencial destacan las bacterias ácido lácticas, debido a su presencia natural en el tracto gastrointestinal de diversas especies de peces, así como, por sus múltiples propiedades beneficiosas. Esta incluye la producción de compuestos antimicrobianos, la mejora en la digestibilidad, y absorción de nutrientes (Bolotin et al., 2001; Ray et al., 2012), y la modulación de la respuesta inmunológica (Lee et al., 2021). En cuanto a los probióticos fúngicos, las levaduras han cobrado relevancia por sus efectos positivos en la salud intestinal y por sus rendimientos productivos. En particular, *Debaryomyces hansenii* destaca como probiótico, debido a que mejora la digestibilidad (Angulo et al., 2020), el crecimiento y, la supervivencia de especies como la trucha arcoíris (*Oncorhynchus mykiss*), el rodaballo (*Scophthalmus maximus*), la lubina europea (*Dicentrarchus labrax*) y la dorada (*Sparus aurata*), además modula la respuesta inmunológica y la microbiota intestinal (Tovar-Ramírez et al., 2004; Angulo et al., 2020; Teles et al., 2022; Sanahuja et al., 2023). Adicionalmente, se han aislado de peces sanos otras levaduras productoras de metabolitos secundarios con actividad antimicrobiana, como *Candida tropicalis* (Siangpro et al., 2023), sin embargo, su efecto *in vivo* no se ha estudiado.

En este contexto, el cultivo del pejelagarto (*Atractosteus tropicus*) en el sureste de México representa una alternativa para el desarrollo económico regional debido a su creciente demanda alimenticia, por su alto valor ecológico y cultural (Bussing, 1998; Miller et al., 2009; Márquez-Couturier et al., 2015; Márquez-Couturier & Vázquez-Navarrete, 2015). Diversos estudios han abordado aspectos como su desarrollo ontogénico (Frías-Quintana et al., 2010; Frías-Quintana et

al., 2016), fisiología (Huerta-Ortiz et al., 2018) y nutrición (López et al., 2005; Márquez-Couturier et al., 2006; Álvarez-González et al., 2007; Huerta-Ortiz et al., 2009; Palma-Cancino et al., 2019), con el objetivo de optimizar su cultivo mediante la aplicación de aditivos alimentarios. Sin embargo, aún es escasa la evidencia científica sobre el efecto de la inclusión de probióticos en la dieta, particularmente en las primeras etapas del desarrollo, las cuales se consideradas críticas debido a las altas tasas de canibalismo y agresividad, lo que afecta la supervivencia (Frías-Quintana et al., 2017; Sepúlveda-Quiroz et al., 2024).

Por ello, el presente estudio tuvo como objetivo evaluar el efecto de la inclusión de la bacteria *Lactococcus lactis* en la dieta de larvas de *A. tropicus*, así como la inclusión de las levaduras *Candida tropicalis* y *Debaryomyces hansenii* en la dieta de juveniles, sobre el crecimiento, los parámetros productivos, actividad enzimática digestiva, morfología digestiva, expresión de genes relacionados con la función de barrera intestinal y el sistema inmunológico, así como la composición de la microbiota intestinal.

2. MARCO TEORICO

2.1 Uso de probióticos en acuacultura

En los últimos años, en la acuicultura se incluye a los probióticos como suplementos o aditivos alimenticios en dietas experimentales debido al gran potencial que ejercen sobre la salud y bienestar del huésped. La Organización de las Naciones Unidas para la Agricultura y la Alimentación (FAO), así como la Organización Mundial de la Salud (OMS), en el 2001 definieron a los probióticos como *"microorganismos vivos que cuando se administran en cantidades adecuadas confieren beneficios para la salud del huésped"*. Los probióticos inducen cambios significativos en la fisiología del huésped, estimulan la actividad enzimática digestiva y mejoran la digestión de los alimentos, lo que contribuye a optimizar la absorción de nutrientes y maximiza la energía metabólica (Pérez-Sánchez et al., 2013). Adicionalmente, estos microorganismos tienen la capacidad de desplazar a organismos patógenos, evitan su proliferación, refuerzan el sistema inmunológico, y mejoran la función intestinal del huésped mediante la modulación de la microbiota intestinal (Ibrahem, 2015).

Los probióticos más utilizados son las bacterias ácido lácticas. Estudios previos han demostrado que *Lactococcus lactis* favorece la ganancia de peso, la tasa de supervivencia y modula la microbiota intestinal en el pez besugo (*Pagrus major*) (Dawood et al., 2016). Asimismo, *L. lactis* mejora el crecimiento en *Seriola dumerili* (Linh et al., 2018).

Sin embargo, también se ha reportado que las levaduras poseen un gran potencial como probiótico. Una de sus principales ventajas es la síntesis de poliaminas, las cuales tienen un papel crucial en los enterocitos (Tovar-Ramírez et al., 2004). Además, las levaduras probióticas pueden mejorar la nutrición del huésped mediante la contribución de la actividad enzimática. Este mecanismo mejora la digestibilidad, absorción y asimilación de los nutrientes aportados en los alimentos (Gómez & Balcazar, 2008; Pérez-Sánchez et al., 2013; Raghuwanshi et al., 2018).

Una de las levaduras probióticas más estudiadas en la acuicultura es *Saccharomyces cerevisiae* debido a sus múltiples beneficios asociados a la salud de organismos como *Labeo rohita* en donde promueve el aumento de peso y mejora la tasa de crecimiento especifica (Jahan et al., 2021). En

juveniles del esturión beluga (*Huso huso*), esta levadura modificó la composición de la microbiota intestinal, incrementó la población de bacterias lácticas, mejoró la ganancia de peso, la tasa de crecimiento especifico y la conversión alimenticia (Hoseinifar et al., 2011).

Adicionalmente, *Debaryomyces hansenii* se destaca como una levadura emergente y con gran potencial para su aplicación en la acuicultura, debido a los efectos positivos que ejerce sobre los peces evaluados. En larvas de lobina (*Dicentrarchus labrax*), la suplementación con 1.1% de *D. hansenii* favoreció el crecimiento y supervivencia (Tovar-Ramírez et al., 2004). En *Seriola rivoliana*, mejoró la ganancia de peso, la supervivencia y favoreció en el desarrollo del tracto digestivo y redujo la incidencia de malformaciones esqueléticas, un problema común en las larvas de esta especie (Teles et al., 2022). Además, se ha reportado que promueve efectos inmunoestimulantes a partir de los compuestos funcionales de su pared celular y la producción de poliaminas (Angulo et al., 2020).

Los probióticos utilizados en la acuicultura se aíslan principalmente del medio acuático o de fermentaciones. No obstante, la evidencia científica reciente destaca el interés por evaluar el efecto de cepas probióticas autóctonas, ya que podrían mostrar mayor eficacia y adaptación en el hospedero (Zhang et al., 2024). Tal es el caso de bacterias autóctonas que muestran mayor capacidad de colonización en el tracto digestivo de los mismos peces de las que se aislaron previamente, en comparación con bacterias probióticas exógenas (Balcázar et al., 2007; Nayak, 2010). Además, estos microorganismos previenen la invasión de patógenos, mantienen la homeostasis del sistema intestinal, mejoran la actividad enzimática digestiva, el crecimiento y la tasa de supervivencia (Gómez & Balcazar, 2008). Por ejemplo, *Candida haemuloni* C27 y *Debaryomyces hansenii* C10 y C28, tres cepas de levaduras aisladas del pez cobia (*Rachycentron canadum*) han demostrado propiedades probióticas *in vitro*, incluyendo la capacidad de adherencia intestinal, la producción de biomasa y la síntesis de poliaminas. No obstante, aún no se reporta su eficiencia *in vivo* en la misma especie (Reinoso et al., 2023). Por otro lado, la bacteria endógena *Bacillus subtilis* aislada de la trucha arcoíris (*Oncorhynchus mykiss*), ha demostrado ser eficaz en la ganancia de peso, crecimiento, modulación de la respuesta inmunológica, desarrollo de células intestinales y absorción de nutrientes en juveniles de esta especie (Wang et al., 2024). Sin embargo, a pesar de estos avances, la investigación sobre probióticos endógenos en la acuicultura sigue

siendo limitada y se requieren estudios adicionales que permitan aislar e identificar nuevas cepas potenciales, optimizar su aplicación y desarrollar estrategias que permitan mejorar la salud y el rendimiento de los peces en cultivo.

2.2 Microbiota intestinal en peces

El interés por el estudio de las comunidades microbianas intestinales y su papel en la fisiología de los peces, ha experimentado un crecimiento exponencial en la última década. Este auge se debe a la creciente evidencia científica que demuestra la influencia de los microorganismos que colonizan el tracto gastrointestinal sobre la salud y el desarrollo de su hospedero (Llewellyn al., 2014b; Yukgehnaish et al., 2020; Medina-Félix et al., 2023). La microbiota es una comunidad de microorganismos conformada por arqueas, bacterias, hongos, virus y protozoos que habitan un ambiente en específico, como la piel, el apartado digestivo o genital (Grice & Segre, 2011; Egerton et al., 2018). Particularmente, la microbiota intestinal es una comunidad diversa y dinámica que establece una relación simbiótica con el hospedero, y puede clasificarse como microbiota transitoria o residente dependiendo de su estabilidad y permanencia en el tracto digestivo (Kim et al., 2007; Ringø & Birkbeck, 1999). Los microorganismos transitorios ingresan al tracto gastrointestinal principalmente por la ingesta de alimentos y se alojan en el intestino por un tiempo limitado, sin embargo, pueden influir en procesos fisiológicos a corto plazo. En cambio, los microorganismos residentes se adhieren y colonizan el intestino, en donde establecen una relación simbiótica con el hospedero. Mediante esta relación simbiótica se ejercen diversos efectos en la fisiología y salud del huésped (Zhang et al., 2016).

En los peces, la composición y función de la microbiota intestinal es influenciada por una amplia gama de factores ambientales, fisiológicos y genéticos (Gajardo et al., 2016). La diversidad microbiana depende particularmente de una interacción compleja de variables intrínsecas y extrínsecas. Mientras que, en los factores extrínsecos se incluyen variables abióticas como el pH y la temperatura del medio ambiente, factores bióticos como la disponibilidad de nutrientes y la estacionalidad de cada especie (Sullam et al., 2012; Llewellyn et al., 2014; Xia et al., 2014; Ye et al., 2014; Zhang et al., 2016). El tipo de dieta, carnívora, omnívora o herbívora, es un factor intrínseco y es uno de los factores cruciales que influye directamente en la composición de la microbiota intestinal. A su vez, las comunidades microbianas desempeñan un papel crucial en la

nutrición del pez, a través de la secreción de enzimas digestivas extracelulares como las proteasas, amilasas, celulasas, fitasas y lipasas, que proveen al hospedero la capacidad para degradar una variedad de sustratos, facilitando la digestión y absorción de nutrientes (Ray et al., 2012). Así mismo, participan en la síntesis de vitaminas y ácidos grasos poliinsaturados que proporcionan efectos positivos en la fisiología digestiva, refuerzan el sistema inmunológico y contribuyen a mejorar la salud general del hospedero (Nayak, 2010; Whiteside, 2015; Yukgehnaish et al., 2020; Medina-Félix et al., 2022).

La microbiota intestinal de los peces está compuesta principalmente por filos Actinobacteria, Bacteroidetes, Fusobacteria, Firmicutes y Proteobacteria. Estos grupos bacterianos representan hasta el 90% de la comunidad microbiana en especies tanto de agua dulce como marina (Larsen et al., 2014; Eichmiller et al., 2016; Hennersdorf et al., 2016; Liu et al., 2016). Las Actinobacterias son conocidas por su capacidad de producir metabolitos secundarios con propiedades antimicrobianas que contribuyen a la defensa del huésped (Wu et al., 2012). Las bacterias del filo Proteobacteria, cumplen funciones esenciales en la digestión de los peces (Nayak, 2010). Por otra parte, la disminución de bacterias del grupo Bacteroidetes se ha asociado a desequilibrios metabólicos e intestinales (Meng et al., 2018). Sin embargo, la diversidad y abundancia de la microbiota en organismos de cultivo difiere de la de los peces silvestres, ya que estos últimos suelen albergar comunidades más diversas debido a la amplia variedad de recursos alimenticios disponibles en su medio natural (Sun et al., 2021).

La microbiota intestinal del salmón del Atlántico (*Salmo salar*) está compuesta principalmente por Proteobacteria, Firmicutes, Fusobacteria y Actinobacteria (Gajardo et al., 2016). No obstante, se ha reportado que el proceso de esmoltificación ejerce un efecto enérgico en la composición de la microbiota intestinal lo que genera ciertos cambios en la abundancia de los microorganismos presentes (Wang et al., 2021). En el caso de la microbiota intestinal de la carpa herbívora (*Ctenopharyngodon idellus*), se muestra una notable diversidad bacteriana, donde destacan los filos Proteobacteria, Firmicutes, Fusobacteria, Bacteroides y Chloroflexi (Wu et al., 2012). Particularmente, Firmicutes y Bacteroides son más abundantes debido a que desempeñan un papel crucial en la digestión de la celulosa, un componente principal de la dieta de esta especie (Yu & Morrison, 2004; Wu et al., 2012).

La composición de la microbiota intestinal de la trucha arcoíris (*Oncorhynchus mykiss*), es significativamente afectada debido a los cambios fisiológicos que el pez enfrenta a lo largo de su ciclo de vida, influenciados por las etapas de desarrollo, las migraciones y la disponibilidad de alimento, que a su vez, inciden en el sistema inmunólogo y en su fisiología (Ingerslev et al., 2014). Sin embargo, se ha logrado determinar que la microbiota central de esta especie, está dominada por los filos Bacteroidetes, Proteobacteria, Firmicutes y Actinobacteria (Desai et al., 2012; Navarrete et al., 2012).

Estos estudios evidencian que la microbiota intestinal no es estática, y que cada organismo presenta una microbiota única. En este contexto, la acuicultura busca optimizar la producción sostenible de alimentos acuáticos mediante el uso de probióticos, los cuales pueden favorecer la salud y bienestar de los organismos acuáticos al inducir cambios beneficiosos en su microbiota intestinal.

La suplementación con *Lactobacillus rhamnosus* en larvas del pez cebra (*Danio rerio*) generó cambios significativos en la composición de su microbiota intestinal, en donde se observó un incremento en la abundancia de los filos Firmicutes (62.6%) y Proteobacterias (34.8%). Estos cambios en la microbiota, regularon positivamente la expresión de genes relacionados al apetito (*cb1*: receptor cannabinoide 1; *npy:* neuropéptido *y*), y promovieron cambios morfológicos significativos a nivel intestinal, lo que, se tradujo en mayor capacidad de absorción y digestión de nutrientes (Falcinelli et al., 2015).

En relación con la microbiota del pejelagarto (*A. tropicus*), Méndez-Pérez et al. (2020) reportaron la composición de la microbiota intestinal en juveniles y adultos. Este estudio reveló diferencias en la composición bacteriana entre sexos y entre individuos de origen silvestre y de cautiverio, proporcionando una línea base para comprender la dinámica de la microbiota intestinal en esta especie. Se encontró que en juveniles de cultivo predominan los filos Firmicutes, Proteobacteria y Bacteroidetes; en hembras silvestres, Proteobacteria y Firmicutes, mientras que, en hembras de cautiverio y machos adultos, *Fusobacteria*. A nivel género, *Cetobacterium* fue el más abundante en todos los grupos, seguido de *Serriata*, *Edwarsiella* y *Paludibacter*. Aunque se observaron diferencias en la composición de filos y géneros, la diversidad y riqueza de la microbiota no presentaron variaciones significativas, lo que sugiere una distribución homogénea de la diversidad microbiana intestinal.

2.3. Métodos de detección para la microbiota intestinal

La caracterización de comunidades microbianas complejas, como la microbiota intestinal es posible gracias al uso de herramientas de vanguardia como la metagenómica. Esta poderosa herramienta permite investigar el contenido genético de todos los organismos en una muestra (Yukgehnaish et al., 2020). Al utilizar la secuenciación masiva, es posible secuenciar el genoma completo de los diferentes microorganismos que componen una comunidad a partir de la extracción de su ADN total, sin necesidad de cultivar a las especies que conforman dicha comunidad (Wooley et al., 2010; Nielsen et al., 2014; Whiteside et al., 2015). Aunque la microbiota de los organismos de acuicultura está lejos de ser completamente descrita, el uso de herramientas metagenómicas ha permitido avances significativos en la comprensión de su estructura y dinámica (Martínez-Porchas & Vargas-Albores, 2015). En las últimas décadas, el desarrollo de la metagenómica y la secuenciación de alto rendimiento junto con herramientas bioinformáticas, han revolucionado el estudio de la microbiota intestinal, permitiendo identificar su composición, diversidad y abundancia con mayor precisión (Whiteside et al., 2015).

Por otra parte, la secuenciación de amplicones se basa en la amplificación de regiones específicas del ADN o genes de interés mediante una PCR con cebadores dirigidos a dichos genes. Estos fragmentos, denominados amplicones, se secuencian en una *flowcell* y, posteriormente, se comparan con una base de datos mediante herramientas bioinformáticas para su identificación y clasificación taxonómica (Matthjis et al., 2016).

Para este propósito, los genes mayormente analizados son el gen 16S rRNA de procariotas (bacterias y arqueas), y el gen 18S rRNA de eucariotas (hongos y levaduras) (Janda & Abbott, 2007; Not et al., 2009; Salipante et al., 2013). La secuenciación del gen 16S rRNA ha permitido identificar microorganismos previamente desconocidos en diversos ambientes (Lozupone & Knight, 2007). En particular, las regiones hipervariables V3 y V4 son las más utilizadas para la caracterización de la microbiota bacteriana en los peces (Lane et al., 1985; Frank et al., 2008; Wu et al., 2010). La secuenciación de amplicones, es una técnica más rápida, menos exigente en el procesamiento y económicamente menos costosa que la secuenciación metagenómica de próxima generación. Además, es altamente sensible para detectar poblaciones microbianas de baja abundancia y es ideal para estudiar cambios en comunidades bacterianas o arquéales, a bajo

distintas condiciones experimentales a lo largo del tiempo. No obstante, su cobertura funcional es menor, en comparación con la metagenómica, lo que limita la posibilidad de inferir perfiles fisiológicos, bioquímicos o metabólicos para comprender las interacciones entre el huésped-hospedero.

2.4 *Atractosteus tropicus* Gill, 1863

El pejelagarto (*Atractosteus tropicus*) es una especie dulceacuícola que se distribuye en el Sureste de México, que habita en ríos, lagunas y humedales del estado de Tabasco, Campeche y Veracruz hasta América Central y Costa Rica (Bussing, 1998; Miller et al., 2009). Forma parte de un grupo de peces primitivos que pertenece a la familia Lepisosteidae, la cual se compone de dos géneros: *Lepisosteus* y *Atractosteus*. Este último comprende únicamente de tres especies: *Atractosteus spatula, A. tropicus* y *A. tristoechus,* donde en México, se encuentran únicamente las especies, *A. spatula y A. tropicus* (Bussing, 1998).

Es considerado un "fósil viviente" debido a la conservación de sus características morfológicas y fisiológicas primitivas que datan del Mesozoico, en el periodo Cretácico (Wiley, 1976). Morfológicamente es alargado y cilíndrico, y posee un hocico alargado con dientes caninos. Es completamente carnívoro, y tiene la capacidad de tomar oxígeno atmosférico (Márquez-Couturier et al., 2015). Su posición filogenética lo convierte en una especie clave para el estudio de procesos ecológicos y evolutivos, por tales razones, el pejelagarto es considerado un organismo modelo de gran interés para la investigación científica (Márquez-Couturier et al., 2015; Burggren et al., 2016; Martínez et al., 2021). *A. tropicus* es crucial para los ecosistemas acuáticos de la región, desempeña un papel ecológico fundamental. Actúa regulando eficazmente las poblaciones de peces y anfibios, manteniendo el equilibrio de las poblaciones (Alfaro et al., 2008). Además de su función ecológica, la especie posee una importancia económica significativa debido a su alta demanda para consumo y la elaboración de artesanías (Bussing, 1998; Aguilera et al., 2002; Márquez-Couturier & Vázquez-Navarrete, 2015; Márquez-Couturier et al., 2015). Sin embargo, la degradación de su hábitat y la pesca incontrolada han llevado a la disminución de sus poblaciones silvestres (Aguilera et al., 2002; Márquez-Couturier et al., 2015). Por ello, su cultivo en sistemas de acuicultura sustentable se presenta como una alternativa viable para su conservación y producción. Sus características biológicas, como su rápido crecimiento, la capacidad para respirar aire atmosférico

y su adaptabilidad a dietas formuladas, lo convierte en un candidato idóneo para la acuicultura sustentable en la región (Aguilera et al., 2002; Márquez-Couturier et al., 2015; Burggren et al., 2016; Martínez et al., 2021; Córdova et al., 2022).

En los últimos años se ha incrementado el número de las investigaciones científicas dirigidas a optimizar su cultivo desde la etapa larval hasta la etapa adulta. Estos estudios abarcan la alimentación, desarrollo y requerimientos nutricionales (López et al., 2005; Márquez-Couturier et al., 2006; Álvarez-González et al., 2007; Huerta-Ortiz et al., 2009; Huerta-Ortiz et al., 2018; Sáenz de Rodrigáñez et al., 2018; Palma-Cancino et al., 2019; Maldonado et al., 2020), así como, la formulación de dietas microparticuladas para evaluar el efecto en el desarrollo del tracto digestivo y la actividad de enzimas digestivas durante su ontogenia inicial (Márquez-Couturier et al., 2006; Frías-Quintana et al., 2010; Frías-Quintana et al., 2016; Frías-Quintana et al., 2017; Huerta-Ortiz et al., 2018). Además, la caracterización de marcadores nutrigenómicas por secuenciación transcriptómica durante el desarrollo larvario de *A. tropicus* (Martínez-Burguete et al., 2021). Así mismo, se han incluido diversos prebióticos como aditivos en dietas experimentales. En particular, se ha descrito que, en juveniles de pejelagarto, la suplementación con manano-oligosacáridos (MOS) mejora la ganancia de peso, eficiencia proteica y tasa de conversión alimenticia (Nájera-Arzola et al., 2018). La inclusión de Fructooligosacaridos (FOS) incrementa el área de absorción de nutrientes en el intestino, la actividad enzimática digestiva y la expresión de genes asociados al fortalecimiento de la función de barrera intestinal (Sepúlveda-Quiroz et al., 2020). En larvas la suplementación de inulina favorece la supervivencia y la actividad enzimática digestiva (De la Cruz-Marín et al., 2023). Y la inclusión de FOS y MOS mejoran la supervivencia, la actividad enzimática digestiva, los parámetros de crecimiento y la expresión de genes relacionados al reforzamiento de la función de la barrera intestinal y el sistema inmunológico (Maytorena-Verdugo et al., 2022; Pérez-Jiménez et al., 2022).

Adicionalmente, se evaluó el efecto de la inclusión del probiótico *Debaryomyces hansenii* en dietas experimentales en juveniles de *A. tropicus*. Los resultados de este estudio indicaron que las altas dosis utilizadas de *D. hansenii* (10^{14}, 10^{15} y 10^{16} UFC/g) causaron efectos adversos en los organismos (Hernández-López et al., 2021). Estos hallazgos subrayaron la importancia de determinar la dosis óptima de administración de cada probiótico, así como la especie a la cual se

administra y la etapa de desarrollo, ya que dosis excesivas pueden resultar tóxicas o inducir una inmunosupresión en el organismo.

Con la finalidad de optimizar la eficiencia de las dietas experimentales para el cultivo del pejelagarto y de mejorar el bienestar a los organismos en cultivo, además de abordar la escasez de información sobre el uso de probióticos y de cepas autóctonas con potencial probiótico en *A. tropicus*. Este estudio tuvo como objetivo evaluar el efecto de la administración de las cepas autóctonas *Candida tropicalis* y *Lactococcus lactis*, así como de la cepa probiótica *Debaryomyces hansenii*, sobre crecimiento, supervivencia, actividad enzimática digestiva, morfología digestiva, expresión de genes asociados a la función de barrera intestinal y el sistema inmunológico, y composición de la microbiota intestinal en larvas y juveniles de pejelagarto (*Atractosteus tropicus*).

3. JUSTIFICACIÓN

La acuicultura es una actividad clave para la producción alimentaria a nivel mundial. Sin embargo, el crecimiento intensivo de esta práctica ha generado desafíos significativos, como el aumento de enfermedades y el uso excesivo de antimicrobianos, lo que plantea riesgos para la sostenibilidad ambiental y la seguridad alimentaria. Ante este panorama, la industria acuícola ha adoptado el uso de los probióticos como una estrategia para mejorar las practicas acuícolas, al promover la salud de los organismos, reducir la dependencia de antibióticos, y mejorar la productividad y rentabilidad. En particular, los probióticos autóctonos han demostrado ventajas destacables debido a su mejor adaptación al hospedero y al entorno local, lo que maximiza su eficacia.

En este contexto, el pejelagarto (*A. tropicus*), un pez importante y emblemático del sureste de México, posee un alto valor ecológico, cultural, social y económico en la región. Aunque el cultivo de esta especie ha experimentado un notable desarrollo en la última década, aún enfrenta limitaciones relacionadas con la optimización de prácticas sostenibles. En ese sentido, el uso de probióticos, particularmente autóctonos, presenta un gran potencial para el desarrollo e investigación.

Por consiguiente, en este estudio se evaluó el impacto de la administración de las cepas autóctonas *Lactococcus lactis y Candida tropicalis*, así como de la cepa probiótica *Debaryomyces hansenii* en larvas y juveniles de pejelagarto (*Atractosteus tropicus*), considerando su efecto sobre el crecimiento, fisiología y morfología digestiva, y la composición de la microbiota intestinal.

Los resultados de este estudio no solo contribuyen a cerrar brechas en el conocimiento sobre el uso de probióticos en la etapa larval y juvenil de *A. tropicus*, sino que también podrían fortalecer la acuicultura de la especie e incluso la de otras especies acuáticas de la región, ampliando el impacto positivo de esta tecnología en la industria acuícola.

4. PREGUNTAS DE INVESTIGACIÓN

I. ¿Cuál es el efecto de la inclusión de la bacteria autóctona *Lactococcus lactis* sobre la supervivencia, el crecimiento, la actividad de enzimas digestivas, la morfología digestiva, y la expresión de genes asociados a la función de barrera intestinal y al sistema inmunológico en larvas de *A. tropicus*?

II. ¿Cuál es el efecto de la inclusión de las levaduras *Candida tropicalis* y *Debaryomyces hansenii* sobre la supervivencia, el crecimiento, la actividad de enzimas digestivas, la morfología digestiva y la expresión de genes asociados a la función de barrera intestinal y al sistema inmunológico en juveniles de *A. tropicus*?

III. ¿Cómo está constituida la microbiota intestinal de larvas y juveniles de pejelagarto criados en condiciones de cautiverio?

IV. ¿Cómo influye la suplementación de *L. lactis*, *C. tropicalis*, y *D. hansenii* en dietas experimentales en la composición de la microbiota intestinal de pejelagarto a lo largo de su etapa larval y juvenil?

5. HIPOTESIS

La adición individual de *Lactococcus lactis, Candida tropicalis y Debaryomyces hansenii*, en la dieta de larvas y juveniles de pejelagarto (*Atractosteus tropicus*) promoverá un incremento en el crecimiento y la supervivencia. Esta estrategia también mejorará la actividad enzimática digestiva, fortalecerá el sistema inmunológico y favorecerá en una microbiota intestinal beneficiosa.

6. OBJETIVOS

6.1 Objetivo general

Determinar los efectos de la administración de las cepas *Lactococcus lactis*, *Candida tropicalis* y *Debaryomyces hansenii* sobre crecimiento, supervivencia, actividad enzimática digestiva, morfología digestiva, expresión de genes asociados a la función de barrera intestinal y el sistema inmunológico, y composición de la microbiota intestinal en larvas y juveniles de pejelagarto (*Atractosteus tropicus*).

6.2 Objetivos específicos

1. Evaluar el efecto antagónico *in vitro* de *L. lactis*, *C. tropicalis* y *D. hansenii*, contra bacterias patógenas de peces.
2. Evaluar el efecto de la inclusión en la dieta de la bacteria autóctona *L. lactis* sobre el crecimiento, indicadores de productividad acuícola, supervivencia, actividad enzimática digestiva, morfología digestiva y expresión de genes asociados a la función de barrera intestinal y el sistema inmunológico en larvas de *A. tropicus.*
3. Determinar el efecto de la inclusión en la dieta de las levaduras *C. tropicalis* y *D. hansenii* sobre el crecimiento, indicadores de productividad acuícola, supervivencia, actividad enzimática digestiva, morfología digestiva y expresión de genes asociados a la función de barrera intestinal y el sistema inmunológico en juveniles de *A. tropicus*.
4. Caracterizar la composición de la microbiota bacteriana residente en el intestino de larvas y juveniles de *A. tropicus* mediante análisis metagenómicos.
5. Analizar los cambios en la composición de la microbiota intestinal de larvas y juveniles de *A. tropicus* tras la inclusión en la dieta de *L. lactis*, *C. tropicalis* y *D. hansenii* mediante análisis metagenómicos.
6. Estimar las funciones metabólicas de la microbiota intestinal en larvas de *A. tropicus* alimentadas con la bacteria autóctona *L. lactis* mediante análisis de predicción basado en la composición genética.

7. MATERIALES Y METODOS

7.1 Formulación y elaboración de dietas experimentales

Las dietas experimentales se formularon según el protocolo de Frías-Quintana et al., (2016). Para el estudio en larvas, se formularon tres dietas experimentales con diferentes concentraciones de *Lactococcus lactis* PH3-05 (10^4, 10^6 y 10^8 UFC/g) y una dieta control (Tabla 1), siguiendo el protocolo de Sepúlveda-Quiroz et al., (2020). Para el estudio en juveniles de *A. tropicus*, se formularon tres dietas suplementadas con diferentes concentraciones de *Candida tropicalis* (10^4, 10^5 y 10^6 UFC/g) y tres dietas con diferentes concentraciones de *Debaryomyces hansenii* (10^3,10^5 y 10^7 UFC/g), además de una dieta control sin inclusión de levadura (Tabla 2).

Las dietas se elaboraron siguiendo la metodología de Álvarez-González et al., (2001). Los ingredientes se pesaron en una balanza analítica con capacidad de 2000 g (Ohaus mod. CS2000, China). Los macronutrientes se mezclaron durante 15 min en una batidora industrial (Bathamex, 178716, México); posteriormente, se añadieron los micronutrientes (pre-mezclas de vitaminas, minerales y vitamina C) y, a continuación, se añadieron los ingredientes líquidos (aceite de soya y vitamina E). Finalmente, se agregaron 200 mL de agua por kilogramo de dieta y se incorporaron los probióticos mediante la disolución de la biomasa en 200 mL de agua estéril. La mezcla resultante se colocó en un molino 1HP (Torrey, M-22RI, México) con una criba de 5 mm para la obtención del alimento peletizado. Los pellets se secaron a 40 °C durante 15 h en un horno de convencción (Coriat, HC-35-D, México). Las dietas resultantes se molieron manualmente y se tamizaron en tamaños de partículas específicas para las diferentes etapas de desarrollo de las larvas (500-800 µm) y juveniles (3-5 mm). Posteriormente se almacenaron en bolsas de plástico selladas herméticamente a –20 °C hasta su uso.

Tabla 1. Composición de las dietas experimentales suplementadas con diferentes concentraciones de *L. lactis* y una dieta control para larvas de *A. tropicus*.

Ingredientes	Dieta control	*Lactococcus lactis* (UFC/g)		
		10^4	10^6	10^8
Harina de pescado[a]	350	350	350	350
Harina de cerdo[a]	270.9	270.9	270.9	270.9
Harina de ave[a]	150	150	150	150
Almidón[b]	100	100	100	100
Aceite de pescado[a]	36.5	36.5	36.5	36.5
Lactococcus lactis	0	1	0.01	0.001
Harina de trigo[c]	37.6	36.6	37.59	37.6
Grenetina[d]	20	20	20	20
Premix vitaminas y minerales[e]	15	15	15	15
Lecitina de soya[f]	15	15	15	15
Vitamina C[g]	5	5	5	5
Composición química (g 100 g^{-1} de dieta de materia seca)				
Proteína	50.1	50.2	49.8	49.9
Lípidos	14.2	14.1	13.9	14.2
Fibra	10.1	10.2	9.9	10.0
Análisis proximales				
Cenizas	14.0	13.9	14.1	14.2
Humedad	8.1	8.0	8.4	8.2
ENF	11.6	11.6	12.3	11.7

[a]Marine and agricultural proteins S.A. de C.V., Guadalajara, Jalisco; [b] Pronat Ultra, Mérida, Yucatán, México; [c] Ragasa Industries S.A. de C.V.; [d]Vitamin premix composition g/mg or International Units per kg of diet: Vitamin A, 10,000,000 IU; Vitamin D3, 2,000,000 IU; Vitamin E, 100,000 IU; Vitamin K3, 4.0 g; Thiamine B1, 8.0 g; Riboflavin B2, 8.7 g; Pyridoxine B6, 7.3 g; Vitamin B12, 20.0 mg; Niacin, 50.0 g; Pantothenic acid, 22.2 g; Inositol, 0.15 mg; Nicotinic Acid, 0.16 mg; Folic Acid, 4.0 g; Biotin, 500 mg; Vitamin C, 10.0 g; Choline 0.3 mg, Excipient q.s. 2 g; Manganese, 10 g; Magnesium, 4.5 g; Zinc, 1.6 g; Iron, 0.2 g; Copper, 0.2 g; Iodine, 0.5 g; Selenium, 40 mg; Cobalt 60 mg. Excipient q.s. 1.5 g; [e]ROVIMIX® STAY-C® 35 –DSM, Guadalajara, Mexico; [f]D´gari, food and diet products relámpago, S.A. de C.V. NFE = Nitrogen-free extract:100-(% protein-% etherel extrac-% ash-%fiber).

Tabla 2. Composición de las dietas experimentales suplementadas con *C. tropicalis*, *D. hansenii* y una dieta control para juveniles de *A. tropicus*.

		Candida tropicalis **(UFC/g)**			***Debaryomyces hansenii*** **(UFC/g)**		
Ingredientes (g kg^{-1})	**Dieta control**	**10^4**	**10^5**	**10^6**	**10^3**	**10^5**	**10^7**
Harina de pescado[a]	305.4	305.4	305.4	305.4	305.4	305.4	305.4
Harina de ave	150	150	150	150	150	150	150
Harina de cerdo[a]	150	150	150	150	150	150	150
Harina de soya[a]	150	150	150	150	150	150	150
Aceite de pescado[a]	34.95	34.95	34.95	34.95	34.95	34.95	34.95
Almidón	124.7	124.7	124.62	117.2	124.7	124.55	109.7
Lecitina de soya[b]	10	10	10	10	10	10	10
Aceite de soya[c]	34.95	34.95	34.95	34.95	34.95	34.95	34.95
Candida tropicalis	0	0.00075	0.075	7.5	0	0	0
Debaryomyces hansenii	0	0	0	0	0.0015	0.15	15
Premix de minerales[d]	5	5	5	5	5	5	5
Premix de vitaminas[e]	10	10	10	10	10	10	10
Vitamina C[e]	5	5	5	5	5	5	5
Grenetina[f]	20	20	20	20	20	20	20
	Análisis proximales (g kg^{-1} de materia seca)						
Proteína	45	45	45	45	45	45	45
Extracto de éter (%)	13.82	15.00	15.25	16.17	15.76	16.41	13.08
Cenizas (%)	10.80	11.04	11.10	11.51	11.76	10.68	11.17

[a]Marine and agricultural proteins S.A. de C.V., Guadalajara, Jalisco; [b] Pronat Ultra, Merida, Yucatan, Mexico; [c] GALMEX SA de CV, Villahermosa, Tabasco, México; [d] D'gari, food and diet products relámpago, S.A. de C.V. [e] Vitamin premix composition g/mg or International Units per kg of diet: Vitamin A, 10,000,000 IU; Vitamin D3, 2,000,000 IU; Vitamin E, 100,000 IU; Vitamin K3, 4.0 g; Thiamine B1, 8.0 g; Riboflavin B2, 8.7 g; Pyridoxine B6, 7.3 g; Vitamin B12, 20.0 mg; Niacin, 50.0 g; Pantothenic acid, 22.2 g; Inositol, 0.15 mg; Nicotinic Acid, 0.16 mg; Folic Acid, 4.0 g; Biotin, 500 mg; Vitamin C, 10.0 g; Choline 0.3 mg, Excipient q.s. 2 g; Manganese, 10 g; Magnesium, 4.5 g; Zinc, 1.6 g; Iron, 0.2 g; Copper, 0.2 g; Iodine, 0.5 g; Selenium, 40 mg; Cobalt 60 mg. Excipient q.s. 1.5 g; [f] Pronat Ultra, Yucatán, México; [g] ROVIMIX® STAY-C® 35–DSM, Guadalajara, México. NFE = Nitrogen-free extract: 100 - (% protein - % ether extract - % ash - %fiber).

7.2 Viabilidad de las cepas incorporadas en las dietas experimentales

Se cuantificaron las UFC/g de las cepas incorporadas en cada dieta experimental al inicio y final del experimento, con el fin de confirmar las concentraciones administradas y evaluar su viabilidad durante el ensayo. De cada dieta se obtuvo una muestra representativa utilizando el método de cuarteo (Campos & Campos, 2017). Se pesó 1 g de la dieta y se resuspendió en 9 mL de solución salina (0.85%). Se realizaron diluciones exponenciales de 10^{-1} hasta 10^{-10}, y se inocularon 100 µL de cada dilución en placas de Agar Papa Dextosa (PDA, Difco) adicionadas con antibióticos (Amikacina y Cloranfenicol) para disminuir la carga bacteriana. El inoculo se dispersó de manera homogénea con ayuda de un asa de Drigalsky estéril. Las placas se incubaron 24 h a 32 °C. Finalmente se contaron las colonias presentes en las cajas de las diluciones con 10 a 30 UFC y se calculó el número de UFC/g de alimento con la siguiente formula:

$$\text{UFC/ml o UFC/g} = \frac{No.\ de\ colonias\ por\ placa\ X\ el\ factor\ de\ dilución}{ml\ de\ la\ muestra\ sembrada}$$

Las bacterias viables se cuantifican como unidades formadoras de colonias (UFC), que indican el número total de colonias vivas dentro del volumen sembrado.

7.3 Diseño experimental

Este estudio se dividió en dos bioensayos independientes determinados a evaluar el efecto de la inclusión de las cepas en dos etapas del desarrollo de *A. tropicus*. Los especímenes de *A. tropicus* fueron obtenidos de la granja acuícola "Otot Ibam" del municipio de Comalcalco, Tabasco, México. Los peces fueron transportados en bolsas de plástico aireadas. A su llegada al Laboratorio de Fisiología y Recursos Acuáticos (LAFIRA), las bolsas se mantuvieron a flote y se aclimataron gradualmente a la temperatura de las tinas experimentales en un periodo de 3 horas.

7.3.1 Ensayo en etapa larval de *A. tropicus*

Se utilizaron 1,200 larvas, de 5 días post-eclosión (DAH), con un peso promedio de 0.027 ± 0.00 g y longitud total de 1.81 ± 0.18 cm. Se distribuyeron 100 larvas por tina, en 12 tinas de 70 L

provistas de un sistema de recirculación accionado por una bomba de agua de 0.5 HP y conectado a un reservorio de 1,500 L y un filtro biológico. La calidad del agua se monitoreó dos veces al día para mantener una temperatura promedio de 27.1 ± 0.8 °C, oxígeno disuelto de 5.7 ± 0. 2 mg/L y el pH alrededor de 7.3 ± 7.5, empleando un oxímetro (YSI 85, USA) y un potenciómetro (HANNA HI 991001, Romania).

Las larvas se alimentaron cuatro veces al día (7:00, 11:00, 15:00 y 19:00 h) hasta saciedad aparente. Se implementó un plan de Co-alimentación: desde el día 5DAH hasta el día 10 DAH, se suministró nauplios de *Artemi*a sp. junto con las dietas experimentales y biomasa de *Artemia* sp. previamente mezclada con las dietas experimentales. A partir del día 11 DAH hasta el día 21 DAH, se ofreció únicamente las dietas experimentales hasta el final del experimento. Después de cada alimentación, se realizó la limpieza de cada tina y el recambio de agua parcial (30 %) mediante el método de sifoneo. Todos los tratamientos se evaluaron por triplicado.

7.3.2 Sacrificio y toma de muestras de larvas

Al finalizar la Co-alimentación (día 10 DHA) se midió la talla y peso de todas las larvas en estudio. Para ello se empleó una balanza analítica (Ohaus HH120, con precisión de 120 ± 0.01 g; Shenzhen, China) y la longitud total se determinó a través de fotografías analizadas en el software ImageJ 1.5. Al finalizar el experimento (21 DAH), se recolectaron todas las larvas por tratamiento y se determinó el peso y longitud. Posteriormente, las larvas fueron sacrificadas mediante shock térmico. Se recolectaron tres larvas por tina y se lavaron con agua inyectable y se conservaron en tubos libres de RNAsa para los análisis metagenómicos: tres larvas preservadas en solución RNAlater (Ambion) para los análisis de expresión génica, tres larvas se preservaron en solución Davidson para el análisis histológico y, tres más recolectadas para la cuantificación de la actividad enzimática. Todas las muestras se preservaron a –80 °C hasta su uso.

7.3.3 Ensayo en etapa juvenil de *A. tropicus*

Se utilizaron 378 juveniles de *A. tropicus* de 38 días post-eclosión (DAH) con un peso promedio de 0.22 ± 0.08 g y una longitud total de 3.77 ± 0.29 cm. Los peces se distribuyeron aleatoriamente en 21 tinas (18 peces por tina) con capacidad de 70 L, conectadas a un sistema de recirculación accionado por una bomba de agua de 1HP, conectado a un reservorio de 1,500 L con un filtro biológico y un filtro de arena automatizado, además de una lampara de luz ultravioleta para

minimizar la descarga bacteriana posible. La temperatura promedio de las tinas fue de 27.1 ± 0.8 °C, la concentración de oxígeno disuelto de 5.7 ± 0.2 mg/L y el pH de 7.3 ± 7.5. Estos parámetros fueron monitoreados diariamente con un oxímetro (YSI 85, USA) y un potenciómetro (HANNA HI 991001, Romania).

El bioensayo tuvo una duración de 45 días. Durante los primeros cinco días, los organismos fueron sujetos a una habituación al alimento, para ello, se alimentaron con una mezcla compuesta por las dietas experimentales y alimento comercial. El primer día se administró una dieta compuesta por 80% de alimento comercial y 20% de dieta experimental, consecutivamente cada día se administró una proporción mayor a la dieta experimental de 70/30, 60/40, 50/50 y 40/60, respectivamente. Posteriormente a la habituación, se administró únicamente las dietas experimentales y la dieta control durante los días restantes del experimento. Todos los tratamientos se realizaron por triplicado. Los peces fueron alimentados cuatro veces al día (7:00, 11:00, 15:00 y 19:00 h) hasta saciedad aparente. La limpieza de cada tina y recambio parcial del agua (10%) se realizó después de cada alimentación por el método de sifoneo.

7.3.4 Sacrificio y toma de muestras de juveniles

Se realizaron biometrías cada 15 días para determinar el peso y longitud total de todos los organismos en estudio. Al final del bioensayo todos los peces fueron pesados y medidos, posteriormente fueron anestesiados con aceite de clavo (0.1 mL L^{-1}) y sacrificados mediante un corte cefálico. Se extrajo el hígado, estomago e intestino de todos los peces, mediante una incisión en la zona ventral, para la determinación de índices somáticos.

Se recolectaron los intestinos de tres peces por tina, se lavaron con agua inyectable y se conservaron en tubos libres de RNAsa para los análisis metagenómicos; tres intestinos por tina se preservaron en solución RNAlater (Ambion) para los análisis de expresión génica; tres estómagos y tres intestinos por tina se congelaron para la evaluación de la actividad enzimática. Todas las muestras se preservaron a - 80°C.

El presente estudio se realizó bajo el acuerdo de la Declaración de Helsinki. Y de acuerdo al protocolo autorizado por la Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (SAGARPA), México, NOM-062-ZOO-1999.2001.

7.6 Evaluación de parámetros productivos y supervivencia

Al final de ambos experimentos (juveniles y larvas), se realizó una biometría con el número total de organismos para determinar el peso con una balanza analítica (Ohaus HH120, Shenzhen, China) y la longitud total (cm) mediante fotografías analizadas en el software Image 1.5.

Los datos obtenidos de talla y peso fueron utilizados para determinar los siguientes parámetros productivos: alimento consumido (FI): ingesta total de alimento por unidad experimental / días de experimento; ganancia de peso (AWG): [(peso final – peso inicial) / peso inicial] × 100; tasa de crecimiento especifica (SGR): [(log (peso final) – log (peso inicial) / días)] × 100; tasa de conversión alimenticia (FCR): alimento consumido (g) / peso ganado (g); tasa de conversión proteica (PER): peso ganado / proteína ingerida; factor de condición (K): [(peso húmedo (g) × (g) / longitud total^3 (cm)] × 100; y supervivencia (S): (número final / número inicial) × 100. En el caso del experimento con juveniles de *A. tropicus* también se evaluaron los siguientes índices: índice hepatosomático (HSI): (peso del hígado (g) / × 100) / total de peso corporal (g); índice viscerosomático (VSI): (peso de las vísceras (g) × 100 / peso corporal total (g).

7.7 Evaluación de actividad de enzimas digestivas

7.7.1 *Larvas*

Se realizó un pool de tres larvas (sin cabeza y cola) por réplica y se homogeneizaron en Tris-HCl 50 mM, pH 7; posteriormente se centrifugaron a 14,000 *g* a 4 °C por 15 min. El sobrenadante se resguardó a – 80 °C.

7.7.2 *Juveniles*

El extracto multienzimático se realizó a partir de un pool de tres estómagos y tres intestinos por replica y se homogenizaron en solución Tris-HCl 50 mM, pH 7, posteriormente se centrifugaron a 14, 000*g* a 4 °C por 15 min. El sobrenadante se resguardó a – 80 °C hasta su uso.

La cuantificación de la proteína soluble presente en los sobrenadantes, se llevó a cabo mediante el método de Bradford (1976). La actividad enzimática de proteasa acida, proteasa alcalina, tripsina, quimotripsina, lipasa y α-amilasa se determinaron siguiendo las especificaciones descritas a continuación:

- Proteasa acida. Se cuantificó utilizando hemoglobina al 1% en buffer glicina-HCl 0.1 M (pH 2) y se incubó por 10 min (Anson, 1938).
- Proteasa alcalina. Se determinó de acuerdo con la metodología de Walter (1984), utilizando caseína al 1% en una solución de 100 mM Tris-HCl, 10 mM $CaCl_2$ (pH 9), con incubación de 20 min.
- Tripsina. Se cuantificó mediante la técnica de Erlanger et al., (1961), utilizando 1 mM BAPNA (Nα-Benzoil-DL-Arginina-P-nitroanilida) como sustrato en buffer Tris-HCl 50 mM, $CaCl_2$ 10 mM (pH 8.2). Se utilizó un volumen final de 250 µL compuesto por 135 µL de sustrato y 15 µL de extracto enzimático diluido 1:3. Se incubo 30 min y se midió la absorbancia a 410 nm en una micro placa (xMark, Biorad, Hercules, CA).
- Quimotripsina. Se evaluó utilizando 1.25 mM SAPNA como sustrato en Tris-HCl-50 mM (pH 8), y se incubó por 30 min. La absorbancia se midió a 410 nm de acuerdo a la técnica de Del Mar et al., (1979).
- Lipasa. Su actividad se determinó con el método de Gjellesvik et al., (1992), utilizando 4-nitrofenil palmitato como sustrato en una solución de Tris-HCl, 0.5 M (pH7), taurocolato de sodio 6 mM y 5 µL de extracto enzimático. Se incubó por 10 min y la absorbancia se cuantificó a 415 nm.
- α-amilasa. Se cuantificó utilizando almidón al 2% como sustrato en citrato de sodio (125 µL), NaCl 0.05 M (pH 7.5), con incubación a 37 °C durante 60 min. La absorbancia se midió a 600 nm (Robyt & Whelan, 1968).

Todos los valores de actividad enzimática se expresaron como U mg proteína^{-1}.

7.8 Extracción de ARN y síntesis de cDNA

Se realizó la extracción del ARN total de los intestinos de tres peces por replica, mediante la técnica de Trizol (Invitrogen, Waltham, MA) de acuerdo con las instrucciones del fabricante. La concentración y pureza de las muestras se determinó mediante la relación de la absorbancia a 260/280 nm utilizando un espectrofotómetro (Jenway GenovaNano, Cole-Parmer, Staffordshire, Reino Unido). La integridad del ARN se verificó mediante la visualización de electroforesis en un gel de agarosa/formaldehído al 1%. El ARN (1 µg) se transcribió de forma inversa a cDNA mediante un kit de transcripción inversa de cDNA de alta capacidad (ThermoScientific, Waltham, MA) en un volumen final de 20 µL siguiendo las recomendaciones del fabricante.

7.9 Cuantificación de expresión de genes

Se cuantificó la expresión de los genes *muc-2* (proteína de la capa de mucosa intestinal) y *zo-1* (proteínas de unión estrecha), relacionados con la integridad de la barrera intestinal; y de los genes *il-8* (citocina proinflamatoria), *il-10* (citocina antiinflamatoria) y lisozima (proteína bacteriolítica), componentes del sistema inmunológico. Se utilizó el gen *β-actina* como gen de referencia endógena (Jiménez-Martínez et al., 2022). Los cebadores empleados se muestran en la Tabla 3. Las mezclas de qPCR se realizaron con 5 µL del kit comercial *Eva Green* (BioRad, Hercules, CA), 4.5 µl de cDNA (5 ng/ µl) y 0.25 µM de cada cebador, en un volumen final de 10 µL. Las reacciones de qPCR se realizaron en un termociclador en tiempo real CFX96TM (BioRad, Hercules, CA) bajo las siguientes condiciones: un ciclo de desnaturalización a 95 °C durante 10 min, seguido de 40 ciclos de 15 s a 95 °C y 1 minuto a 60 °C. Los cambios relativos en la expresión génica se calcularon empleando el método $2^{\Delta\Delta Ct}$ (Livak & Schmittgen, 2001).

Tabla 3. Cebadores utilizados para el análisis qPCR.

Gen	Secuencia de cebadores (5´- 3')	Eficiencia de amplificación (%)	Tamaño de amplicon (bp)	Referencias
muc-2	FW: GGCCTCCTCAAGAGCACGGTG RV:TCTGCACGCTGGAGCACTCAATG	90.94	100	Nieves-Rodriguez et al., (2018)
zo-1	FW: TGTGCCTCAGATCACTCCAC RV: AAAGGCAGAGGGTTGGCTTC	98.58	123	Pérez-Jiménez et al., (2022)
zo-2	FW: TACCCATGGAAAATGTGCCTCA RV: CGGGGTCTCTTCACGGTAA	95.29	88	Pérez-Jiménez et al., (2022)
lyz	FW: CACTGCAGCCATCAATCACAAC RV: ATTAGTCAGCAGCTTGCTGCAG	89.91	100	Nieves-Rodriguez et al., (2018)
il-8	FW: ATATTCACTGGTGGGCGGAG RV: GTGCGGCCTGAGATTGTTT	94.18	369	Pérez-Jiménez et al., (2022)
il-10	FW: TTATAAAGCCATGGGGGAGCTG RV: CTGCACAGTCTGCCTCTAGT	94.47	91	Este estudio
β-actin	FW:GAGCTATGAGCTGCCTGAGTGG RV:GTGGTCTCATGAATGCCACAGG	97.10	119	Jiménez-Martínez et al., 2022

7.10 Aislamiento de ADN de la microbiota intestinal

Se realizó la extracción de ADN genómico de los intestinos de tres peces por replica, con el kit QIAGEN DNeasy PowerLyzer PowerSoil (Hilden, Germany). Los intestinos por replica se colocaron en la matriz *Lysing Matrix A* (MP Biomedicals TM Santa Ana, CA, EE. UU.) con el tampón de lisis proporcionado por el kit y se homogenizaron en el quipo FrastPrep-24TM 5G (MP BiomedicalsTM, Santa Ana, CA, EEI.UU.). Los pasos posteriores de la técnica de extracción se realizaron siguiendo el protocolo del fabricante (QIAGEN). La concentración de ADN se cuantificó utilizando el flurómetro Qubit 3.0 y el kit de ensayo *ds* DNA BR (Invitrogen de Thermo Fisher Scientific). La integridad del ADN se verificó mediante electroforesis en gel de agarosa al 1%.

7.11 Preparación de bibliotecas y secuenciación del gen rRNA 16s

La región hipervariable V4 del gen 16s rRNA de las muestras de ADN bacteriano se amplificó mediante PCR para la preparación de las bibliotecas. Se utilizaron los cebadores específicos del

gen V4-515f: 5′ GTGCCAGCMGCCGCGGTAA -3′ y V4-806r:5′–GGACTACHVGGGTWTCTAA T-3′ descritos por Caporaso et al., (2011). La amplificación por PCR consistió en un paso inicial de desnaturalización de 3 min a 98°C, seguido de 25 ciclos de desnaturalización de 15 s a 94°C, seguido de 15 s a 51°C, un paso de extensión de 15 s a 72°C y una extensión final de 5 min a 72°C. Se realizó una segunda PCR utilizando el índice de Nextera XT (Ilumina, San Diego, CA, EE.UU.) bajo las siguientes condiciones: 30 s a –95 °C, 30 s a 61°C y 5 min a 72°C. A todos los productos de PCR se les realizó un paso de limpieza optimizado, utilizando perlas Agencourt AMPure XP de acuerdo con el protocolo publicado por Ilumina. Las bibliotecas V4 se secuenciaron con el kit *MiSeq Reagent Kit v3* (300 ciclos) usando la plataforma MiniSeq (Illumina, San Diego, CA, EE.UU.). Se realizaron 2 × 150 ciclos de secuenciación de extremos emparejados.

7.12 Análisis bioinformáticos

Las lecturas obtenidas a partir de la secuenciación de la región hipervariable V4 del gen 16S rRNA se procesaron mediante el software QIIME v.2022 (Quantitative Insights Into MicrobialEcology) (Boylen et al., 2019). Para la eliminación de ruido se utilizó el complemento "*deblur denoise-16S*"de QIIME2, con el cual se depuraron un total de 605, 588 lecturas sin procesar. Posteriormente, las secuencias fueron recortadas a una longitud de 150 pares de bases, filtradas en función de los puntajes de calidad y se eliminaron las quimeras con Deblur.

La asignación taxonómica de un total de 201 variantes de secuencia de amplicón (ASV) se realizó utilizando la base de datos del clasificador SILVA 132, basada en un 99% de identidad. Las lecturas no asignadas y los ASV con una frecuencia inferior a <4 lecturas se eliminaron de los análisis posteriores.

Para analizar la diversidad alfa y beta entre los tipos de muestras, se utilizó la función "*qiime diversity core-metrics-phylogenetic*", la cual requiere una rarefacción a una profundidad de muestreo especificada por usuario antes del cálculo de las métricas de diversidad. En este caso, los tamaños de las bibliotecas se ajustaron mediante submuestreo, a una profundidad de 26, 962

lecturas para evitar sesgos por tamaños de muestra desiguales. Se generó una curva de rarefacción utilizando ASV para estimar la riqueza de especies (diversidad alfa) con el complemento "*rarefacción alfa de diversidad qiime*".

Los índices de diversidad alfa de la comunidad microbiana se analizaron con el índice de Shannon-Weaver para la biodiversidad y los estimadores de abundancia Chao 1 y ACE. Todos los índices se calcularon en QIIME2 con diversidad q2. Las comparaciones por pares de los valores de diversidad alfa se realizaron mediante ANOVA unidireccional ($p< 0.05$). Para comparar la estructura general de la comunidad bacteriana entre diferentes tipos de muestras (diversidad beta), se calcularon las matrices de disimilitud y similitud utilizando las distancias de Bray-Curtis y Jaccard, así como la matriz de distancia filogenética basada en el índice Unifrac ponderado. A partir de las matrices, se realizó un análisis de coordenadas principales (PCoA), en QIIME, y la visualización de los resultados se llevó a cabo mediante EMPeror (Vázquez-Baeza et al., 2013). Finalmente, la comparación por pares de las distancias de diversidad beta en el tracto digestivo, se realizó mediante un análisis de varianza multivariante de permutación (PERMANOVA) con 4, 999 permutaciones y un valor de significancia de $p< 0.05$.

7.13 Análisis estadísticos

Los análisis estadísticos de los resultados obtenidos, se realizaron de manera independiente por bioensayo, según la etapa de desarrollo evaluada en el pez y la cepa empleada (levaduras y bacteria) y se compararon con la dieta control (DC).

Se realizaron pruebas de normalidad (Kolmogorov-Smirnov) y la homocedasticidad (Bartlett) sobre los datos de crecimiento, actividad enzimática digestiva y las mediciones histológicas, las cuales cumplieron los supuestos requeridos. En consecuencia, se realizó un ANOVA de una vía seguido de la prueba de post hoc de Tukey. Para los análisis de expresión génica, se utilizó la prueba de Kruskal-Wallis y la prueba de post hoc de Nemenyi para identificar diferencias significativas. Todos los datos se analizaron utilizando el software GraphPad Prism 8 (GraphPad Software, La Jolla, CA) con un valor de significancia de $p<0.05$.

8. CRONOGRAMA

	Semestre	I	II	III	IV	V	VI	VII	VIII
Actividades	Revisión de bibliografía.	x	x	x	x	x	x	x	X
	Asignaturas.	x	x						
	Objetivo 1 Evaluar el efecto antagónico *in vitro* de *C. tropicalis*, *D. hansenii* y *L. lactis* contra bacterias patógenas de peces								
	Cultivo de cepas probióticas y viabilidad.		x			x			
	Formulación y realización de las dietas experimentales.		x			x			
	Bioensayo de larvas y juveniles con la administración de dietas experimentales y obtención de ejemplares para los análisis posteriores.			x		x			
	Objetivo 2 Determinar el efecto de la inclusión en la dieta de las levaduras *C. tropicalis* y *D. hansenii* sobre el crecimiento, indicadores de productividad acuícola, supervivencia, actividad enzimática digestiva, morfología digestiva y expresión de genes asociados a la función de barrera intestinal y el sistema inmunológico en juveniles de *A. tropicus.*			x	x	x	x		
	Obtención de resultados de parámetros productivos.			x	x	x	x		
	Obtención de resultados de análisis metagenómicos, expresión de genes y actividad enzimática.				x	x	x		
	Objetivo 3 Evaluar el efecto de la inclusión en la dieta de la bacteria autóctona *L. lactis* sobre el crecimiento, indicadores de productividad acuícola, supervivencia, actividad enzimática digestiva, morfología digestiva y expresión de genes asociados a la función de barrera intestinal y el sistema inmunológico en larvas de *A. tropicus.*				x	x	x		
	Objetivo 4 Caracterizar la composición de la microbiota bacteriana residente en el intestino de larvas y juveniles de *A. tropicus* mediante análisis metagenómicos. **Objetivo 5** Estimar las funciones metabólicas de la microbiota intestinal en larvas de *A. tropicus* alimentadas con la bacteria autóctona L. lactis mediante análisis de predicción basado en la composición genética.				x	x	x		
	Análisis estadísticos.				x	x	x		
	Redacción de artículos.			x	x	x	x		
	Someter primer artículo.					x	x		
	Someter segundo artículo.							x	
	Estancia de investigación en CIAD-Hermosillo, Sonora.				x	x			
	Examen pre-doctoral.					x	x		
	TOEFL.			x				x	X
	Examen de grado.								X
	Redacción de Tesis.	x	x	x	x	x	x	x	X

9. REFERENCIAS CITADAS

Aguilera, C., Mendoza, R., Rodríguez, G., & Márquez, G. (2002). Morphological Description of Alligator Gar and Tropical Gar Larvae, with an Emphasis on Growth Indicators. *Transactions Of The American Fisheries Society*, 131(5), 899-909. https://doi.org/10.1577/1548-8659(2002)131

Alfaro, R. M., González, C. A., & Ferrara, A. M. (2008). Gar biology and culture: status and prospects. *Aquaculture Research*, 39(7), 748-763. https://doi.org/10.1111/j.1365-2109.2008.01927.x

Álvarez-González, C. A., Ortiz-Galindo, J., Dumas, S., Martínez-Díaz, S., Hernández-Ceballos, D., Del Alamo, T., Moreno-Legorreta, M. & Peña-Martínez, R. (2001). Effect of stocking density on the growth and survival of spotted sand bass *Paralabrax maculatofasciatus* larvae in a closed recirculating system. *Journal of the World Aquaculture Society*, 32(1):151-159.

Álvarez-González, C., Contreras, W., Castillo, K., Santana, O. & Gallegos R. (2007). Evaluation of commercial diets on tropical gar *Atractosteus tropicus* growth. In: Memories of Aquaculture America 2007, San Antonio, Texas, EUA. pp 835.

Angulo, M., Reyes-Becerril, M., Medina-Córdova, N., Tovar-Ramírez, D. & Angulo, C. (2020). Probiotic and nutritional effects of *Debaryomyces hansenii* on animals. *Applied Microbiology and Biotechnology*, 104: 7689-7699. https://doi.org/10.1007/s00253-020-10780-z

Anson, M. (1938). The estimation of pepsin, trypsin, papain, and chatepsin with hemoglob. *The Journal of General Physiology*, 22: 79-89.

Balcázar, J. L., De Blas, I., Ruiz-Zarzuela, I., Vendrell, D., Calvo, A. C., Márquez, I., Gironés, O. & Muzquiz, J. L. (2007). Changes in intestinal microbiota and humoral immune response following probiotic administration in brown trout (*Salmo trutta*). *British Journal Of Nutrition*, 97(3), 522-527. https://doi.org/10.1017/s0007114507432986

Bolotin, A. Wincker, P., Mauger, S., Jaillon, O., Malarme, K., Weissenbach, J., Ehrlich, S.D. & Sorokin, A. (2001). The Complete Genome Sequence of the Lactic Acid Bacterium *Lactococcus lactis* ssp. *lactis* IL1403. *Genome Research*, *11*, 731–753. https://doi.org/10.1101/gr.gr-1967r

Bolyen, E., Rideout, J. R., Dillon, M. et al. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, *37*(8), 852-857. https://doi.org/10.1038/s41587-019-0209-9

Bradford, M. (1976). A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle of protein-dye binding. *The Journal Analytical Biochemistry*, 72: 248-254.

Burggren, W. W., Bautista, G. M., Coop, S. C., Couturier, G. M., Delgadillo, S. P., García, R. M., & González, C. A. A. (2016). Developmental cardiorespiratory physiology of the air-breathing tropical gar, *Atractosteus tropicus*. AJP Regulatory Integrative and Comparative Physiology, 311(4), R689-R701. https://doi.org/10.1152/ajpregu.00022.2016

Bussing, A. (1998). Peces de las aguas Continentales de Costa Rica. 2da Edición. Universidad de Costa Rica, San José, Costa Rica. 468 pp.

Campos, M.M. & Campos, C.R. (2017). Applications of quartering method in soils and foods. *International Journal of Engineering Research and Applications*, *7*: 35-39. https://doi.org/10.9790/9622-0701023539

Caporaso, J., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F., Costello, E., Fierer, N., Peña, A., Goodrich, J., Gordon, J., Huttley, G., Kelley, S., Knights, D., Koenig, E., Ley, E., Lozupone, C., McDonald, D., Muegge, B., Pirrung, M. Reeder, J., Sevinsky, J., Turnbaugh, P., Walters, W., Widmann, J., Yatsunenko, T., Zaneveld, J. & Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, 7(5), 335-336. https://doi.org/10.1038/nmeth.f.303

Carrias, A., Ran, C., Terhune, J. & Liles, M. (2012). Bacteria and bacteriophages as biological agents for disease control in aquaculture. In: Austin B (ed) Infectious Disease in Aquaculture, pp. 353–393. Elsevier, Cambridge, UK.

Córdova-de la Cruz, S. E. C., Riesco, M. F., Martínez-Bautista, G., Calzada-Ruiz, D., Martínez-Burguete, T., Peña-Marín, E. S., Álvarez-González, C. A., & Fernández, I. (2022). Larval development in tropical gar (*Atractosteus tropicus*) is dependent on the embryonic thermal regime: ecological implications under a climate change context. *Fishes*, 7(1), 16. https://doi.org/10.3390/fishes7010016

Dawood, M.A., Koshio, S., Ishikawa, M., Yokoyama, S., Basuini, E., Hossain, M.S., Nhu, T.H., Dossou, S. & Moss, A.S. (2016). Effects of dietary supplementation of *Lactobacillus rhamnosus* or/and *Lactococcus lactis* on the growth, gut microbiota and immune responses of red sea bream, *Pagrus major*. *Fish and Shellfish Immunology*,49:275–285. https://doi.org/10.1016/j.fsi.2015.12.047.

De la Cruz-Marín, E., Martínez-García, R., López-Hernández, J. F., Méndez-Marín, O., De la Rosa-García, S. C., Peña-Marín, E. S., Tovar-Ramírez, D., Sepúlveda-Quiroz, C. A., Pérez-Jiménez, G. M., Jiménez-Martínez, L. D., Asencio-Alcudia, G. G. & Álvarez-González, C. A. (2023). Inulin Supplementation in Diets for Tropical Gar (*Atractosteus tropicus*) Larvae: Effects on Growth, Survival, and Digestive and Antioxidant Enzyme Activities. *Aquaculture Journal*, 3(1), 43-55. https://doi.org/10.3390/aquacj3010006

Del Mar, E., Largman, C., Brodrick, J. & Geokas, M. (1979). A sensitive new substrate for chymotrypsin. *The Journal Analytical Biochemistry*, 99: 316-320. https://doi.org/10.1016/S0003-2697(79)80013-5

Desai, R., Links, G., Collins, A., Mansfield, S., Drew, D., Van Kessel, G. & Hill, E. (2012). Effects of plant-based diets on the distal gut microbiome of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 350: 134–142. https://doi.org/10.1016/j.aquaculture.2012.04.005

Egerton, S., Culloty, S., Whooley, J., Stanton, C., & Ross, R. P. (2018). The Gut Microbiota of Marine Fish. *Frontiers In Microbiology*, 9. https://doi.org/10.3389/fmicb.2018.00873

Eichmiller, J., Hamilton, M., Staley, C., Sadowsky, J. & Sorensen, W. (2016) Environment shapes the fecal microbiome of invasive carp species. *Microbiome* 4: 44. https://doi.org/10.1186/s40168-016-0190-1

Erlanger, B., Kokowsky, N. & Cohen, W. (1961). The preparation and properties of two new chromogenic substrates of trypsin. *Archives of Biochemistry and Biophysics*, 95: 271-278.

Falcinelli, S., Picchietti, S., Rodiles, A., Cossignani, L., Merrifield, D., Taddei, A., Maradonna, F., Olivotto, I., Gioacchini, G. & Carnevali, O. (2015). *Lactobacillus rhamnosus* lowers zebrafish lipid content by changing gut microbiota and host transcription of genes involved in lipid metabolism. *Scientific Reports*, 5: 9336. https://doi.org/10.1038/srep09336

FAO/WHO. (2001). *Report of a Join FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria: Córdoba, Argentina, 1-4 October 2001*. Food and Agriculture Organization of the

United Nations and World Health Organization. Retrieved from: https://openknowledge.fao.org/server/api/core/bitstreams/382476b3-4d54-4175-803f-2f26f3526256/content

Frank, J. A., Reich, C. I., Sharma, S., Weisbaum, J. S., Wilson, B. A. & Olsen, G. J. (2008). Critical evaluation of two primers commonly used for amplification of bacterial 16S RRNA genes. *Applied and Environmental Microbiology*, 74(8), 2461-2470. https://doi.org/10.1128/aem.02272-07

Frías-Quintana, C. & Álvarez-González, C. (2010). Design of microdiets for the larviculture of tropical gar *Atractosteus tropicus*, Gill 1863. *Universidad y Ciencia*, 26(2): 265–282

Frías-Quintana, C. A., Domínguez-Lorenzo, J., Álvarez-González, C. A., Tovar-Ramírez, D. & Martínez-García, R. (2016). Using cornstarch in microparticulate diets for larvicultured tropical gar (*Atractosteus tropicus*). *Fish Physiology and Biochemistry*, 42(2): 517–528. https://doi.org/10.1007/s10695-015-0156-4

Frías-Quintana, C., Álvarez-González, C., Tovar-Ramírez, D., Martínez-García, R., Camarillo-Coop, S. Peña, E. & Galaviz, M. (2017). Use of potate starch in diets of tropical gar (*Atractosteus tropicus*,Gill 1863) larvae. *Fishes*, 2(1):3. https://doi.org/10.3390/fishes2010003

Gajardo, K., Rodiles, A., Kortner, T., Krogdahl, A., Bakke, A., Merrifield, D. & Sorum, H. (2016). A high-resolution map of the gut microbiota in Atlantic salmon (*Salmo salar*): A basis for comparative gut microbial research. *Scientific Reports*, 6 (1):30893.

Gjellesvik, D., Lombardo, D. & Walther, B. (1992). Pancreatic baile salt dependent lipase from cod (*Gadus morhua*): purification and properties. *Biochemica et Biophysica Acta*, 1124(2): 123-124.

Gómez, D. & Balcázar, L. (2008). A review on the interactions between gut microbiota and innate immunity of fish. *FEMS Immunology and Medical Microbiology*, 52: 145–154. https://doi.org/10.1111/j.1574-695X.2007.00343.x.

Grice, E. A., & Segre, J. A. (2011). The skin microbiome. *Nature Reviews Microbiology*, *9*(4), 244-253. https://doi.org/10.1038/nrmicro2537

Hennersdorf, P., Kleinertz, S., Theisen, S., Abdul-Aziz, M., Mrotzek, G., Palm, H. & Saluz, H. (2016). Microbial Diversity and Parasitic Load in Tropical Fish of Different Environmental Conditions. *PLOS One*, 11(3): e0151594. https://doi.org/10.1371/journal.pone.0151594

Hernández-López, I. A., Tovar-Ramírez, D., De La Rosa-García, S. C., Alvarez-Villagomez, C. S., Asencio-Alcudia, G. G., Martínez-Burguete, T., Galaviz, M. A., Guerrero-Zárate, R., Martínez-García, R., Peña-Marín, E. S. & Álvarez-González, C. A. (2021). Dietary live yeast (*Debaryomyces hansenii*) provides no advantages in tropical gar, *Atractosteus tropicus* (Actinopterygii: Lepisosteiformes: Lepisosteidae), juvenile aquaculture. *Acta Ichthyologica Et Piscatoria*, *51*(3), 311-320. https://doi.org/10.3897/aiep.51.67095

Hoseinifar, S. H., Mirvaghefi, A. & Merrifield, D. L. (2011). The effects of dietary inactive brewer's yeast *Saccharomyces cerevisiae* var. *ellipsoideus* on the growth, physiological responses and gut microbiota of juvenile beluga (*Huso huso*). *Aquaculture*, 318(1-2), 90-94. https://doi.org/10.1016/j.aquaculture.2011.04.043

Huerta-Ortiz, M., Álvarez-González, C., Márquez-Couturier, G., Contreras-Sánchez, W., Civera-Cerecedo, R. & Goytortúa-Bores, E. (2009). Sustitución total de aceite de pescado con aceite vegetal en larvas del pejelagarto *Atractosteus tropicus*. *Kuxulkab*',15(28): 51-58.

Huerta-Ortiz, M., Álvarez-González, C., Civera-Ceredo, R., Martínez-García, R., Camarillo, S., Goytortúa, E., Peña, E. & Pérez, A. (2018). Optimum level of dietary lipids for growth, chemical composition and apparent digestibility of lipids for *Atractosteus tropicus*. *Latin American Journal of Aquatic Research*, 46(5): 1073-1082.

Ibrahem, M. (2015). Evolution of probiotics in aquatic world: Potential effects, the current status in Egypt and recent prospectives. *Journal of Advanced Research*, 6: 765-791.

Ingerslev, H., von Gersdorff, L., Lenz, M., Larsen, N., Dalsgaard, I., Boye, M. & Madsen, L. (2014). The development of the gut microbiota in rainbow trout (*Oncorhynchus mykiss*) is affected by first feeding and diet type. *Aquaculture*, 424-425: 24-34. https://doi.org/10.1016/j.aquaculture.2013.12.032

Janda, J. M. & Abbott, S. L. (2007). 16S RRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. *Journal of Clinical Microbiology*, 45(9), 2761-2764. https://doi.org/10.1128/jcm.01228-07

Jahan, N., Islam, S.M., Rohani, Md., Hossain, M. & Shahjahan, Md. (2021). Probiotic yeast enhances growth performance of rohu (*Labeo rohita*) through upgrading hematology, and intestinal microbiota and morphology. *Aquaculture*, 545: 737243. https://doi.org/10.1016/j.aquaculture.2021.737243

Jiménez-Martínez, L., Morales, V., Frías-Quintana, C., Castillo, A., Ascencio-Alcudia, G., Álvarez-Villagomez, C., et al. 2021. Quality evaluation of reference gene expression on different tissues in adults of tropical gar *Atractosteus tropicus*. *Pakistan Journal of Zoology*, 54: 363-372. doi: https://doi.org/10.17582/journal.pjz/20200913180928

Kim, D., Brunt, J. & Austin, B. (2007). Microbial diversity of intestinal contents and mucus in rainbow trout (*Oncorhynchus mykiss*). *Journal of Applied Microbiology*, 102: 1654–1664. https://doi.org/10.1111/j.1365-2672.2006.03185.x.

Lane, D. J., Pace, B., Olsen, G. J., Stahl, D. A., Sogin, M. L. & Pace, N. R. (1985). Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. *Proceedings Of The National Academy Of Sciences*, 82(20), 6955-6959. https://doi.org/10.1073/pnas.82.20.6955

Larsen, A., Mohammed, H. & Arias, C. (2014). Comparison of DNA extraction protocols for the analysis of gut microbiota in fishes. *FEMS Microbiology Letters*, 362: fnu031. https://doi.org/10.1093/femsle/fnu031.

Lee, S.H., Beck, B.R., Hwang, S. & Song, S.K. (2021). Feeding olive flounder (*Paralichthys olivaceus*) with *Lactococcus lactis* BFE920 expressing the fusion antigen of *Vibrio* OmpK and FlaB provides protection against multiple *Vibrio* pathogens: A universal vaccine effect. *Fish and Shellfish Immunoogy*, *114*, 253–262. https://doi.org/10.1016/j.fsi.2021.05.007

Llewellyn, M., McGinnity, P., Dionne, M., Letourneau, J., Thonier, F., Carvalho, G., Creer, S. & Derome, N. (2014a). The biogeography of the atlantic salmon (*Salmo salar*) gut microbiome. *The ISME Journal*, *10*(5): 1280-1284. https://doi.org/10.1038/ismej.2015.189

Llewellyn, M. S., Boutin, S., Hoseinifar, S. H., & Derome, N. (2014b). Teleost microbiomes: the state of the art in their characterization, manipulation and importance in aquaculture and fisheries. *Frontiers In Microbiology*, 5. https://doi.org/10.3389/fmicb.2014.00207

Linh, T.H., Nagai, S., Nagasaka, N., Okane, S. & Taoka, Y. (2018). Effect of *Lactococcus lactis* K-C2 on the growth performance, amino acid content and gut microflora of amberjack *Seriola dumerili*. *Fisheries Science*, 84: 1051–1062. https://doi.org/10.1007/s12562-018-1243-0

Liu, H., Guo, X., Gooneratne, R., Lai, R., Zeng, C., Zhan, F. & Wang, W. (2016). The gut microbiota and degradation enzyme activity of wild freshwater fishes influenced by their trophic levels. *Scientifics Reports*, 6: 24340. https://doi.org/10.1038/srep24340

Livak, K. J. & Schmittgen, T. D. (2001). Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. *Methods*, *25*(4), 402-408. https://doi.org/10.1006/meth.2001.1262

López, S., Márquez, G., Contreras, W. & Álvarez, C. (2005). Evaluation of commercial diets on growth and survival of tropical gar *Atractosteus tropicus* juveniles in captivity. In: Memories de Aquaculture America, New Orleans, Lousiana, EUA. pp 8.

Lozupone, C., & Knight, R. (2007). Global Patterns in Bacterial Diversity. *Proceedings of the National Academy of Sciences of the United States of America*, 104(27), 11436-11440. https://doi.org/10.1073/pnas.0611525104

Maldonado, A., De la Cruz, J., Gómez, D., López, L., Fernández, A., Peña, E. & Álvarez-González, C. (2020). Cultivo de pejelagarto (*Atractosteus tropicus*). Prototipo sustentable de cultivo de peces nativos para el autoconsumo. Centro del Cambio Global y de la Sustentabilidad, A. C.

Márquez-Couturier, G., Álvarez-González, C., Contreras-Sánchez, W., Hernández-Vidal, U., Hernández-Franyutti, A., Mendoza-Alfaro, R. & Goytortúa-Bores, E. (2006). Avances en la Alimentación y Nutrición del Pejelagarto *Atractosteus tropicus*. Avances En Nutrición Acuícola VIII, 446–523.

Márquez-Couturier, G., Vázquez-Navarrete, C., Contreras-Sánchez, W. & Álvarez-González, C. (2015). Acuicultura Tropical Sustentable: Una estrategia para producción y conservación del pejelagarto (*Atractosteus tropicus*) en Tabasco, México (2nd ed.), Villahermosa, Tabasco. Universidad Juárez Autónoma de Tabasco. 87 Pp.

Márquez-Couturier, G. & Vázquez-Navarrete, C.J. (2015). Estado del arte de la biología y cultivo de pejelagarto (*Atractosteus tropicus*). *Agroproductividad* 8: 44-51.

Martínez-Burguete, T., Peña-Marín, E. S., García-Gasca, A., Alvarez-González, C. A., & Llera-Herrera, R. (2021). Nutrigenomic marker discovery by de novo transcriptomic sequencing during early development of the tropical gar (*Atractosteus tropicus*). *Aquaculture Research*, 52(8), 3829-3842. https://doi.org/10.1111/are.15228

Martínez, G., Peña, E., Martínez, R., Camarillo, S., Burggren, W., & Álvarez, A. (2021). Survival, growth, and development in the early stages of the tropical gar *Atractosteus tropicus*: Developmental critical windows and the influence of temperature, salinity, and oxygen availability. *Fishes*, 6(1), 5. https://doi.org/10.3390/fishes6010005

Martínez-Porchas, M. & Martínez-Córdova, L. (2012). World Aquaculture: Environmental Impacts and Troubles-hooting Alternatives. *The Scientific World Journal*, 2012: 389623. https://doi.org/10.1100/2012/389623

Martínez-Porchas, M. & Vargas-Albores, F. (2015). Microbial metagenomics in aquaculture: a potential tool for a deeper insight into the activity. *Reviews in Aquaculture*, 7:1-15. https://doi.org/10.1111/raq.1202

Matthijs, G., Souche, E., Alders, M., Corveleyn, A., Eck, S., Feenstra, I., Race, V., Sistermans, E. A., Sturm, M., Weiss, M. M., Yntema, H. G., Bakker, E., Scheffer, H. & Bauer, P. (2016). Guidelines for diagnostic next-generation sequencing. *European Journal of Human Genetics*, 24(1), 2-5. https://doi.org/10.1038/ejhg.2015.226

Maytorena-Verdugo, C. I., Peña-Marín, E. S., Alvarez-Villagómez, C. S., Pérez-Jiménez, G. M., Sepúlveda-Quiroz, C. A., & Alvarez-González, C. A. (2022). Inclusion of Mannan-Oligosaccharides in Diets for Tropical Gar *Atractosteus tropicus* Larvae: Effects on Growth, Digestive Enzymes, and Expression of Intestinal Barrier Genes. *Fishes*, 7(3), 127. https://doi.org/10.3390/fishes7030127

Medina-Félix, D. M., Garibay-Valdez, E., Vargas-Albores, F. & Martínez-Porchas, M. (2022). Fish disease and intestinal microbiota: a close and indivisible relationship. *Reviews in Aquaculture*, *15*(2), 820-839. https://doi.org/10.1111/raq.12762

Méndez-Pérez, R., García-López, R., Bautista-López, J. S. B.-L., Vázquez-Castellanos, J., Alvarez-González, C., Peña-Marín, E., Baltierra-Trejo, E., Adams-Schroeder, R., Domínguez-Rodríguez, V., Melgar-Valdés, C., Martínez-García, R., Moya, A. & Gómez-Cruz, R. (2020). High-throughput sequencing of the 16S rRNA gene to analyze the gut microbiome in juvenile and adult tropical gar (*Atractosteus tropicus*). *Latin American Journal of Aquatic Research*, 48(3): 456-479.

Meng, X., Li, S., Qin, C., Zhu, Z., Hu, W., Yang, L., Lu, R., Li, W., & Nie, G. (2018). Intestinal microbiota and lipid metabolism responses in the common carp (*Cyprinus carpio* L.) following Copper exposure. *Ecotoxicology and Environmental Safety*, *160*, 257-264. https://doi.org/10.1016/j.ecoenv.2018.05.050

Meek, R., Vyas, H. & Piddock, L. (2015). Nonmedical uses of antibiotics: time to restrict their use?. *PLoS Biology* ,13(10): e1002266.

Miller, R.R. (2009). Los Peces Dulceacuícolas de México. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad. México, D.F. 94-96 pp.

Nájera-Arzola I. C., Álvarez-González, C. A., Frías-Quintana, C. A., Peña, E., Martínez-García, R., Camarillo-Coop, S., Méndez-Marín, O. & Gisbert, E. (2018). Evaluación de oligosacáridos de manano (MOS) en dietas balanceadas para juveniles de pejelagarto (*Atractosteus tropicus*). *Hidrobiológica*, 28 (3): 239-246. https://doi.org/10.24275/uam/izt/dcbs/hidro/2018v28n3/pena

Navarrete, P., Magne, F., Araneda, C., Fuentes, P., Barros, L., Opazo, R., Espejo, R. & Romero, J. (2012). PCR-TTGE analysis of 16S rRNA from rainbow trout (*Oncorhynchus mykiss*) gut microbiota reveals host-specific communities of active bacteria. *PLOS One*: e31335, 1–10. https://doi.org/10.1371/journal.pone.0031335

Nayak, S. (2010). Role of gastrointestinal microbiota in fish. *Aquaculture Research*, 41:1553-1573. https://doi.org/10.1111/j.1365-2109.2010.02546.x

Nielsen, B., Almeida, M., Juncker, A. & Rasmussen, S. et al. (2014). Identification and assembly of genomes and genetic elements in complex metagenomic samples without using reference genomes. *Nature Biotechnology*, 32:822-828. https://doi.org/10.1038/nbt.2939

Nieves-Rodríguez, K., Álvarez-González, C.A., Peña-Marín, E., Vega-Villasante, F., Martínez-García, R., Camarillo-Coop, S., et al. 2018. Effect of β-glucans in diets on growth, survival, digestive enzyme activity, and immune system and intestinal barrier gene expression for tropical gar (*Atractosteus tropicus*) juveniles. *Fishes*, 3: 27. https://doi.org/10.3390/fishes3030027

Not, F., Del Campo, J., Balagué, V., De Vargas, C. & Massana, R. (2009). New Insights into the Diversity of Marine Picoeukaryotes. *PLOS One*, 4(9), e7143. https://doi.org/10.1371/journal.pone.0007143

Palma-Cancino, D., Martínez-García, R., Álvarez-González, C., Camarillo-Coop, S. & Peña-Marín, E. (2019). Esquemas de alimentación para larvicultura de pejelagarto (*Atractosteus tropicus* Gill): crecimiento, supervivencia y canibalismo. *Ecosistemas y recursos agropecuarios*, 6(17): 273-281. https://doi.org/10.19136/era.a6n17.2092

Pérez-Jiménez, G. M., Peña-Marín, E. S., Maytorena-Verdugo, C. I., Sepúlveda-Quiroz, C. A., Jiménez-Martínez, L. D., De la Rosa-García, S., Asencio-Alcudia, G. G., Martínez, R., Tovar-Ramírez, D., Galaviz, M. A., Martínez-Burguete, T., Alvarez-González, C. A. & Alvarez-Villagomez, C. S. (2022). Incorporation of Fructooligosaccharides in Diets Influence Growth Performance, Digestive Enzyme Activity, and Expression of Intestinal Barrier Function Genes

in Tropical Gar (*Atractosteus tropicus*) Larvae. *Fishes*, *7*(3), 137. https://doi.org/10.3390/fishes7030137

Pérez-Sánchez, T., Ruiz-Zarzuela, I., De Blas, I. & Balcázar, J. L. (2013). Probiotics in Aquaculture: A Current assessment. *Reviews in Aquaculture*, *6*(3), 133-146. https://doi.org/10.1111/raq.12033

Raghuwanshi, S., Misra, S., Sharma, R. & Bisen, P. S. (2018). Probiotics: Nutritional Therapeutic Tool. *Journal of Probiotics & Health*, *06*(01). https://doi.org/10.4172/2329-8901.1000194

Ray, A., Ghosh, K. & Ringø, E. (2012). Enzyme-producing bacteria isolated from fish gut: a review. *Aquaculture Nutrition*, 18: 465– 492. https://doi.org/10.1111/j.1365-2095.2012.00943.x

Reinoso, S., Gutiérrez, M. S., Domínguez-Borbor, C., Argüello-Guevara, W., Bohórquez-Cruz, M., Sonnenholzner, S., Nova-Baza, D., Mardones, C. & Navarrete, P. (2023). Selection of Autochthonous Yeasts Isolated from the Intestinal Tracts of Cobia Fish (*Rachycentron canadum*) with Probiotic Potential. *Journal Of Fungi*, 9(2), 274. https://doi.org/10.3390/jof9020274

Ringø, E., Sperstad, S., Myklebust, R., Refstie, S. & Krogdahl, Å. (2006). Characterisation of the microbiota associated with intestine of Atlantic Cod (*Gadus morhua* l.). *Aquaculture*, 261(3), 829-841. https://doi.org/10.1016/j.aquaculture.2006.06.030

Ringø, E. & Birkbeck, T. (1999). Intestinal microflora of fish larvae and fry. *Aquaculture research*, 30: 73–93.

Robyt, J. & Whelan, W. (1968). Amylases. In: Radley JA (ed) Starch and its Derivates. Champan and Hall, England.

Teles, A., Álvarez-González, C. A., Llera-Herrera, R., Gisbert, E., Salas-Leiva, J., Del Carmen Rodríguez-Jaramillo, M. & Tovar-Ramírez, D. (2022). *Debaryomyces hansenii* CBS 8339 promotes larval development in *Seriola rivoliana*. *Aquaculture*, *560*, 738587. https://doi.org/10.1016/j.aquaculture.2022.738587

Tovar-Ramírez, D., Infante, J. Z., Cahu, C., Gatesoupe, F. & Vázquez-Juárez, R. (2004). Influence of dietary live yeast on European sea bass (*Dicentrarchus labrax*) larval development. *Aquaculture*, *234*(1-4), 415-427. https://doi.org/10.1016/j.aquaculture.2004.01.028

Saenz de Rodrigáñez, M., Aguilar, F., Alarcón, F., Pedrosas, R., Peña, E., Martínez, R., Guerrero, R., Matamoros, W. & Álvarez, C. (2018). Alimentos microencapsulados para el cultivo de larvas

de pejelagarto (*Atractosteus tropicus*). *Revista de Biología Tropical*, 66(3): 1298-1313. https://doi.org/10.15517/rbt.v66i3.31727

Salipante, S. J., SenGupta, D. J., Rosenthal, C., Costa, G. L., Spangler, J., Sims, E. H., Jacobs, M. A., Miller, S. I., Hoogestraat, D. R., Cookson, B. T., McCoy, C. O., Matsen, F. A., Shendure, J., Lee, C. C., Harkins, T. T. & Hoffman, N. G. (2013). Rapid 16S RRNA Next-Generation sequencing of polymicrobial clinical samples for diagnosis of complex bacterial infections. *PLOS One*, 8(5), e65226. https://doi.org/10.1371/journal.pone.0065226

Sanahuja, I., Ruiz, A., Firmino, J.P., Reyes-López, F.E., Ortiz-Delgado, J.B., Vallejos-Vidal, E., Tort, L., Tomar-Ramirez, D., Cerezo, I., Moriñigo, M., Sarasquete, C. & Gisbert, E. (2023). *Debaryomyces hansenii* supplementation in low fish meal diets promotes growth, modulates microbiota, and enhances intestinal condition in juvenile marine fish. *Journal of Animal Science and Biotechnology*, 14: 1. https://doi.org/10.1186/s40104-023-00895-4

Sepúlveda-Quiroz, C. A., Peña-Marín, E. S., Pérez-Morales, A., Martínez-García, R., Álvarez-Villagómez, C. S., Maytorena-Verdugo, C. I., Camarillo-Coop, S., Vissio, P. G., Sirkin, D. I. P., Tovar-Ramírez, D., Galaviz, M. A. & Álvarez-González, C. A. (2020). Fructooligosaccharide supplementation in diets for tropical Gar (*Atractosteus tropicus*) juvenile: Effects on morphophysiology and intestinal barrier function. *Aquaculture Research*, 52(1), 37-50. https://doi.org/10.1111/are.14867

Sepúlveda-Quiroz, C., Alvarez-Villagomez, C., Álvarez-González, C., Jiménez-Martínez, L., Pérez-Jiménez, G., Asencio-Alcudia, G., Mendoza-Porras, O., López-Hernández, J., Pérez-Morales, A., Galaviz-Espinoza, M., Tovar-Ramírez, D., Ramírez-Martínez, C., & Martínez-García, R. (2024). Intra-cohort cannibalism and aggressive behavior in tropical gar (*Atractosteus tropicus*) larvae: Effects of stocking density and starvation. *Aquaculture*, 593, 741324. https://doi.org/10.1016/j.aquaculture.2024.741324

Siangpro, N., Chuakrut, S., Sirimanapong, W., Tanasupawat, S., Phongsopitanun, W., Meksiriporn, B., Boonnorat, J., Sarin, S., Kucharoenphaibul, S. & Jutakanoke, R. (2023). *Lactiplantibacillus argentoratensis* and *Candida tropicalis* Isolated from the Gastrointestinal Tract of Fish Exhibited Inhibitory Effects against Pathogenic Bacteria of Nile Tilapia. *Veterinary Sciences*, *10*(2), 129. https://doi.org/10.3390/vetsci10020129

Sullam, K., Essinger, S., Lozupone, C., O'connor, M., Rosen, G., Knight, R., Kilham, S. & Russell, J. (2012). Environmental and ecological factors that shape the gut bacterial communities of fish: a meta-analysis. *Molecular Ecology*, *21*(13): 3363-3378. https://doi.org/10.1111/j.1365-294X.2012.05552.x.

Sun, P., Zhang, H., Jiang, Y., Gao, Q., Tang, B., Ling, J. & Yuan, X. (2021). Relationships between the Gut Microbiota of Juvenile Black Sea Bream (*Acanthopagrus schlegelii*) and Associated Environment Compartments in Different Habitats. *Microorganisms*, *9*(12), 2557. https://doi.org/10.3390/microorganisms9122557

Vázquez-Baeza, Y., Pirrung, M., González, A. & Knight, R. (2013). EMPeror: a tool for visualizing high-throughput microbial community data. *GigaScience*, *2*(1). https://doi.org/10.1186/2047-217x-2-16

Walter, H. (1984). Proteinases: Methods with hemoglobin, casein and azocoll as substrates. *In Methods of Enzymatic Analysis*: Verlag Chemie: Weinheim, Germany, 270-277.

Wang, J. J., Jaramillo-Torres, A., Li, Y., Kortner, T. M., Gajardo, K., Brevik, Ø. J., Jakobsen, J. N. & Krogdahl, Å. (2021). Microbiota in intestinal digesta of Atlantic salmon (*Salmo salar*), observed from late freshwater stage until one year in seawater, and effects of functional ingredients: a case study from a commercial sized research site in the Arctic region. *anim microbiome*, *3*(1). https://doi.org/10.1186/s42523-021-00075-7

Wang, J., Fan, D., Zhao, R., Lu, T., Li, S. & Wang, D. (2024). Effects of Dietary Supplementation with Endogenous Probiotics *Bacillus subtilis* on Growth Performance, Immune Response and Intestinal Histomorphology of Juvenile Rainbow Trout (*Oncorhynchus mykiss*). *Fishes*, 9(6), 229. https://doi.org/10.3390/fishes9060229

Whiteside, S., Razvi, H., Dave, S., Reid, G. & Burton, J. (2015). The microbiome of the urinary tract-a role beyond infection. *Nature Reviews Urology*, 12: 81-90.

Wichmann, F., Udikovic-N., Andrew, S. & Handelsman, J. (2014). Diverse antibiotic resistance genes in dairy cow manure. *Journal of American Society for Microbioloty*, 5: e01017-13.

Wiley, E. O. 1976. The phylogeny and biogeography of fossil and recent gars (Actinopterygii: Lepisosteidae). University of Kansas. Museum Natural History. USA. 64: 1-111 pp.

Wooley, C., Godzik, A. & Friedberg, I. (2010). A primer on metagenomics. *PLOS One*, *6: e1000667*. https://doi.org/10.1371/journal.pcbi.1000667

Wu, S., Gao, T., Zheng, Y., Wang, W., Cheng, Y. & Wang, G. (2010). Microbial diversity of intestinal contents and mucus in yellow catfish (*Pelteobagrus fulvidraco*). *Aquaculture*, 303(1-4), 1-7. https://doi.org/10.1016/j.aquaculture.2009.12.025

Wu, S., Wang, G., Angert, E. R., Wang, W., Li, W. & Zou, H. (2012). Composition, Diversity, and Origin of the Bacterial Community in Grass Carp Intestine. *PLOS One*, 7(2): e30440. https://doi.org/10.1371/journal.pone.0030440.

Xia, J., Lin, G., Fu, G., Wan, Z., Lee, M., Wang, L., Liu, X. & Yue, G. (2014). The intestinal microbiome of fish under starvation. *BMC Genomics*, 15(1): 266. https://doi.org/10.1186/1471-2164-15-266

Ye, L., Amberg, J., Chapman, D., Gaikowski, M. & Liu, W. (2014). Fish gut microbiota analysis differentiates physiology and behavior of invasive Asian carp and indigenous American fish. *The Multidisciplinary Journal of Microbial Ecology*, 8: 541–551. https://doi.org/10.1038/ismej.2013.181.

Yu, Z. & Morrison, M. (2004). Improved extraction of PCR-quality community DNA from digesta and fecal samples. *BioTechniques*, 36(5): 808-812. https://doi.org/10.2144/04365ST04

Yukgehnaish, K., Kumar, P., Sivachandran, P., Marimuthu, K., Arshad, A., Paray, B. & Arockiaraj, J. (2020). Gut microbiota metagenomics in aquaculture: factors influencing gut microbiome and its physiological role in fish. *Reviews in Aquaculture*, 12(3), 1903-1927. https://doi.org/10.1111/raq.12416

Zhang, M., Sun, Y., Liu, Y., Qiao, F., Chen, L., Liu, W.-T., Du, Z. & Li, E. (2016). Response of gut microbiota to salinity change in two euryhaline aquatic animals with reverse salinity preference. *Aquaculture*, 454: 72-80. https://doi.org/10.1016/j.aquaculture.2015.12.014

Zhang, Z., Zhang, H., Yang, D., Hao, Q., Yang, H., Meng, D., De Vos, W. M., Guan, L., Liu, S., Teame, T., Gao, C., Ran, C., Yang, Y., Yao, Y., Ding, Q. & Zhou, Z. (2024). *Lactobacillus rhamnosus* GG triggers intestinal epithelium injury in zebrafish revealing host dependent beneficial effects. *iMeta*, 3(2). https://doi.org/10.1002/imt2.181

10. Capítulo II

Inclusion of yeast *Candida tropicalis* and *Debaryomyces hansenii* in diets for tropical gar (*Atractosteus tropicus*) juveniles: effect on growth, digestive enzymatic activity, intestinal barrier gene expressions, and gut microbiota

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Inclusion of yeast *Candida tropicalis* and *Debaryomyces hansenii* in diets for tropical gar (*Atractosteus tropicus*) juveniles: effect on growth, digestive enzymatic activity, intestinal barrier gene expressions, and gut microbiota

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Including probiotic yeasts, such as *Debaryomyces hansenii*, in fish diets improves aquaculture production and fish health. Some fish-isolated yeasts, like *Candida tropicalis*, produce antimicrobial metabolites. However, there have been no reports on their *in vivo* effects. We evaluated the effects of dietary administration of both yeasts on growth, survival, digestive enzyme activity, expression of intestinal barrier genes, and intestinal microbiota composition in *Atractosteus tropicus* juveniles. We administered diets with three concentrations of *C. tropicalis* (10^4, 10^6, and 10^8 CFU g^{-1}), three with *D. hansenii* (10^3, 10^5, and 10^7 CFU g^{-1}), and a control diet without yeast for 45 days. We also evaluated the antagonistic capacity of both yeasts against fish pathogens *in vitro*. Results showed that lower doses of C*. tropicalis* (10^4 CFU g^{-1}) and *D. hansenii* (10^3 CFU g^{-1}) improved growth, survival, and lipase activity. However, *C. tropicalis* compromised intestinal barrier integrity by reducing *zo-1* expression and increasing *il-8* expression while altering the microbiota to favor Desulfovibrionaceae. Conversely, *D. hansenii* enhanced intestinal barrier integrity by increasing the expression of *muc-2*, *zo-1*, and *il-10* genes and decreasing *il-8* expression without altering the microbiota, where *Mycoplasma* predominated. Both yeasts showed antagonistic activity against pathogens. In conclusion, the dietary inclusion of *C. tropicalis* did not favor the intestinal health of the fish. Conversely, *D. hansenii* at a concentration of 10^3 CFU g^{-1} is recommended to improve the growth, digestive function, and health of *A. tropicus* juveniles.

Candida tropicalis; *Debaryomyces hansenii*; yeast probiotic; intestinal microbiota; tropical gar

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Inclusion of yeast *Candida tropicalis* and *Debaryomyces hansenii* in diets for tropical gar (*Atractosteus tropicus*) juveniles: effect on growth, digestive enzymatic activity, intestinal barrier gene expressions, and gut microbiota

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ABSTRACT. Including probiotic yeasts, such as *Debaryomyces hansenii*, in fish diets improves aquaculture production and fish health. Some fish-isolated yeasts, like *Candida tropicalis*, produce antimicrobial metabolites. However, there have been no reports on their *in vivo* effects. We evaluated the effects of dietary administration of both yeasts on growth, survival, digestive enzyme activity, expression of intestinal barrier genes, and intestinal microbiota composition in *Atractosteus tropicus* juveniles. We administered diets with three concentrations of *C. tropicalis* (10^4, 10^6, and 10^8 CFU g^{-1}), three with *D. hansenii* (10^3, 10^5, and 10^7 CFU g^{-1}), and a control diet without yeast for 45 days. We also evaluated the antagonistic capacity of both yeasts against fish pathogens *in vitro*. Results showed that lower doses of C. *tropicalis* (10^4 CFU g^{-1}) and *D. hansenii* (10^3 CFU g^{-1}) improved growth, survival, and lipase activity. However, *C. tropicalis* compromised intestinal barrier integrity by reducing *zo-1* expression and increasing *il-8* expression while altering the microbiota to favor Desulfovibrionaceae. Conversely, *D. hansenii* enhanced intestinal barrier integrity by increasing the expression of *muc-2*, *zo-1*, and *il-10* genes and decreasing *il-8* expression without altering the microbiota, where *Mycoplasma* predominated. Both yeasts showed antagonistic activity against pathogens. In conclusion, the dietary inclusion of *C. tropicalis* did

not favor the intestinal health of the fish. Conversely, *D. hansenii* at a concentration of 10^3 CFU g^{-1} is recommended to improve the growth, digestive function, and health of *A. tropicus* juveniles.

Keywords: *Candida tropicalis*; *Debaryomyces hansenii*; yeast probiotic; intestinal microbiota; tropical gar

INTRODUCTION

The use of probiotics as food additives that exert positive effects at the nutritional, physiological, and immunological levels of aquatic organisms has generated interest in the aquaculture sector (El-Saadony et al. 2021, Rohani et al. 2022), intending to meet consumer demands and provide well-being in culture conditions. Probiotics are those live microorganisms that, when supplemented in food, have beneficial effects on a host by contributing to the balance of the intestinal microbiota (Gram et al. 1999). Probiotic administration's effects are diverse, including cell proliferation and differentiation, immune system enhancement, improved digestive activity, growth, survival, and modulation of the intestinal microbiota. The intestinal microbiota plays a vital role in fish development and is subject to modulation by various factors such as environmental stimuli, diseases, diet type, and developmental stage (Miyake et al. 2015, Talwar et al. 2018). However, the effects of probiotics are strain-specific and depend on the administered concentration and the host (Merrifield et al. 2010). Many probiotics of great interest are primarily Gram-positive and lactic acid bacteria (Verschuere et al. 2000). However, yeasts can be good probiotics (Caruffo et al. 2015) since they are part of the normal microbiota of fish, with physiological effects attributed to the production of various enzymes, polyamines, and the components of their wall such as β-glucans and mannan-oligosaccharides (MOS), which promote the immune response (Lokesh et al. 2012, Navarrete & Tovar-Ramírez 2014). Also, they can modulate the intestinal microbiota's composition and influence the organisms' health status (Vargas-Albores et al. 2021). Yeasts of the genera *Pichia*, *Saccharomyces*, *Candida*, and *Debaryomyces* are most frequently isolated from the intestinal system of fish (Navarrete & Tovar-Ramírez 2014). These yeasts can produce killer toxins that act antagonistically against pathogens, thereby protecting the host from infections (Wang et al. 2018). Several studies have shown that feeding *Debaryomyces hansenii* to

fish, including rainbow trout (*Oncorhynchus mykiss*), turbot (*Scophthalmus maximus*), European bass (*Dicentrarchus labrax*), and gilthead seabream (*Sparus aurata*), can enhance their growth, survival rate, intestinal condition, and function, and also regulate their intestinal microbiota (Tovar-Ramírez et al. 2004, Angulo et al. 2020, Teles et al. 2022, Sanahuja et al. 2023). On the other hand, *Candida tropicalis*, which has been isolated from healthy specimens of Nile tilapia (*Oreochromis niloticus*), produces secondary metabolites with antimicrobial activity (Siangpro et al. 2023), making it a potential probiotic. However, it is essential to analyze the effects of its *in vivo* administration on the physiological and immunological status and the composition of the fish microbiota.

Tropical gar (*Atractosteus tropicus*) is a species native to the southeast region of Mexico and is of ecological and biological importance. *A. tropicus* aquaculture for local and commercial consumption represents a growing economic activity (Márquez-Couturier & Vázquez-Navarrete 2015). However, the use of probiotics as an additive has been scarcely studied, representing a field of potential improvements in the cultivation of this species. This study investigated the impact of including *C. tropicalis* and *D. hansenii* yeasts in diets for *A. tropicus* juveniles on growth, digestive enzyme activity, immune system gene expression, intestinal barrier gene expression, and intestinal microbiota composition. Additionally, we evaluated the antagonistic capacity of both yeasts against fish pathogens *in vitro*.

MATERIALS AND METHODS

Yeast biomass

The *C. tropicalis* strain (PH-04) was provided by the Applied Microbiology Laboratory (DACBiol-UJAT), which was isolated from the intestine of an adult male of *A. tropicus*, and *D. hansenii* (CBS 8339), provided by CIBNOR, SC. The yeast biomass was obtained by stepwise cultivation for 48 h in dextrose yeast peptone broth (yeast extract 10 g, peptone 20 g, dextrose 20 g, distilled water 1,000 mL) in a rotary shaker (Thermo scientific MaxQ 600) at 140 rpm for 48 h at 32°C. The cultures were centrifuged at 2,500 *g* for 40 min, and the cell package was washed twice with sterile solution saline (85%). The colony-forming units (CFU) of the biomasses

obtained were quantified (Hoben & Somasegaran 1982), and the number of CFU per g of wet biomass was reported.

Pathogen antagonism test

The *in vitro* antagonistic capacity of the yeasts *C. tropicalis* and *D. hansenii* against different fish pathogenic bacteria was evaluated. The pathogenic bacteria used were *Aeromona hydrophila* (NCIBM 1134), *A. dhaken*sis (Caim, 1873), *A. ichthiosmia* (Caim, 1876), *Staphylococcus arlettae* (CAIM 1658 and UJAT-02), *Vibrio harveyi* (Caim, 1622), *V. campbelli* (Gibgen, 002) and *Photobacterium damselae* (Caim, 192). The overnight cultures of the bacteria were adjusted to an optical density of 0.6-0.9 at 520 nm (approximately 1×10^{8} CFU mL^{-1}). The adjusted suspension was diluted 1:10 in sterile saline solution (0.85%) to obtain an inoculum of 1×10^{7} CFU mL^{-1}, 3.5 mL of the bacterial suspension was taken and placed in 31.5 mL of Mueller-Hinton Agar (Difco) (45°C), it was homogenized and poured into square Petri dishes (Greiner, Bio-one). After the agar had solidified, the yeast cultures from the 2-day-old culture were punctually inoculated with a calibrated loop (1 µL). The plates were incubated at 30°C for 24 h, and the antagonistic effect of each yeast strain against the specific bacterial strains was quantified by measuring the diameter of the inhibition zones in mm with the standard caliper. The experiments were performed in triplicate (Pantelides et al. 2015).

Preparation of the experimental diets

Seven experimental diets were formulated following the protocol of Frías-Quintana et al. (2016). Three diets were supplemented with *C. tropicalis* 10^{4}, $10^{5,}$ and 10^{6} CFU g^{-1} and *D. hansenii* at 10^{3}, 10^{5}, and 10^{7} CFU g^{-1} concentrations. A control diet without yeast (CD) was also included (Table 1). Yeast incorporation into the diets was achieved by suspending the biomass adjusted in 200 mL of sterile water. The diets were pelleted (3-5 mm), dried at 40°C for 15 h in a convection oven, and stored at 4°C until use. All diets' proximal analysis (ash, lipid, and protein) was conducted according to the Official Methods of Analysis of AOAC International (2000).

Viability of yeasts in experimental diets

At the experiment's beginning and end, the yeast concentration and viability in each experimental diet were confirmed by quantifying CFU per gram. A representative sample from each diet was

obtained using the quartering method (Campos & Campos 2017). One gram of the diet was weighed and suspended in 9 mL of saline solution (0.85%), and exponential dilutions from 10^{-1} to 10^{-10} were prepared. One hundred microliters of the various dilutions were inoculated on Potato Dextrose Agar (PDA, Difco) supplemented with antibiotics (amikacin and chloramphenicol) to reduce bacterial contamination in the formulated foods. The inoculum was evenly spread using a sterile Digralsky loop, and the plates were incubated for 24-48 h at 32°C. Colonies in dilution boxes with 10-30 colonies were counted, and the CFU per gram of diet was calculated.

Experimental design

Three hundred seventy-eight *A. tropicus* juveniles at 38 days post-hatching (DPH) were used, with an average weight of 0.22 ± 0.08 g and a total length of 3.67 ± 0.29 cm, obtained from the aquaculture farm "Otot Ibam", Comalcalco, Tabasco, México. Twenty-one tanks (59 cm in diameter and 49 cm in height) were connected to a recirculation system powered by a 1 HP water pump linked to a 1,500 L reservoir with a biological and automated sand filter and an ultraviolet light lamp to minimize bacterial load. Eighteen fish were placed in each tank. The average temperature was 27.1 ± 0.8°C, dissolved oxygen was 5.7 ± 0.2 mg L^{-1}, and pH was 7.3-7.5, monitored daily using an oximeter (YSI 85, USA) and a potentiometer (HANNA HI 991001, Romania).

During the initial five days, the organisms were acclimated to the experimental diets with commercial feed: 80% commercial feed and 20% experimental diet. Gradually, each day, a higher proportion of the experimental diet was provided (70/30, 60/40, 50/50, and 40/60). Only the experimental and CD, starting from day six, were administered for 45 days. The treatments were conducted in triplicate. Fish were fed four times daily (07:00, 11:00, 15:00, and 19:00 h) until apparent satiety. Cleaning each tank and partial water replacement (10%) after each feeding using a siphoning method.

At the end of the bioassay, all fish were weighed and measured. Subsequently, they were anesthetized with clove oil (0.1 mL L^{-1}) and euthanized by decapitation. An incision was made in the ventral area for the extraction of the liver, visceral package, stomach, and intestine for somatic

index determination. Additionally, three stomachs and three intestines per replicate were frozen for enzymatic activity evaluation. Only the organisms from treatments with the concentrations of each yeast showing the highest growth and survival were used to quantify the expression of genes related to the intestinal barrier function and determine the intestinal microbiota composition. For this purpose, three more intestines per replicate were preserved in RNAlater™ solution (Ambion) for gene expression analyses. Additionally, three intestines per replicate were taken and rinsed with RNase-free water (Sigma), then preserved in RNase-free tubes for metagenomic analyses. All samples were preserved at -80°C.

The study was conducted under the technical specifications for the care and use of laboratory animals, as stated in the Official Mexican Standard NOM-062-ZOO-1999 from the Ministry of Agriculture, Livestock, Rural Development, Fisheries, and Food, and we followed the Declarations of Helsinki recommendations.

Evaluation of growth indexes, survival, and somatic indexes

Every 15 days and at the end of the experiment, weight was determined using an analytical balance (Ohaus HH120, precision 120 ± 0.01 g, Shenzhen, China), and total length was measured using photographs analyzed with Image 1.5 software for all specimens.

At the end of the bioassay, the following productive parameters were determined: feed intake (FI): (feed intake, g dry matter / number of fish/day); AWG: the absolute weight gain: final weight (g) – initial weight (g); SGR: specific growth rate: [(Ln final weight – Ln initial weight)/days] × 100; FCR: feed conversion rate: (feed intake, g dry matter) / (fish wet weight gain, g); PER: the protein efficiency ratio: wet weight gain (g) / protein intake (g); and S: survival: (number of final fish / number of initial fish) × 100.

Before tissues were preserved, the liver, visceral package, and intestines were weighed to calculate the following indexes: hepatosomatic indexes (HSI): (liver weight (g) × 100 / total body weight (g); viscerosomatic indexes (VSI): (viscera weight (g) × 100 / total body weight (g); and the condition factor (K): (final mean body weight / final mean body length^3) × 100).

Enzyme activities quantification

The multi-enzymatic extract was obtained from a pool of three stomachs and three intestines homogenized by replication in 50 mM TRIS-HCl, pH 7, and subsequently centrifuged at 14,000 *g* at 4°C for 15 min. The supernatant was stored at -80°C until use. Quantification of soluble protein was performed using the Bradford method (1976). The activity of trypsin was quantified using the Erlanger et al. (1961) technique with 1 mM BAPNA as substrate (Nα-benzoyl-DL-arginine-P-nitroanilide) in 50 mM Tris-HCl, 10 mM $CaCl_2$, pH 8.2 and the absorbance was measured at 410 nm. Chymotrypsin activity was quantified using 1.25 mM SAAPNA as substrate with 50 mM Tris-HCl, pH 8.2, and the absorbance was measured at 410 nm, following the technique of Delmar et al. (1979). Leucine aminopeptidase activity was determined using 0.1 M leucine p-nitroanilide as substrate, dissolved in DMSO and diluted with 50 mM sodium phosphate, pH 7.2, incubated at 37°C and measured at 405 nm, according to the method of Maroux et al. (1973). α-amylase activity was quantified using 1% starch as substrate in buffer sodium citrate, 0.05 M NaCl, pH 7.5, and the absorbance was measured at 600 nm (Robyt & Whelan 1968). Lipase activity was carried out with a modified method by Gjellesvik et al. (1992), using 4-nitrophenyl palmitate as substrate and 0.5 M Tris-HCl, pH 7.4, 6 mM of sodium taurocholate and 10 µL of extract and the absorbance was measured at 540 nm. All data obtained are shown as unit per milligram of protein (U mg protein^{-1}) according to the following equations: units by mL (U mL^{-1}) = [Δabs × final reaction volume (mL)]×[ε × time (min) × extract volume (mL)]$^{-1}$; specific activity (U mg protein^{-1}) = U mL mg^{-1} of soluble protein.

RNA extraction and reverse transcription

Total RNA was extracted from three intestines per triplicate (nine intestines per treatment), using Trizol (Invitrogen, Waltham, MA) and following the manufacturer's protocol. RNA concentration and purity were assessed using a spectrophotometer (Jenway GenovaNano, Cole-Parmer, Staffordshire, UK). Subsequently, one microgram of RNA was reverse-transcribed into cDNA within a thermocycler (Mastercycle nexus GSX1, Eppendorf, Hamburg, Germany) using the high-capacity cDNA reverse transcription kit (Maxima First Strand cDNA Synthesis Kit for RT-qPCR,

Thermo Scientific, Waltham, MA) in a final volume of 20 µL, following the manufacturer's guidelines.

Gene expression analysis

Five genes were selected for evaluation of gene expression related to the intestinal barrier integrity such as mucus layer protein (*muc-2*) and tight junctions (*zo-1*), and the immune system as cytokine proinflammatory (*il-8*), cytokine anti-inflammatory (*il-10*), and lysozyme (bacteriolytic). The primers used are shown in Table 2. The qPCR reactions were carried out using 10 µL of Eva Green supermix (BioRad, Hercules, CA), 9 µL of cDNA (5 ng μL^{-1}), and 1 µL primers mix (3 mM) in a final volume of 20 µL. The β-actin gene (Jiménez-Martínez et al. 2021) was used as the reference gene. Subsequently, the qPCR was conducted using a CFX96™ Real-Time Thermocycler (BioRad, Hercules, CA). The relative gene expression changes compared to the untreated control were calculated using the ΔΔCt method (Livak & Schmittgen 2001).

DNA isolation from gut microbiota

Genomic DNA extractions were performed using the QIAGEN DNeasy PowerLyzer PowerSoil kit. Three gut tissues were pooled and placed in Lysing Matrix A (MP Biomedicals TM Santa Ana, CA, USA) with lysis buffer from the kit and homogenized per replicate using the FastPrep-24TM 5G Instrument (MP Biomedicals™, Santa Ana, CA, USA). Subsequent steps were carried out according to the manufacturer's protocol (QIAGEN). The DNA concentration was quantified using the Qubit 3.0 Fluorometer and the ds DNA BR Assay kit (Invitrogen by Thermo Fisher Scientific). DNA integrity was verified by 1% agarose gel electrophoresis.

Library preparation and bacterial 16S rRNA gene sequencing

The V4 hypervariable region of the bacterial 16s rRNA gene was amplified by PCR for the library preparation, using gene-specific primers V4-515f: 5′-GTGCCAGCMGCCGCGGTAA-3′ and V4-806r: 5′-GGACTACHVGGGTWTCTAA T-3′ described by Caporaso et al. (2011). PCR amplification consisted of an initial denaturation step for 3 min at 98°C, followed by 25 cycles of denaturation for 15 s at 94°C, annealing for 15 s at 51°C, an extension step at 72°C and a final extension for 5 min at 72°C. A second PCR was performed using Nextera XT index (Illumina, San

Diego, CA, USA) under the following conditions: 30 s at -95°C, 30 s at 61°C, and 5 min at 72°C. All PCR products were followed by an optimized Clean-Up step utilizing Agencourt AMPure XP beads following Illumina's published protocol. Finally, V4 libraries were sequenced with a MiSeq Reagent Kit v3 (300 cycles) using the MiniSeq Platform (Illumina, San Diego, CA, USA), and 2×150 cycles of paired-end sequencing were performed.

Bioinformatics analyses

All the raw sequencing reads of the V4 16S rRNA gene were processed with Quantitative Insights Into Microbial Ecology 2 (QIIME2) v.2022 (Bolyen et al. 2019). Six hundred five thousand five hundred eighty-eight raw reads were denoised using the "Deblur denoise-16S" plug-in of QIIME2, reads were trimmed at 150 bases, filtered based on quality scores, and chimeras were removed using Deblur. The taxonomic assignment of 201 amplicon sequence variants (ASVs) was performed using the classifier SILVA 132 database set at 99% of identity. Unassigned reads and ASVs with frequency <4 reads were eliminated from further analyses.

The "qiime diversity core-metrics-phylogenetic" function was set to analyze alpha and beta diversity among sample types, which requires rarefaction to a user-specified sampling depth before computing diversity metrics. Library sizes were adjusted, rarefying the number of reads with a minimum depth of 26,962 to avoid unequal sample sizes (n = 9, three replicas per treatment). A rarefaction curve was generated using ASVs to estimate species richness (alpha diversity) with the qiime diversity alpha-rarefaction plug-in.

Alpha diversity indices (ASV) of microbial community composition were analyzed with Shannon-Weaver for the biodiversity and the abundance estimator using Chao 1 and ACE; all indices were calculated in QIIME2 with q2-diversity. Pairwise comparisons of alpha diversity indices values were performed using one-way ANOVA ($P < 0.05$). To compare overall microbial community structures between different sample types (beta diversity), dissimilarity and similarity matrices were calculated based on the Bray-Curtis and Jaccard distances and the phylogenetic distance matrix based on the Weighted Unifrac index. Principal coordinate analysis (PCoA) was conducted on the calculated matrixes, performed by QIIME2, and visualized using EMPeror (Vázquez-Baeza et al. 2013). A pairwise comparison of the digestive tract beta diversity distances

was performed using permutation multivariate analysis of variance (PERMANOVA) through 4,999 permutations with a *P*-value of 0.05 to the beta diversity analysis of QIIME2.

Data analysis and statistics

The statistical analyses were conducted independently for each yeast compared to the CD. Normality (Kolmogorov-Smirnov) and homoscedasticity (Bartlett) were tested for all treatments. Differences in growth indexes, survival, and digestive enzyme activities between diets were assessed using one-way ANOVA, followed by Tukey's test. As the gene expression results did not follow a normal distribution, differences in gene expression were determined by the Kruskal-Wallis and Nemenyi methods. All data were statistically analyzed using GraphPad Prism 8 (GraphPad Software, La Jolla, CA) software with a significance value 0.05.

RESULTS

Yeast antagonism against pathogens

The antagonistic properties of the yeasts *C. tropicalis* and *D. hansenii* were evaluated against eight fish pathogens, including both Gram-positive (*Staphylococcus arlettae* strains CAIM 1658 and UJAT-02) and Gram-negative *(Aeromona hydrophila*, *A. dhakensis*, *A. ichistiomia*, *Vibrio campbelli*, *V. harveyi*, and *Photobacterium damselae*) bacteria. Table 3 shows the antagonistic capacity of the yeast *C. tropicalis* in inhibiting the growth of *A. ichistiomia* with inhibition zones of 13.0 ± 1.5 mm, *S. arlettae* (strain Caim, 1658) with inhibition zones of 14.7 ± 1.3 mm, and *V. harveyi* with inhibition zones of 13.5 ± 1.3 mm. Meanwhile, *D. hansenii* only inhibited the growth of *P. damselae*, with inhibition zones of 15.4 ± 1.8 mm.

Viability of yeasts in experimental diets

The CFU number after diet formulation and at the end of the experiment (50 days of storage) indicates that both yeasts were present in concentrations similar to the initial ones throughout the experiment (data not shown).

Growth indexes, survival, and somatic indexes

After 45 days of feeding fish with diets supplemented with *C. tropicalis* yeast at a concentration of 10^4 CFU g^{-1}, the fish exhibited significantly higher weight (3.16 ± 0.48 g) and total length (9.47 ± 0.45 cm) compared to the other two concentrations and the CD ($P < 0.02$) (Fig. 1a-b). On the other hand, fish-fed diets containing *D. hansenii* yeast at a concentration of 10^3 CFU g^{-1} showed the highest weight (3.29 ± 0.12 g) and total length (9.93 ± 0.37 cm), also displaying significant differences compared to the other two concentrations and the CD ($P < 0.02$) (Fig. 1c-d).

The determination of growth indices revealed that feed intake (FI) was lower in fish fed with all three inclusions of *C. tropicalis* compared to the CD ($P < 0.02$). On the other hand, no differences in FI were observed between fish fed with *D. hansenii* at 10^3, 10^5 CFU g^{-1}, and the CD. However, FI was lower in the diet with *D. hansenii* at 10^7 CFU g^{-1} compared to the other treatments ($P < 0.05$). Fish-fed diets supplemented with *C. tropicalis* at 10^4 CFU g^{-1} and *D. hansenii* at 10^3 CFU g^{-1} exhibited the highest AWG and SGR compared to the other treatments and the CD ($P < 0.02$). In this regard, higher survival rates (20.37 ± 3.20 and 25.92 ± 3.20%) were recorded in fish receiving the diet supplemented at the lower concentrations of *C. tropicalis* (10^4 CFU g^{-1}) and *D. hansenii* (10^3 CFU g^{-1}), respectively (Table 4). However, incorporating *C. tropicalis* yeast at different concentrations showed no significant effect ($P < 0.05$). Contrarily, significant differences in survival were observed in fish fed with *D. hansenii*, with a concentration of 10^3 CFU g^{-1} compared to the CD ($P < 0.01$) (Table 4). In contrast, the highest FCR was observed in fish fed with the CD and the higher concentration of *C. tropicalis* (10^5 CFU g^{-1}). The PER values did not show significant treatment differences.

The determination of somatic indices indicated that fish fed with the CD had higher VSI than those supplemented with *D. hansenii* ($P < 0.03$). Fish fed with *C. tropicalis* at 10^5 CFU g^{-1} also showed higher VSI, although this was not significantly different from the CD. The highest HSI value was observed in juveniles treated with *D. hansenii* at 10^3 CFU g^{-1}, although without significant differences (Table 4). The K value of fish fed with the CD was higher compared to fish fed with treatments supplemented with *D. hansenii* ($P < 0.05$). In contrast, no differences in K were observed between fish fed with the CD and those fed with treatments supplemented with *C. tropicalis* (Table 4).

Digestive enzyme activities

Juveniles fed with the *D. hansenii* diet at 10^5 CFU g^{-1} showed the highest specific activity of all evaluated enzymes, including trypsin, chymotrypsin, leucine aminopeptidase, amylase, and lipase, with significant differences compared to the other treatments and the CD ($P < 0.05$) (Table 5).

Regarding supplementation with *C. tropicalis*, it was observed that amylase activity was higher in juveniles fed at 10^5 CFU g^{-1}, while lipase activity was higher in those fed with 10^6 CFU g^{-1}. Significant differences were observed in both cases compared to the remaining treatments and the CD ($P < 0.05$). However, trypsin, chymotrypsin, and leucine aminopeptidase activities were significantly higher in fish fed with the CD compared to those supplemented with yeast ($P < 0.05$) (Table 5).

Intestinal barrier gene expressions

The relative expression of *muc-2*, *zo-1*, *il-8*, *il-10*, and *lyz* in juveniles treated with *C. tropicalis* at 10^4 CFU g^{-1} and *D. hansenii* at 10^3 CFU g^{-1} is shown (Fig. 2). Fish fed with *C. tropicalis* 10^4 CFU g^{-1} showed no changes in the expression of the *muc-2* gene but exhibited a notable decrease in *zo-1* expression ($P < 0.0422$). Although the *lyz* and *il-10* genes did not show changes in their expression levels compared to the control group, a significant increase in the *il-8* gene expression was observed compared to the CD ($P < 0.008$) (Fig. 2a). Furthermore, *D. hansenii* at 10^3 CFU g^{-1} significantly enhanced the expression of genes related to intestinal barrier function, *muc-2*, and *zo-1*, compared to the CD ($P < 0.02$). The evaluation of gene expression associated with the immune system revealed no significant differences in *lyz* expression compared to the CD. On the other hand, the expression of the *il-8* gene decreased significantly compared to the CD ($P < 0.001$), in contrast to the expression of *il-10*, which increased significantly compared to the CD ($P < 0.0001$) (Fig. 2b).

Intestinal microbial diversity

Table 6 shows the alpha-diversity values of the intestinal microbiota of juveniles treated with *C. tropicalis* at 10^4 CFU g^{-1} and *D. hansenii* at 10^3 CFU g^{-1}, along with the CD. The obtained values indicate no significant differences in the diversity of the microbiota present in the intestines of fish fed with *C. tropicalis* and *D. hansenii* compared to those fed with the CD. However, there is a trend toward higher values with *C. tropicalis* (Table 6).

The intestinal microbiota of *A. tropicus* juveniles fed with *C. tropicalis*, *D. hansenii*, and the CD comprised two dominant phyla: Tenericutes and Proteobacteria (Fig. 3). In fish fed with the CD and *D. hansenii*, the phylum Tenericutes predominated (82.43 and 78.80%, respectively), whereas in fish fed with *C. tropicalis*, it was found at a lower percentage of only 23.19%. In contrast, the phylum Proteobacteria was predominant in fish fed with *C. tropicalis* (76.22%), while in fish fed with the CD and *D. hansenii*, it was only found at 17.14 and 20.86%, respectively. Additionally, less than 1% of the microbiota in fish fed with both yeast treatments and the CD belonged to the phylum Firmicutes (CD 0.366%, *C. tropicalis* 0.397%, and *D. hansenii* 0.981%) (Fig. 3).

Eight families were identified, including Mycoplasmataceae, Desulfovibrionaceae, Moraxellaceae, and Neisseriaceae, which were found in the fish of the three treatments (Fig. 4). In fish fed with the CD and *D. hansenii*, the Mycoplasmataceae family predominated with 82 and 77%, respectively, while in fish fed with *C. tropicalis*, it was found at a lower percentage of only 23.19%. In contrast, the Desulfovibrionaceae family was predominantly found in fish fed with the yeast *C. tropicalis* at 75.33%; in contrast, in fish fed with the CD and *D. hansenii*, it was only found at 19.66 and 12.11%, respectively. The Moraxellaceae and Neisseriaceae families were present at percentages below 1% in fish from all treatments. As for the Enterobacteriaceae and Erysipelotrichaceae families, these were only found in fish fed with *D. hansenii*, although at less than 1% (Fig. 4).

Figure 5 shows the genus present in the intestinal microbiota of the evaluated fish. A total of six genera were identified, with *Mycoplasma* being the most abundant of the three treatments. In fish fed with the CD and *D. hansenii*, the genus *Mycoplasma* predominated with 82.03 and 77.42%, respectively, while in fish fed with *C. tropicalis*, it was found at a lower percentage of only 23.19%. Conversely, the genus *Desulfovibrio* was found at a greater percentage in fish fed with *C. tropicalis* (75.13%) than those fed with the CD and *D. hansenii* (12.12 and 19.67%, respectively). The genus *Acinetobacter* was observed in lower proportions, less than 5% in fish fed with the CD, while in fish fed with *C. tropicalis*, it was found at only 0.047%.

Community structure of the bacterial microbiota

The β-diversity index values showed significant differences ($P < 0.05$) in the intestinal microbiota of fish fed with *C. tropicalis* and *D. hansenii* compared to those fed with the CD. The distance

matrix calculated using the Bray-Curtis index indicated a total variability of 99.74% (P = 0.0038). In contrast, the Jaccard index revealed an accumulated variance of 62.37% (P = 0.004) (Fig. 6b). The weighted UniFrac metric recorded a total variance of 99.87% (P = 0.0048) (Fig. 6c). In comparison, the unweighted UniFrac metric showed a total variance of 74.23 % (P = 0.0042) (Fig. 6d).

DISCUSSION

The effect of probiotic yeasts on host growth largely depends on the type and concentration administered. This study showed positive effects on *A. tropicus* juveniles fed with diets supplemented with the lowest doses of *D. hansenii* (10^3 CFU g^{-1}) and *C. tropicalis* (10^4 CFU g^{-1}). These fish exhibited higher weight, total length, AWG, SGR, and S rates than those fed the CD. However, higher levels of both yeasts resulted in similar or worse growth and feed utilization parameters than those observed with the CD. This finding aligns with the results of Hernández-López et al. (2021), who demonstrated that high concentrations of *D. hansenii* (10^{14}, 10^{15}, and 10^{16} CFU g^{-1}) in the diet of *A. tropicus* juveniles have adverse effects on the growth and survival. The negative impact on growth and physiological variables is attributed to the high yeast concentrations, which produce elevated levels of polyamines that can be toxic, impair growth and development, and displace the resident microbiota (Tovar-Ramírez et al. 2004). FI was evaluated in this study to understand these effects further. The results showed that fish fed all three concentrations of *C. tropicalis* and the highest concentration (10^7 FCU g^{-1}) of *D. hansenii* exhibited lower FI values than the CD, which indicates that these yeast-supplemented diets might lead to reduced feed consumption.

Interestingly, despite the lower FI, significant growth improvement and better FCR were observed only in fish fed with the lowest concentrations of both yeasts, suggesting that while higher concentrations of yeasts may reduce FI, the optimal growth-promoting effects are achieved at lower concentrations, possibly due to better nutrient utilization or enhanced metabolic efficiency at these levels. Regarding K, our results showed that fish fed with *C. tropicalis* (10^4 and 10^5 CFU g^{-1}) obtained a lower K value than those fed with CD. Moreover, in the fish fed with the three concentrations of *D. hansenii*, K was significantly lower than CD. Although the K values are below

the CD value, they agree with the values reported in juveniles of *A. tropicus* by Nájera-Arzola et al. (2018), Sepúlveda-Quiroz et al. (2020) and Hernández-López et al. (2021), which could represent a standard value or range for K in juveniles of this species. Positive effects on the growth and survival of fish fed with *D. hansenii* (5.7%) have also been reported in European seabass (*D. labrax*) larvae (Tovar-Ramírez et al. 2004), greater amberjack (*Seriola rivoliana*) (Teles et al. 2022), and gilthead seabream (*S. aurata*) (Sanahuja et al. 2023).

Regarding aquaculture production, higher HSI and VSI values could indicate compromised muscle growth and higher fat storage. In this study, diets supplemented with 10^4 and 10^6 FCU g^{-1} of *C. tropicalis* and all three concentrations of *D. hansenii* showed significantly lower VSI values than the CD. Both yeasts might contribute to reduced visceral fat accumulation, which improves muscle growth quality. However, no significant differences were found in HSI values between fish fed with *C. tropicalis* and *D. hansenii*, compared to the CD, indicating that the yeasts do not significantly affect hepatic fat storage.

In addition to the beneficial effect on growth, this study observed that fish fed with all three concentrations of *C. tropicalis* and the two highest concentrations of *D. hansenii* (10^5 and 10^7 CFU g^{-1}) showed an increase in amylase enzymatic activity. Additionally, lipase activity was increased in fish fed with both yeasts compared to the CD. Fish fed with all three concentrations of *C. tropicalis* and *D. hansenii* at 10^3 and 10^7 CFU g^{-1} showed lower values of trypsin, chymotrypsin, and leucine aminopeptidase activity than those fed the CD. The lower enzymatic activity observed could be due to these yeasts altering the gut environment or microbiota composition, leading to changes in enzyme expression. However, the yeasts might compensate for the reduced enzymatic activity by providing essential nutrients or bioactive compounds that support growth through alternative metabolic pathways, ensuring that overall growth performance remains unaffected.

Since the best results in terms of growth, survival, and digestive enzymatic activity were observed in fish fed with the lowest concentrations of *C. tropicalis* (10^4 CFU g^{-1}) and *D. hansenni* (10^3 CFU g^{-1}) inclusion, these specimens were selected for the evaluation of their effect on the expression of genes related to intestinal barrier function (both physical and immunological) and to determine the composition of the intestinal microbiota. Gene expression results showed that the yeast *C. tropicalis* did not favor intestinal barrier function. However, it did not promote changes

in the expression of the *muc-2* gene (main mucin of the mucus layer), it did lead to a reduction in the expression of the *zo-1* gene (responsible for maintaining tight junctions between intestinal epithelial cells), which likely resulted in increased intestinal permeability, triggering an adverse immune response by stimulating the overexpression of the proinflammatory cytokine *il-8*. In contrast, a favorable response was observed in the fish fed with the diet supplemented with the probiotic yeast *D. hansenii*. The results demonstrated an increase in the expression of *muc-2*, *zo-1*, and the anti-inflammatory cytokine *il-10* (an important marker for the host's health status).

Additionally, it contributed to the reduction of *il-8* compared to the CD. This expression pattern could indicate improved mucus layer synthesis and reinforcement of junctions between epithelial cells, enhancing the intestinal barrier function and improving the immune response. The exact mechanism of immune system regulation promoted by yeasts is not known with certainty; however, it has been highlighted that components of their cell wall, such as β-glucans and mannan oligosaccharides, modulate it (Torrecillas et al. 2014). Particularly in *A. tropicus*, it has been reported that dietary administration of 1.0 and 1.5% β-glucan to juvenile *A. tropicus* promotes positive *il-10* expression (Nieves-Rodríguez et al. 2018), while in the larval stage, the inclusion of 0.4% promotes *lys* expression (Cigarroa-Ruiz et al. 2023).

In this study, we observed that the intestinal microbiota of *A. tropicus* juveniles obtained from induced spawning and reared under controlled conditions was primarily composed of Tenericutes and Proteobacteria. Although a low diversity was found, this is common in cultured fish compared to wild fish because the evaluated fish were in an early developmental stage (juveniles from 38 to 83 DPH). Wild fish typically have a more diverse microbiota than pond-cultured fish because their diet is more varied due to the abundance of natural food resources (Sun et al. 2021). The most dominant phyla in fish microbiota include Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, and Fusobacteria (Arumugam et al. 2011, Arboleya et al. 2012). In freshwater fish, the dominant phyla are typically Proteobacteria, Firmicutes, and Actinobacteria (Nayak, 2010, Talwar et al. 2018, Wang et al. 2018). In the case of *A. tropicus* juveniles isolated from the natural environment, it has been described that their microbiota mainly consists of the phyla Firmicutes, Proteobacteria, and Bacteroidetes.

In contrast, the phylum Actinobacteria is also found in cultured juveniles (Méndez-Pérez et al. 2020). Deng et al. (2019) showed that the most abundant phyla in the intestine of cultured

Acanthopagrus schlegelii are Firmicutes and Proteobacteria, which aligns with our results. In *A. tropicus* juveniles fed with the CD, Tenericutes predominated at 82.43% compared to 17.14% for Proteobacteria. A similar abundance of Tenericutes was also observed in fish fed with *D. hansenii* (78.80%), with 20.86% for Proteobacteria. However, there was an evident change in the microbiota composition in fish fed with *C. tropicalis*, where the Tenericutes-Proteobacteria ratio was reversed (30% Tenericutes and 70% Proteobacteria). This opposite composition in the microbiota could be related to the negative gene expression response observed in fish fed with the yeast *C. tropicalis*, suggesting that this yeast leads to a decrease in beneficial microorganisms and an increase in microorganisms associated with the development of pathologies, a condition known as dysbiosis (Villamil et al. 2020, Yukgehnaish et al. 2020).

Conversely, the predominance of Tenericutes in fish fed with probiotic yeasts has also been reported in other fish species, such as rainbow trout (*O. mykiss*), where the inclusion of *S. cerevisiae* induced a predominance of Tenericutes (83%) compared to Proteobacteria (25%) (Huyben et al. 2018). Bacteria from the phylum Tenericutes are primarily considered mutualistic symbionts in the host's intestine (Wang et al. 2020). Mycoplasmataceae was the most abundant family in the microbiota composition of fish fed with the CD and *D. hansenii*, in contrast to fish fed with *C. tropicalis*. Members of the Mycoplasmataceae family inhabit symbiotically and commensally in rainbow trout, contributing to better digestion by producing lactic and acetic acid as energy sources for intestinal bacteria (Stadtländer et al. 1995). Particularly, *Mycoplasma* was the most abundant genus in fish fed with the CD and with *D. hansenii*, in contrast to fish fed with *C. tropicalis*. *Mycoplasma* is considered a symbiont in the host's intestine, not a pathogen (Huyben et al. 2021). It also predominates in other species, such as Atlantic salmon (*S. salar*) (Huyben et al. 2021, Rasmussen et al. 2021, Zhou et al. 2022) and rainbow trout (Lyons et al. 2017).

Additionally, we observed a significant change in the proportion of the genus *Desulfovibrio* in fish fed with the CD (19%) and *D. hansenii* (12%), compared to those fed with *C. tropicalis* (75%). Desulfovibrionaceae (sulfate-reducing bacteria) have been described as modifying the host's metabolic balance and potentially causing dysfunction in the intestinal barrier, promoting an inflammatory process (Carbonero et al. 2012), which aligns with the gene expression response pattern observed in fish fed with *C. tropicalis*. The positive effect observed in *A. tropicus* juveniles administered *C. tropicalis* (10^4 CFU g^{-1}) in terms of growth and production parameters is likely a

result of the duration of the administration period (45 days). However, negative effects were observed on the expression of genes related to intestinal barrier function (tight junctions and the immune system) and the composition of the intestinal microbiota (dysbiosis). It is unknown whether a more extended administration period could affect fish growth and generate an inflammatory response. Therefore, extending the administration period of *C. tropicalis* to other stages of fish development is recommended to assess the effects that may occur over time.

Given that dysbiosis and compromised intestinal barrier function can lead to increased susceptibility to infections, evaluating the antagonistic activity of the yeasts against fish pathogens is crucial. Some authors report that yeasts from the genera *Saccharomyces*, *Candida*, *Cryptococcus*, *Debaryomyces*, *Pichia*, *Torulopsis*, and *Zygosaccharomyces* release antimicrobials and killer toxins that inhibit the growth and proliferation of certain pathogenic microorganisms (Liu et al. 2013, Wang et al. 2018). In this study, we evaluated the *in vitro* antagonistic capability of *C. tropicalis* and *D. hansenii* against seven pathogenic bacteria, which have been reported as significant pathogens in fish (*Aeromonas hydrophila*, *A. dhakensis*, *A. ichthiosmia*, *Staphylococcus arlettae* (two strains), *Vibrio harveyi*, *V. campbelli*, and *Photobacterium damselae*). Our results confirmed that both yeasts exhibit specific antagonistic activity against pathogens. *C. tropicalis* showed a broad-spectrum antagonistic effect, inhibiting both Gram-negative bacteria (*A. ichthiosmia* and *V. harveyi*) and Gram-positive bacteria (*S. arlettae*), while *D. hansenii* showed highly selective activity (only against *P. damselae*). The broader antagonistic effect observed in *C. tropicalis* could be attributed to its potential higher production of antimicrobial compounds (Siangpro et al. 2023), its ability to compete more effectively for nutrients and space, or the presence of specific killer toxins that might target a wider range of bacterial pathogens.

CONCLUSIONS

The present study demonstrates that incorporating low doses of the yeasts *C. tropicalis* (10^4 CFU g^{-1}) and *D. hansenii* (10^3 CFU g^{-1}) into the diet improves the growth, survival, and lipase activity in juveniles of *A. tropicus*. The inclusion of *C. tropicalis* in diets, even at low concentrations, induces a shift in the structure of the intestinal microbiota, promoting an increase in the Desulfovibrionaceae family, which suggests a relationship with the downregulation of *zo-1*

expression and an increase in *il-8* expression. In contrast, yeast *D. hansenii* positively affects the expression of intestinal barrier genes, increasing *muc-2*, *zo-1*, and *il-10* expression while decreasing the *il-8* expression. However, it is not accompanied by changes in the composition of the core microbiota, where *Mycoplasma* predominates. Both yeasts showed specific *in vitro* antagonistic activity against pathogens, with *C. tropicalis* exhibiting a broad-spectrum antagonistic effect. Therefore, the dietary inclusion of *C. tropicalis* did not produce favorable outcomes for the intestinal health of the fish. On the other hand, including 10^3 CFU g^{-1} of *D. hansenii* in diets is recommended to promote better development, health, and disease resistance in *A. tropicus* juveniles.

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Ethics approval

The animals used in this study were handled following the technical specifications for the care and use of laboratory animals, as stated in the Official Mexican Standard NOM-062-ZOO-1999 from the Ministry of Agriculture, Livestock, Rural Development, Fisheries, and Food.

Author contribution

G.M. Pérez Jiménez: methodology, writing original draft, writing, review and editing; S. De la Rosa García: conceptualization, resources, formal analysis, writing original draft, writing, review and editing; M. Martínez Porcha: formal analysis, writing, review and editing; C.A. Sepúlveda-Quiroz: methodology, formal analysis, writing, review and editing; E. Garibay Valdez: methodology, formal analysis, writing, review and editing; D. Tovar Ramirez: formal analysis, review and editing; L.D. Jiménez Martínez: methodology, writing, review and editing; C.S.

Alvarez Villagomez: conceptualization, formal analysis, resources, original draft, writing, review and editing; C.A. Alvarez Gonzalez: formal analysis, writing, review and editing.

Conflict of interest

All authors declared they have no conflict of interest.

REFERENCES

Angulo, M., Reyes-Becerril, M., Medina-Córdova, N., Tovar-Ramírez, D. & Angulo, C. 2020. Probiotic and nutritional effects of *Debaryomyces hansenii* on animals. Applied Microbiology and Biotechnology, 104: 7689-7699. doi: 10.1007/s00253-020-10780-z

Association of Official Analytical Chemists (AOAC). Official methods of analysis. AOAC, Arlington.

Arboleya, S., Binetti, A., Salazar, N., Fernández, N., Solís, G., Hernández-Barranco, A., et al. 2012. Establishment and development of intestinal microbiota in preterm neonates. FEMS Microbiology Ecology, 79: 763-772. doi: 10.1111/j.1574-6941.2011.01261.x

Arumugam, M., Raes, J., Pelletier, E., Paslier, D., Yamada, T., Mende, D., et al. 2011. Enterotypes of the human gut microbiome. Nature, 473: 174-180. doi: 10.1038/nature09944

Bolyen, E., Rideout, J., Dillon, M., Bokulich, N., Abnet, C., Al-Ghalith, G., et al. 2019. Reproducible, interactive, scalable, and extensible microbiome data science using QIIME 2. Nature Biotechnology, 37: 852-857. doi: 10.1038/s41587-019-0209-9

Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72: 248-254. doi: 10.1006/abio.1976.9999

Campos, M.M. & Campos, C.R. 2017. Applications of quartering method in soils and foods. International Journal of Engineering Research and Applications, 7: 35-39. doi: 10.9790/9622-0701023539

Caporaso, J., Lauber, C., Walters, W., Berg-Lyons, D., Lozupone, C., Turnbaugh, P., et al. 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Journal of Biological Sciences, 108: 4516-4522. doi: 10.1073/pnas.1000080107

Carbonero, F., Benefiel, A., Alizadeh-Ghamsari, A. & Gaskins, H. 2012. Microbial pathways in colonic sulfur metabolism and links with health and disease. Frontiers in Physiology, 3: 00448. doi: 10.3389/fphys.2012.00448

Caruffo, M., Navarrete, N., Salgado, O., Díaz, A., López, P., García, K., et al. 2015. Potential probiotic yeasts isolated from the fish gut protect zebrafish (*Danio rerio*) from a *Vibrio anguillarum* challenge. Frontiers in Microbiology, 6: 1093. doi: 10.3389/fmicb.2015.010933

Cigarroa-Ruiz, L., Toledo-Solís, F., Frías-Gómez, S., Guerrero-Zárate, R., Camarillo-Coop, S., Álvarez-Villagómez, C., et al. 2023. Addition of β-glucans in diets for tropical gar (*Atractosteus tropicus*) larvae: effects on growth, digestive enzymes and gene expression of intestinal epithelial integrity and immune system. Fish Physiology and Biochemistry, 49: 612-626. doi: 10.1007/s10695-023-01207-3

Delmar, E., Largman, C., Brodrick, J. & Geokas, M. 1979. A sensitive new substrate for chymotrypsin. Analytical Biochemistry, 99: 316-320. doi: 10.1016/s0003-2697(79)80013-5

Deng, H., Liu, F., Li, G., Bao, W., Zou, S., Zheng, P., et al. 2019. Response of gut bacterial community in black sea bream to different feather meal in feed. Oceanologia et Limnologia Sinica, 50: 1309-1317. doi: 10.11693/hyhz20190400072

El-Saadony, M., Alagawany, M., Patra, A., Kar, I., Tiwari, R., Dawood, M., et al. 2021. The functionality of probiotics in aquaculture: an overview. Fish and Shellfish Immunology, 117: 36-52. doi: 10.1016/j.fsi.2021.07.007

Erlanger, B., Kokowsky, N. & Cohen, W. 1961. The preparation and properties of two new chromogenic substrates of trypsin. Archives of Biochemistry and Biophysics, 95: 271-278. doi: 10.1016/0003-9861(61)90145-x

Frías-Quintana, C., Domínguez-Lorenzo, J., Álvarez-González, C.A., Tovar-Ramírez, D. & Martínez-García, R. 2016. Using cornstarch in microparticulate diets for larvicultured tropical gar (*Atractosteus tropicus*). Fish Physiology and Biochemistry, 42: 517-528. doi: 10.1007/s10695-015-0156-4

Gjellesvik, D., Lombardo, D. & Walther, B. 1992. Pancreatic bile salt dependent lipase from cod (*Gadus morhua*): Purification and properties. Biochimica et Biophysica Acta, 1124: 123-134. doi: 10.1016/0005-2760(92)90088-d

Gram, L., Melchiorsen, J., Spanggaard, B., Huber, I. & Nielsen, T. 1999. Inhibition of *Vibrio anguillarum* by *Pseudomonas fluorescens* AH2, a possible probiotic treatment of fish. Applied and Environmental Microbiology, 65: 969-973. doi: 10.1128/AEM.65.3.969-973.1999

Hernández-López, I., Tovar-Ramírez, D., De La Rosa-García, S., Álvarez-Villagomez, C., Asencio-Alcudia, G., Martínez-Burguete, T., et al. 2021. Dietary live yeast (*Debaryomyces hansenii*) provides no advantages in tropical gar, *Atractosteus tropicus* (Actinopterygii: Lepisosteiformes: Lepisosteidae), juvenile aquaculture. Acta Ichthyologica et Piscatoria, 51: 311-320. doi: 10.3897/aiep.51.67095

Hoben, H. & Somasegaran, P. 1982. Comparison of the pour, spread, and drop plate methods for enumeration of *Rhizobium* spp. in inoculants made from presterilized peat. Applied and Environmental Microbiology, 44: 1246-1247.

Huyben, D., Grobler, T., Mattew, C., Bou, M., Ruyter, G. & Glencross, B. 2021. Requirement for omega-3 long-chain polyunsaturated fatty acids by Atlantic salmon is relative to the dietary lipid level. Aquaculture, 531: 736895. doi: 10.1016/j.aquaculture.2020.735805

Huyben, D., Sun, L., Moccia, R., Kiessling, A., Dicksved, J. & Lundh, T. 2018. Dietary live yeast and increased water temperature influence the gut microbiota of rainbow trout. Journal of Applied Microbiology, 124: 1377-1392. doi: 10.1111/jam.13738.

Jiménez-Martínez, L., Morales, V., Frías-Quintana, C., Castillo, A., Ascencio-Alcudia, G., Álvarez-Villagomez, C., et al. 2021. Quality evaluation of reference gene expression on different tissues in adults of tropical gar *Atractosteus tropicus*. Pakistan Journal of Zoology, 54: 363-372. doi: 10.17582/journal.pjz/20200913180928

Liu, G., Chi, Z., Wang, G., Wang, Z. & Li, Y. 2013. Yeast killer toxins, molecular mechanisms of their action and their applications. Critical Reviews in Biotechnology, 35: 222-234. doi: 10.3109/07388551.2013.833582

Livak, K. & Schmittgen, T. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ Method. Methods, 25: 402-408. doi: 10.1006/meth.2001.1262

Lokesh, J., Fernandes, J., Korsnes, K., Bergh, Ø., Brinchmann, M. & Kiron, V. 2012. Transcriptional regulation of cytokines in the intestine of Atlantic cod fed yeast derived mannan oligosaccharide or β-glucan and challenged with *Vibrio anguillarum*. Fish and Shellfish Immunology, 33: 626-631. doi: 10.1016/j.fsi.2012.06.017

Lyons, P., Turnbull, J., Dawson, K. & Crumlish, M. 2017. Phylogenetic and functional characterization of the distal intestinal microbiome of rainbow trout *Oncorhynchus mykiss* from both farm and aquarium settings. Journal of Applied Microbiology, 122: 347-363. doi: 10.1111/jam.13347

Maroux, S., Louvard, D. & Barath, J. 1973, The aminopeptidase from hog intestinal brush border. Biochimica et Biophysica Acta - Enzymology, 321: 282-295. doi: 10.1016/0005-2744(73)90083-1

Márquez-Couturier, G. & Vázquez-Navarrete, C. 2015. Estado del arte de la biología y cultivo de pejelagarto (*Atractosteus tropicus*). Agroproductividad, 8: 44-51.

Méndez-Pérez, R., García-López, R., Bautista-López, J., Vázquez-Castellanos, J., Álvarez-González, C.A., Peña-Marín, E., et al. 2020. High-throughput sequencing of the 16S rRNA gene to analyze the gut microbiome in juvenile and adult tropical gar (*Atractosteus tropicus*). Latin American Journal of Aquatic Research, 48: 456-479. doi: 10.3856/vol48-issue3-fulltext-2419

Merrifield, D., Dimitroglou, A., Foey, A., Davies, S., Baker, R., Bøgwald, J., et al. 2010. The current status and future focus of probiotic and prebiotic applications for salmonids. Aquaculture, 302: 1-18. doi: 10.1016/j.aquaculture.2010.02.007

Miyake, S., Ngugi, D. & Stingl, U. 2015. Diet strongly influences the gut microbiota of surgeonfishes. Molecular Ecology, 24: 656-672. doi: 10.1111/mec.13050

Najera-Arzola, I., Álvarez-González, C.A., Frías-Quintana, E. & Peña, E. 2018. Evaluation of Mannan oligosaccharides (MOS) in balanced diets for tropical gar juveniles (*Atractosteus tropicus*). Hidrobiológica, 28: 239-246. doi: 10.24275/uam/izt/dcbs/hidro/2018v28n3/pena

Navarrete, P. & Tovar-Ramírez, D. 2014. Use of yeasts as probiotics in fish aquaculture. In: Hernández, M. & Pérez-Rostro, C. (Eds.). Sustainable aquaculture technique. IntechOpen, London, pp. 135-171.

Nayak, S. 2010. Probiotics and immunity: A fish perspective. Fish and Shellfish Immunology, 29: 2-14. doi: 10.1016/j.fsi.2010.02.017

Nieves-Rodríguez, K., Álvarez-González, C.A., Peña-Marín, E., Vega-Villasante, F., Martínez-García, R., Camarillo-Coop, S., et al. 2018. Effect of β-glucans in diets on growth, survival, digestive enzyme activity, and immune system and intestinal barrier gene expression for tropical gar (*Atractosteus tropicus*) juveniles. Fishes, 3: 27. doi: 10.3390/fishes3030027

Pantelides, I.S., Christou, O., Tsolakidou, M.D., Tsaltas, D. & Ioannou, N. 2015. Isolation, identification and *in vitro* screening of grapevine yeasts for the control of black aspergilli on grapes. Biological Control, 88: 46-53.

Pérez-Jiménez, G., Peña-Marín, E., Maytorena-Verdugo, C., Sepúlveda-Quiroz, C., Jiménez-Martínez, L., De La Rosa-García, S., et al. 2022. Incorporation of fructooligosaccharides in diets influence growth performance, digestive enzyme activity, and expression of intestinal barrier function genes in tropical gar (*Atractosteus tropicus*) larvae. Fishes, 7: 137. doi: 10.3390/fishes7030137

Rasmussen, J., Villumsen, K., Duchêne, D., Puetz, L., Delmont, T., Sveier, H., et al. 2021. Genome-resolved metagenomics suggests a mutualistic relationship between *Mycoplasma* and salmonid hosts. Communications Biology, 4: 579. doi: 10.1038/s42003-021-02105-1

Robyt, J. & Whelan, W. 1968. The α-amylases and the β-amylases. In: Radley, J.A. (Ed.). Starch and its derivates. Chapman and Hall, London, pp. 432-497.

Rohani, F., Islam, M., Hossain, K., Ferdous, Z., Siddik, A., Nuruzzaman, M., et al. 2022. Probiotics, prebiotics and synbiotics improved the functionality of aquafeed: upgrading growth, reproduction, immunity and disease resistance in fish. Fish and Shellfish Immunology, 120: 569-589. doi: 10.1016/J.FSI.2021.12.037

Sanahuja, I., Ruiz, A., Firmino, J.P., Reyes-López, F.E., Ortiz-Delgado, J.B., Vallejos-Vidal, E., et al. 2023. *Debaryomyces hansenii* supplementation in low fish meal diets promotes growth, modulates microbiota, and enhances intestinal condition in juvenile marine fish. Journal of Animal Science and Biotechnology, 14: 1. doi: 10.1186/s40104 -023-00895-4

Sepúlveda-Quiroz, C.A., Peña-Marín, E.S., Pérez-Morales, A., Martínez-García, R., Alvarez-Villagomez, C.S., Maytorena-Verdugo, C.I., et al. 2020. Fructooligosaccharide supplementation in diets for tropical gar (*Atractosteus tropicus*) juvenile: Effects on

morphophysiology and intestinal barrier function. Aquaculture Research, 52: 37-50. doi: 10.1111/are.14867

Siangpro, N., Chuakrut, S., Sirimanapong, W., Tanasupawat, S., Phongsopitanun, W., Meksiriporn, B., et al. 2023. *Lactiplantibacillus argentoratensis* and *Candida tropicalis* isolated from the gastrointestinal tract of fish exhibited inhibitory effects against pathogenic bacteria of Nile tilapia. Veterinary Sciences, 10: 129. doi: 10.3390/vetsci10020129

Stadtländer, C., Lotz, W., Körting, W. & Kirchhoff, H. 1995. Piscine gill epithelial cell necrosis due to *Mycoplasma* mobile strain 163 K: Comparison of in-vivo and *in-vitro* infection. Journal of Comparative Pathology, 112: 351-359. doi: 10.1016/s0021-9975(05)80016-7

Sun, P., Zhang, H., Jiang, Y., Gao, Q., Tang, B., Ling, J., et al. 2021. Relationships between the gut microbiota of juvenile black sea bream (*Acanthopagrus schlegelii*) and associated environment compartments in different habitats. Microorganisms, 9: 2557. doi: 10.3390/microorganisms9122557

Talwar, C., Nagar, S., Lal, R. & Negi, R. 2018. Fish gut microbiome: Current approaches and future perspectives. Indian Journal of Microbiology, 58: 397-414. doi: 10.1007/s12088-018-0760-y

Teles, A., Álvarez-González, C.A., Llera-Herrera, R., Gisbert, E., Salas-Leiva, J., Rodríguez-Jaramillo, M., et al. 2022. *Debaryomyces hansenii* CBS 8339 promotes larval development in *Seriola rivoliana*. Aquaculture, 560: 738587. doi: 10.1016/j.aquaculture.2022.738587

Torrecillas, S., Montero, D. & Izquierdo, M. 2014. Improved health and growth of fish fed mannan oligosaccharides: potential mode of action. Fish and Shellfish Immunology, 36: 525-544. doi: 10.1016/j.fsi.2013.12.029

Tovar-Ramírez, D., Infante, J., Cahu, C., Gatesoupe, F. & Vázquez-Juárez, R. 2004. Influence of dietary live yeast on European sea bass (*Dicentrarchus labrax*) larval development. Aquaculture, 234: 415-427. doi: 10.1016/j.aquaculture.2004.01.028

Vargas-Albores, F., Martínez-Córdova, L., Hernández-Mendoza, A., Cicala, F., Lago-Lestón, A. & Martínez-Porchas, M. 2021. Therapeutic modulation of fish gut microbiota, a feasible strategy for aquaculture? Aquaculture, 544: 737050. doi: 10.1016/j.aquaculture.2021.737050

Vázquez-Baeza, Y., Pirrung, M., González, A. & Knight, R. 2013. EMPeror: a tool for visualizing high-throughput microbial community data. Gigascience, 2: 16. doi: 10.1186/2047-217x-2-16

Verschuere, L., Rombaut, G., Sorgeloos, P. & Verstraete, W. 2000. Probiotic bacteria as biological control agents in aquaculture. Microbiology and Molecular Biology Reviews, 64: 655-671. doi: 10.1128/MMBR.64.4.655-671.2000

Villamil, S., Huerlimann, R. & Jerry, D. 2020. Microbiome diversity and dysbiosis in aquaculture. Reviews in Aquaculture, 13: 1077-1096. doi: 0.1111/raq.12513

Wang, A., Ran, C., Ringø, E. & Zhou, Z. 2018. Progress in fish gastrointestinal microbiota research. Reviews in Aquaculture, 10: 626-640. doi: 10.1111/raq.12191

Wang, Y., Huang, J., Zhou, Y., Moissl-Eichinger, C., Finn, R., Danchin, A., et al. 2020. Phylogenomics of expanding uncultured environmental Tenericutes provides insights into their pathogenicity and evolutionary relationship with Bacilli. BMC Genomics, 21: 408. doi: 10.1186/s12864-020-06807-4

Yukgehnaish, K., Kumar, P., Sivachandran, P., Marimuthu, K., Arshad, A., Paray, B., et al. 2020. Gut microbiota metagenomics in aquaculture: factors influencing gut microbiome and its physiological role in fish. Reviews in Aquaculture, 12: 1903-1927. doi: 10.1111/raq.12416

Zhou, C., Yang, S., Ka, W., Gao, P., Li, Y., Joseph, S., et al. 2022. Association of gut microbiota with metabolism in rainbow trout under acute heat stress. Frontiers in Microbiology, 13: 846336. doi: 10.3389/fmicb.2022.846336

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Table 1. Composition of experimental diets supplemented with the yeasts and the control diet.

Ingredients (g kg^{-1})	Control diet	*Candida tropicalis*			*Debaryomyces hansenii*		
		10^4	10^5	10^6	10^3	10^5	10^7
Fish meal[a]	305.4	305.4	305.4	305.4	305.4	305.4	305.4
Poultry meal[a]	150	150	150	150	150	150	150
Pork meal[a]	150	150	150	150	150	150	150
Soybean meal[a]	150	150	150	150	150	150	150
Fish oil[a]	34.95	34.95	34.95	34.95	34.95	34.95	34.95
Starch[b]	124.7	124.7	124.62	117.2	124.7	124.55	109.7
Soy lecithin[b]	10	10	10	10	10	10	10
Soybean oil[c]	34.95	34.95	34.95	34.95	34.95	34.95	34.95
Candida tropicalis	0	0.00075	0.075	7.5	0	0	0
Debaryomyces hansenii	0	0	0	0	0.0015	0.15	15
Mineral premix[d]	5	5	5	5	5	5	5
Vitaminic premix[d]	10	10	10	10	10	10	10
Vitamin C[e]	5	5	5	5	5	5	5
Grenetin[f]	20	20	20	20	20	20	20
Proximate composition (g kg^{-1} dry matter)							
Protein	45	45	45	45	45	45	45
Ether extract	13.82	15.00	15.25	16.17	15.76	16.41	13.08
Ash	10.80	11.04	11.10	11.51	11.76	10.68	11.17

[a]Marine and agricultural proteins SA de CV, Guadalajara, Jalisco; [b]Pronat Ultra, Merida, Yucatan, Mexico; [c]Ragasa Industries SA de CV; [d]Vitamin premix composition g mg^{-1} or international units per kg of diet: vitamin A, 10,000,000 IU; vitamin D3, 2,000,000 IU; vitamin E, 100,000 IU; vitamin K3, 4.0 g; Thiamine B1, 8.0 g; riboflavin B2, 8.7 g; pyridoxine B6, 7.3 g; vitamin B12, 20.0 mg; Niacin, 50.0 g; pantothenic acid, 22.2 g; Inositol, 0.15 mg; nicotinic acid, 0.16 mg; folic acid, 4.0 g; biotin, 500 mg; vitamin C, 10.0 g; choline 0.3 mg, excipient q.s. 2 g; manganese, 10 g; magnesium, 4.5 g; zinc, 1.6 g; iron, 0.2 g; copper, 0.2 g; iodine, 0.5 g; selenium, 40 mg; cobalt 60 mg. Excipient q.s. 1.5 g; [e]ROVIMIX® STAY-C® 35 -DSM, Guadalajara, Mexico; [f]D´gari, food and diet products relámpago, S.A. de C.V.

Table 2. Primers used for qPCR analysis.

Target Gene	Gene function	Primer Sequence (5'- 3')	Amplification Efficiency (%)	Amplicon size (bp)	Reference
muc-2	mucus layer protein (mucina 2)	FW: GGCCTCCTCAAGAGCACGGTG RV:TCTGCACGCTGGAGCACTCAATG	90.94	100	Nieves-Rodríguez et al. (2018)
zo-1	tight junction protein	FW: TGTGCCTCAGATCACTCCAC RV: AAAGGCAGAGGGTTGGCTTC	98.58	123	Pérez-Jiménez et al. (2022)
lyz	bacteriolytic	FW: CACTGCAGCCATCAATCACAAC RV: ATTAGTCAGCAGCTTGCTGCAG	89.91	100	Nieves-Rodríguez et al. (2018)
il-8	proinflammatory cytokine	FW: ATATTCACTGGTGGGCGGAG RV: GTGCGGCCTGAGATTGTTT	94.18	369	Pérez-Jiménez et al. (2022)
il-10	anti-inflam-matory cytokine	FW: TTATAAAGCCATGGGGGAGCTG RV: CTGCACAGTCTGCCTCTAGT	94.47	91	This study
β-actin	cytoskeletic actin	FW:GAGCTATGAGCTGCCTGAGTGG RV:GTGGTCTCATGAATGCCACAGG	97.10	119	Jiménez-Martínez et al. (2021)

Table 3. Antagonistic activity of *Candida tropicalis* and *Debaryomyces hansenii* against pathogenic fish strains.

Bacterial test	Zone of inhibition in diameter (mm)	
	Candida tropicalis	*Debaryomyces hansenii*
Aeromona hydrophila (NCIBM1134)	ND	ND
Aeromona dhakensis (CAIM 1873)	ND	ND
Aeromona ichtiosmia (CAIM 1876)	13.0 ± 1.5	ND
Staphylococcus arlettae (CAIM 1658)	14.7 ± 1.3	ND
Staphylococcus arlettae (UJAT-02)	ND	ND
Vibrio harveyi (CAIM 1622)	13.5 ± 1.3	ND
Vibrio campbelli (CAIM 159)	ND	ND
Photobacterium damselae (CAIM 192)	ND	15.4 ± 1.8

The data is expressed as mean ± standard deviation. ND: antibacterial activity not detected.

Table 4. Growth performance, survival rate, and somatic indexes of *A. tropicus* juveniles fed diets supplemented with different concentrations of *C. tropicalis* and *D. hansenii* compared with the control diet..

	Control diet	*Candida tropicalis* (CFU g^{-1})			Control diet	*Debaryomyces hansenii* (CFU g^{-1})		
		10^4	10^5	10^6		10^3	10^5	10^7
FI (g d^{-1})	10.67 ± 0.33[a]	9.48 ± 0.40[b]	8.49 ± 0.23[c]	9.39 ± 0.23[b]	10.67 ± 0.33[a]	10.60 ± 0.33[a]	10.71 ± 0.21[a]	9.42 ± 0.21[b]
AWG (g $fish^{-1}$)	2.00 ± 0.34[b]	3.07 ± 0.20[a]	1.48 ± 0.13[bc]	1.20 ± 0.21[c]	2.00 ± 0.34[b]	3.07 ± 0.08[a]	2.13 ± 0.04[b]	1.89 ± 0.01[b]
SGR (% d^{-1})	5.07 ± 0.34[b]	5.97 ± 0.13[a]	4.50 ± 0.16[bc]	4.09 ± 0.32[c]	5.07 ± 0.34[b]	5.97 ± 0.05[a]	5.22 ± 0.04[b]	4.99 ± 0.01[b]
FCR	5.50 ± 0.96[b]	2. 95 ± 0.19[c]	5.61 ± 0.49[b]	8.09 ± 1.42[a]	5.50 ± 0.96[a]	3.55 ± 0.10[b]	5.13 ± 0.11[a]	5.04 ± 0.02[a]
PER	1.24 ± 0.06	1.03 ± 0.02	0.97 ± 0.19	1.16 ± 0.01	1.24 ± 0.06	1.25 ± 0.13	1.37 ± 0.31	1.26 ± 0.30
S (%)	14.81 ± 3.20	20.37 ± 3.20	14.81 ± 3.20	16.66 ± 5.55	14.81 ± 3.20[a]	25.92 ± 3.20[a]	14.81 ± 3.20[b]	20.37 ± 3.20[ab]
HSI	2.93 ± 1.72	4.49 ± 0.00	2.74 ± 0. 27	3.34 ± 1.69	2.93 ± 1.72	5.58 ± 1.17	4.62 ± 0.17	3.38 ± 0.48
VSI	15.55 ± 2.42[at]	13.03 ± 0.29[b]	17.36 ± 1.36[a]	7.59 ± 0.18[c]	15.55 ± 2.42[a]	10.47 ± 0.80[b]	11.22 ± 0.91[b]	10.08 ± 0.28[b]
K	0.47 ± 0.05	0.38 ± 0.01	0.37 ± 0.05	0.47 ± 0.02	0.47 ± 0.05[a]	0.34 ± 0.04[b]	0.33 ± 0.02[b]	0.35 ± 0.01[b]

Values are mean ± standard deviation. Significant differences within the treatments were compared separately for *C. tropicalis* and *D. hansenii* are indicated by different letters between experimental and control diets ($P < 0.05$). FI: feed intake; AWG: absolute weight gain; SGR: specific growth rate; FCR: feed conversion ratio; PER: the protein efficiency ratio; S: survival; HSI: hepatosomatic index; VSI: viscerosomatic index; K: condition factor

Table 5. Digestive enzymatic activities of *A. tropicus* juveniles fed diets supplemented with *C. tropicalis* and *D. hansenii* compared with the control diet.

Activities (U mg $protein^{-1}$)	Control diet	*Candida tropicalis* (CFU g^{-1})			Control diet	*Debaryomyces hansenii* (CFU g^{-1})		
		10^4	10^5	10^6		10^3	10^5	10^7
Trypsin	1.75 ± 0.08[a]	1.06 ± 0.05[b]	0.86 ± 0.01b	0.88 ± 0.00b	1.75 ± 0.08[b]	1.01 ± 0.01[d]	2.19 ± 0.02[a]	1.33 ± 0.05[c]
Chymotrypsin	40.72 ± 0.12[a]	28.76 ± 0.47[b]	14. 67 ± 2.15[c]	10.91 ± 0.82[d]	40.72 ± 0.12[b]	12.75 ± 0.37[d]	48. 15± 0.49[a]	38.87 ± 0.41[c]
Leucine aminopeptidase	254.36 ± 2.01[c]	108.64 ± 5.72[a]	80.39 ± 4.51[c]	68.18 ± 2.69[d]	254.36 ± 2.01[b]	90.15 ± 6.89[c]	444.69 ± 31.69[a]	163.98 ± 6.82[c]
Amylase	22.14 ± 0.35[c]	56.37 ± 4.03[b]	71. 02 ± 4.34[a]	24.27 ± 4.59[c]	22.14 ± 0.35[c]	20.25 ± 4.22[c]	100.75 ± 0.79[a]	71.23 ± 1.71[b]
Lipase	3.67 ± 0.75[b]	5.98 ± 0.92[b]	5.61 ± 0.07[b]	8.02 ± 0.61[a]	3.67 ± 0.75[c]	8.55 ± 1.13[a]	9.28 ± 0.13[a]	8.64 ± 1.15[a]

Values are mean ± standard deviation. Significant control diets ($P < 0.05$). FI: feed intake; AWG: absolute weight gain; SGR: specific growth rate; FCR: feed conversion ratio; PER: the protein efficiency ratio; S: survival; HSI: hepatosomatic index; VSI: viscerosomatic index; K: condition factor.

Table 6. Alpha-diversity indices of bacterial communities present in the intestine of *A. tropicus* juveniles fed with *C. tropicalis* (10^4 CFU g^{-1}), *D. hansenii* (10^3 CFU g^{-1}), and control diet.

Treatments	Richness		Diversity
	Chao 1	ACE	Shannon
Control diet	64. 41 ± 7.14	64. 53 ± 7.47	2. 83 ± 0. 07
Candida tropicalis (10^4 CFU g^{-1})	83. 11 ± 7. 81	83. 3 ± 8. 05	2. 94 ± 0. 13
Debaryomyces hansenii (10^3 CFU g^{-1})	58. 94 ± 21. 19	59. 05 ± 21. 36	2. 85 ± 0. 22

Values are mean ± standard deviation. No significant differences were observed between treatments ($P > 0.05$).

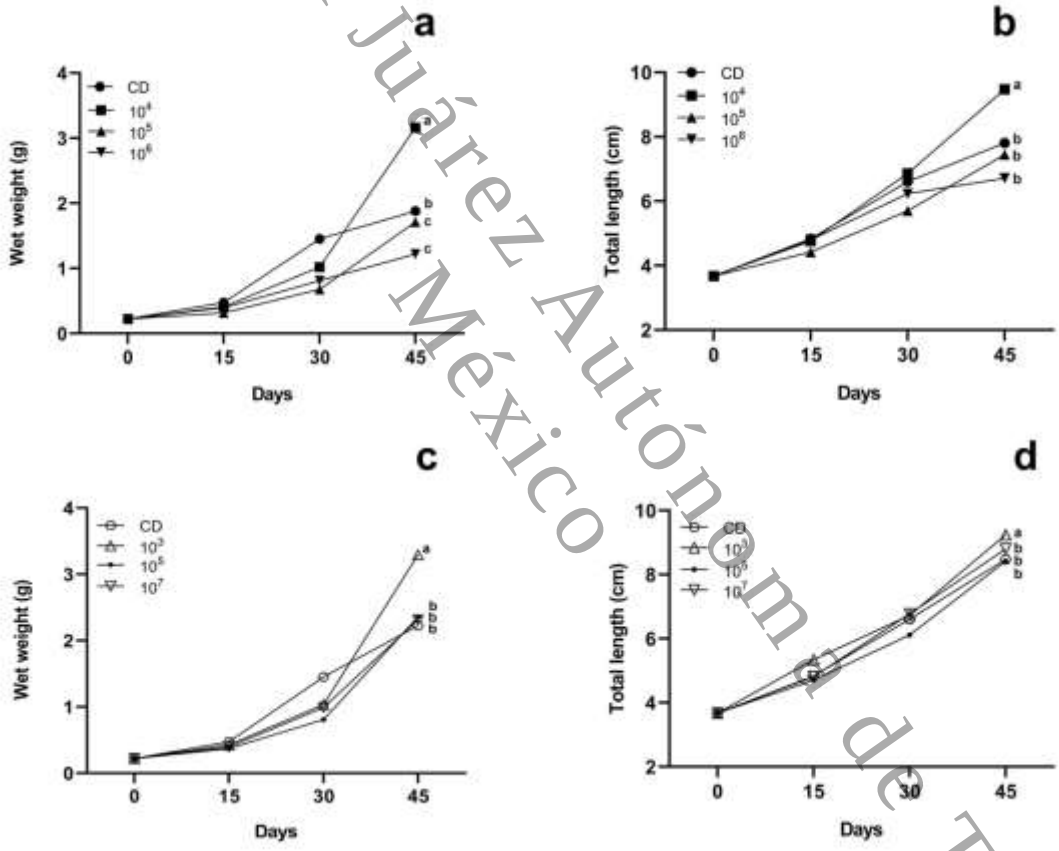

Figure 1. Growth in weight (g) and total length (cm) of *A. tropicus* juveniles fed with a-b) *C. tropicalis* (10^4, 10^5, 10^6 CFU g^{-1}), c-d) *Debaryomyces hansenii* (10^3, 10^5, 10^7 CFU g^{-1}), and the control diet (CD). Values are expressed as mean ± standard deviation. Significant differences among diets are indicated by different letters ($P < 0.05$).

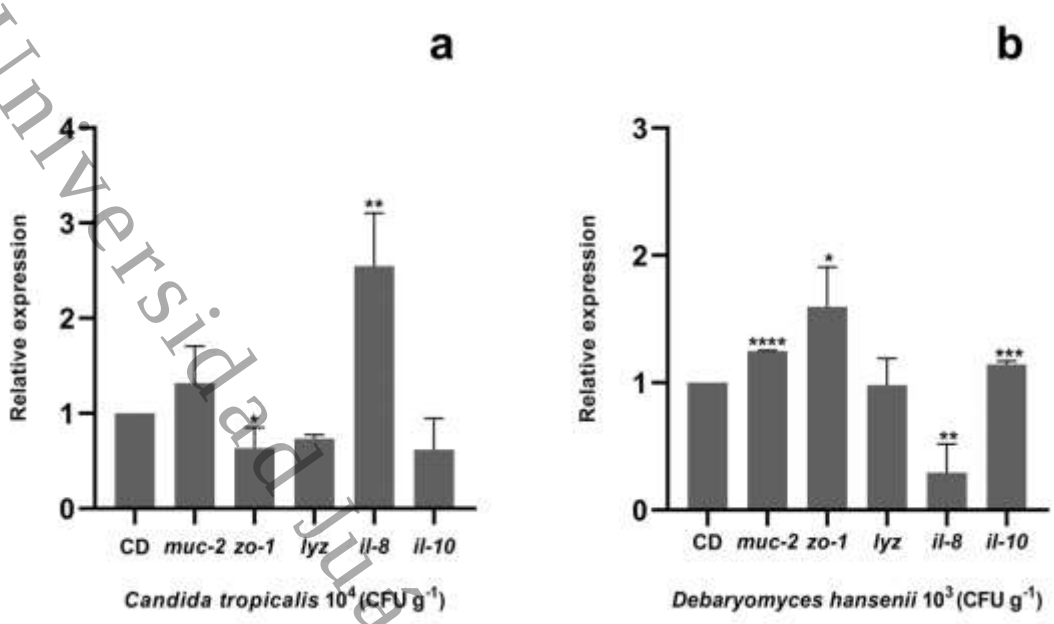

Figure 2. Relative expression levels of the intestinal barrier and immune system genes in *A. tropicus* juveniles fed with a) *C. tropicalis* (10^4 CFU g^{-1}) and b) *D. hansenii* (10^3 CFU g^{-1}), compared to the control diet (CD). Statistical analysis was conducted separately for *C. tropicalis* and *D. hansenii*. Data are presented as fold-change relative to control diet samples (set to 1). Values are mean ± standard deviation. Significant differences ($P < 0.05$) are indicated by asterisks, a) *$P < 0.0422$, **$P < 0.0084$; b) *$P < 0.0297$, **$P < 0.0056$, ***$P < 0.0009$, ****$P < 0.0001$.

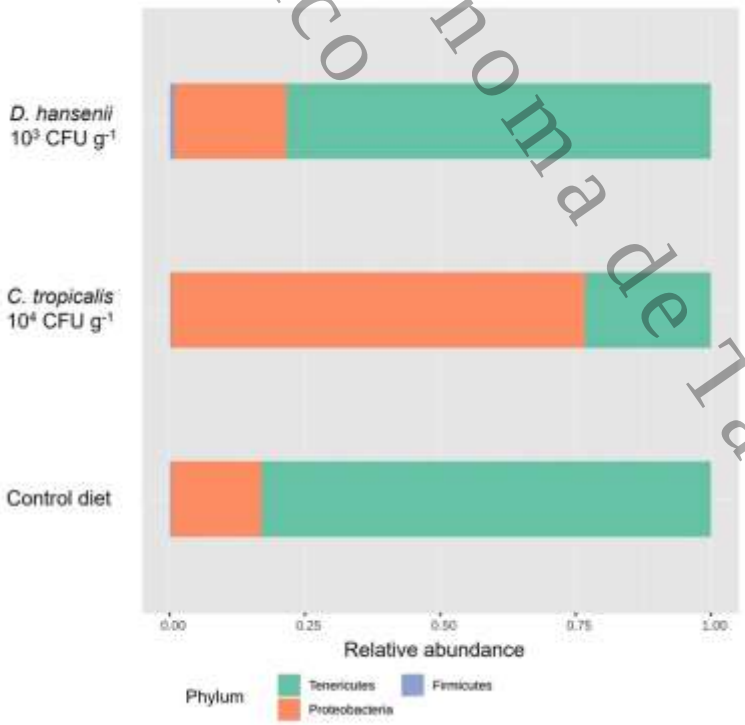

Figure 3. Composition and relative abundance of bacterial phyla present in the intestinal microbiota of *A. tropicus* juveniles fed with *C. tropicalis* (10^4 CFU g^{-1}), *D. hansenii* (10^3 CFU g^{-1}), and the control diet.

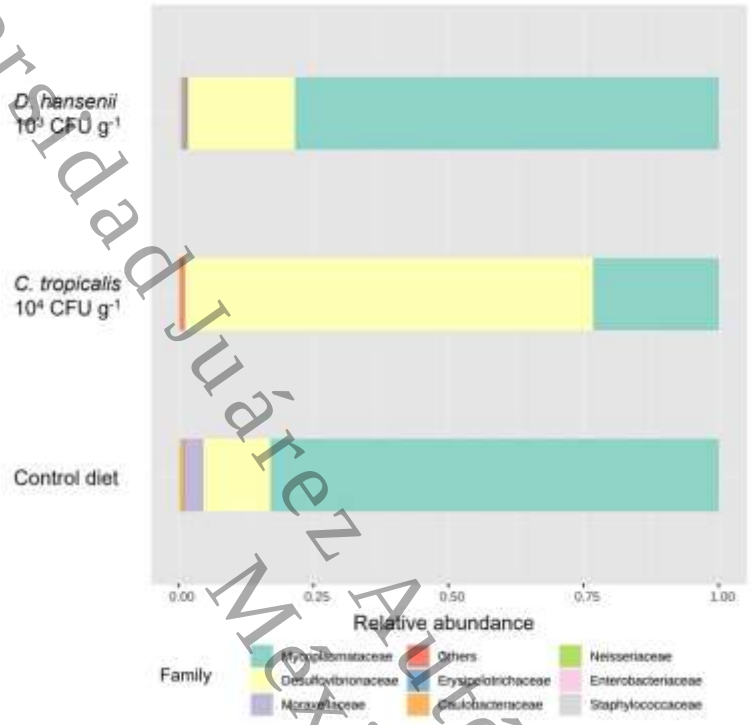

Figure 4. Composition and relative abundance of intestinal bacterial communities at family level in *A. tropicus* juveniles fed with *C. tropicalis* (10^4 CFU g^{-1}), *D. hansenii* (10^3 CFU g^{-1}), and the control diet.

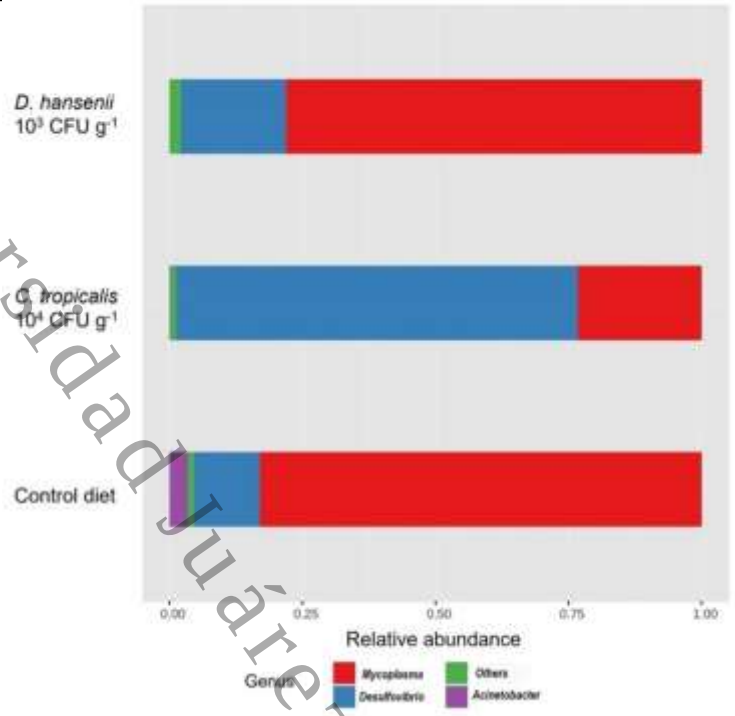

Figure 5. Composition and relative abundance of intestinal bacterial communities at genus level in *A. tropicus* juveniles fed with *C. tropicalis* (10^4 CFU g^{-1}), *D. hansenii* (10^3 CFU g^{-1}), and the control diet (CD).

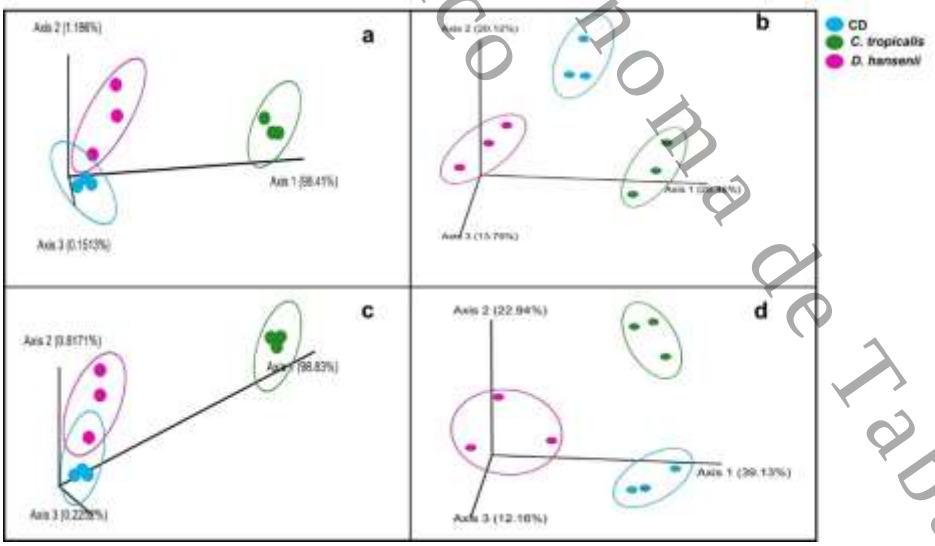

Figure 6. Beta diversity indices through Principal Coordinates Analysis based on distances of a) Bray-Curtis, b) Jaccard, c) Weighted Unifrac, and d) Unweighted Unifrac among the bacterial communities identified in the intestines of *A. tropicus* juvenile after the administration of *C. tropicalis* (10^4 CFU g^{-1}) and *D. hansenii* (10^3 CFU g^{-1}) compared to the control diet (CD).

11. Capítulo II

The Indigenous Probiotic *Lactococcus lactis* PH3-05 Enhances the Growth, Digestive Physiology, and Gut Microbiota of the Tropical Gar (*Atractosteus tropicus*) Larvae.

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The Indigenous Probiotic *Lactococcus lactis* PH3-05 Enhances the Growth, Digestive Physiology, and Gut Microbiota of the Tropical Gar (*Atractosteus tropicus*) Larvae.

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Microorganisms isolated from the intestinal microbiota of fish have demonstrated probiotic effects. Considering their beneficial impact in aquaculture, it is essential to develop feeding strategies that ensure the growth and health of aquaculture animals using isolated strains from fish. This study evaluated the effects of the administration of *Lactococcus lactis* PH3-05, a probiotic bacterium isolated from tropical gar adult (*Atractosteus tropicus*), on the growth, survival, digestion, intestinal morphology, gene expression and intestinal microbiota of larvae of the same species. The results showed that supplementation with *L. lactis* PH3-05 significantly improved growth, survival and digestive enzyme activity. In addition, this supplementation stimulated the expression of genes associated with the mucosal barrier and the anti-inflammatory response. Although no significant changes were observed in the overall composition of the intestinal microbiota, an increase in the abundance of Lactobacillus was recorded in the group treated with *L. lactis*. These findings suggest that *L. lactis* has potential as an indigenous probiotic to improve the health and growth of tropical gar larvae and could be implemented for native species of the region.

Probiotics; tropical gar; microbiome; physiology; gene expression; *Lactococcus lactis*

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Article

The indigenous probiotic *Lactococcus lactis* PH3-05 enhances the growth, digestive physiology, and gut microbiota of the tropical gar (*Atractosteus tropicus*) larvae

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Simple Summary: Microorganisms isolated from the intestinal microbiota of fish have demonstrated probiotic effects. Considering their beneficial impact in aquaculture, it is essential to develop feeding strategies that ensure the growth and health of aquaculture animals using isolated strains from fish. This study evaluated the effects of the administration of *Lactococcus lactis* PH3-05, a probiotic bacterium isolated from tropical gar adult (*Atractosteus tropicus*), on the growth, survival, digestion, intestinal morphology, gene expression and intestinal microbiota of larvae of the same species. The results showed that supplementation with *L. lactis* PH3-05 significantly improved growth, survival and digestive enzyme activity. In addition, this supplementation stimulated the expression of genes associated with the mucosal barrier and the anti-inflammatory response. Although no significant changes were observed in the overall composition of the intestinal microbiota, an increase in the abundance of Lactobacillus was recorded in the group treated with *L. lactis*. These findings suggest that *L. lactis* has potential as an indigenous probiotic to improve the health and growth of tropical gar larvae and could be implemented for native species of the region.

Abstract: Probiotics in aquaculture hold promise for enhancing fish health and growth. Indigenous probiotics, due to their increased specificity and affinity for their host, may offer isolated and potentially amplified benefits. This study investigated the effects of Lactococcus lactis PH3-05 previously isolated from adult of tropical gar (Atractosteus tropicus), on the growth, survival, digestive enzyme activity, intestinal morphology, expression of barrier and immune genes, and intestinal microbiota composition in larvae of tropical gar. Larvae were fed with live L. lactis PH3-05 concentrations of 104, 106, and 108 CFU/g for 15 days alongside a control diet without probiotics. Higher concentrations of L. lactis PH3-05 (106 and 108 CFU/g) positively influenced larval growth, increasing hepatocyte area and enterocyte height. The 106 CFU/g dose significantly enhanced survival (46%) and digestive enzyme activity. Notably, the 108 CFU/g dose stimulated increased expression of muc-2 and il-10 genes, suggesting enhanced mucosal barrier function and anti-inflammatory response. Although L. lactis PH3-05 did not significantly change the diversity, structure, or Phylum level composition of intestinal microbiota, which was constituted by Proteobacteria, Bacteroidota, Chloroflexi, and Firmicutes, an increase in Lactobacillus abundance was observed in fish fed with 106 CFU/g, suggesting enhanced probiotic colonization. These results demonstrate that administering L. lactis PH3-05 at 106 CFU/g promotes growth, survival and digestive health in A. tropicus larvae, establishing it as a promising indigenous probiotic candidate for aquaculture applications.

Keywords: Probiotics; tropical gar; microbiome; physiology; gene expression; *Lactococcus lactis*

Introduction

The growing demand for fish products has propelled aquaculture to the forefront of global food production to meet human needs [1]. In this context, ensuring the growth of fish during their culture, particularly in the technification processes, is crucial, which necessitates safeguarding the health of the organisms [2-4]. Therefore, the recent utilization of pre-and probiotics in balanced feeds as a more suitable alternative to boost the immune system and optimize the intestinal microbiome is significant. This approach has been reported to enhance digestive physiology, increase survival, and improve the health of organisms in culture [5-8]. The most commonly used probiotic bacteria in aquaculture, many of them indigenous, belong to the genera *Bifidobacterium*, *Enterococcus*, *Bacillus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus* and *Weissella*, which have been isolated from the intestinal system of various fish species [9-11].

Among all the probiotic bacterial species, *Lactococcus lactis* stands out as one of the most promising. It is a lactic acid bacterium commonly found in the gastrointestinal tract of many fish species and possesses characteristics that make it a suitable probiotic. It can produce antimicrobial compounds, improve digestibility and nutrient absorption, and modulate immune responses, all of which contribute to improved fish health [12-15]. Additionally, this bacterium produces bacteriocins and enhances the biosynthesis of essential amino acids, further promoting fish health [16-18]. Given these characteristics, *L. lactis* holds great potential as an excellent probiotic candidate to promote the well-being of aquatic organisms.

Based on the above, several studies have shown that the inclusion of *L. lactis* has beneficial effects on survival, weight gain, disease resistance by improving the immune system, increasing mucosal production as well as enhancing intestinal microbial richness and diversity in different fish species, such as bastard halibut (*Paralichthys olivaceaus*), greater amberjack (*Seriola dumerili*), orange-spotted grouper (*Epinephelus coioides*), common carp (*Cyprinus carpio*) and red seabream (*Pagrus major*) [19-23].

In Southeastern Mexico, tropical gar (*Atractosteus tropicus*), a native ancestral fish species of economic and ecological importance, has been commercially cultivated for over 20 years [24]. As part of the development of balanced feeds and their optimization, the inclusion of various prebiotics (inulin, fructooligosaccharide, mannan-oligosaccharides, and β-glucans) has been evaluated, which have shown diverse beneficial effects in larvae and juveniles [25-30]. Additionally, in juvenile *A. tropicus*, only the probiotic yeast *Debaryomyces hansenii* has been evaluated, where no positive effects on growth and physiological variables were observed when included in high concentrations (10^{14}, 10^{15} and 10^{16} CFU/Kg feed) in the balanced feed [31]. However, the use of probiotics during the larval stage in *A. tropicus* has not been evaluated. It is important to note that in *A. tropicus* the larval stage shows variable survival rates, which may be due to several factors, such as the low adaptation to balanced food in the weaning process and the high cannibalism that occurs during this period [32-34]. On the other hand, a significant study is the one conducted by [35], who characterized the intestinal microbial composition of juvenile and adult *A. tropicus*, where some bacterial species with probiotic potential were identified, such as *Cetobacterium*, *Aeromonas hydrophila* P5, *Aeromonas sobria* CP DC28 and *Lactococcus lactis*. This last bacterium was isolated and characterized as a suitable probiotic. Thus, this study evaluated the effects of the addition of *Lactococcus lactis* in weaning food on the growth, survival, digestive enzyme activity, gut morphology and gene expression of *A. tropicus* larvae.

2. Materials and Methods

2.1. Indigenous bacteria of *A. tropicus*

Lactotoccus lactis PH3-05 was provided by the Applied Microbiology Laboratory (DACBiol-UJAT), which was isolated from the intestine of an adult male of *A. tropicus* (Gene Bank OK178269).

2.2. Obtaining the bacterial biomass

Biomass was obtained by a 72h culture in Brain Heart Infusion broth (BHI, Difco) with shaking at 140 rpm at 32 °C. The culture was centrifuged at 4,000 rpm for 40 min, and the cell pellet was washed twice with sterile saline. The Colony-Forming Units (CFU) of the biomass obtained were quantified [36], and the number of CFU per *g* of wet biomass was reported.

2.3. Preparation of experimental diets

Four experimental diets were formulated following the protocol of [27]. Three diets were supplemented with *L. lactis* PH3-05 at 10^4, 10^6, and 10^8 CFU/g concentrations and a control diet without probiotics (CD) (Table 1). The diets were prepared following the methodology of [37], and the bacteria were incorporated into the diets by dissolving the biomass in 200 mL of sterile water. The pelleted diets were dried at 40 °C for 15 h in a conventional oven and stored at 4 °C until use. All diets were analyzed for proximate analysis (humidity, ash, lipid, and protein) according to [38].

Table 1. Composition of experimental diets with different concentrations of *Lactococcus lactis* and control diet.

Ingredients (g/Kg)	*Lactococcus lactis* PH3-05 CFU/g			
	Control diet	**10^4**	**10^6**	**10^8**
Fish meal[a]	350	350	350	350
Pork meal[a]	270.9	270.9	270.9	270.9
Poultry meal[a]	150	150	150	150
Starch[b]	100	100	100	100
Fish oil[a]	36.5	36.5	36.5	36.5
Lactococcus lactis	0	0.001	0.01	0.1
Wheat meal[c]	37.6	36.6	37.59	37.6
Grenetin[d]	20	20	20	20
Vit-min premix[e]	15	15	15	15
Soy lecithin[f]	15	15	15	15
Vitamin C[g]	5	5	5	5
Chemical composition (g 100 g diet DM)				
Crude protein	50.1	50.2	49.8	49.9
Crude lipid	14.2	14.1	13.9	14.2
Fiber	10.1	10.2	9.9	10.0
Ashes	14.0	13.9	14.1	14.2
Humidity	8.1	8.0	8.4	8.2
NFE	11.6	11.6	12.3	11.7

[a]Marine and agricultural proteins S.A. de C.V., Guadalajara, Jalisco; [b] Pronat Ultra, Merida, Yucatan, Mexico; [c] GALMEX SA de CV, Villahermosa, Tabasco, México; [d]D´gari, food and diet products relámpago, S.A. de C.V. [e]Vitamin premix composition g/mg or International Units per kg of diet: Vitamin A, 10,000,000 IU; Vitamin D3, 2,000,000 IU; Vitamin E, 100,000 IU; Vitamin K3, 4.0 g; Thiamine B1, 8.0 g; Riboflavin B2, 8.7 g; Pyridoxine B6, 7.3 g; Vitamin B12, 20.0 mg; Niacin, 50.0 g; Pantothenic acid, 22.2 g; Inositol, 0.15 mg; Nicotinic Acid, 0.16 mg; Folic Acid, 4.0 g; Biotin, 500 mg; Vitamin C, 10.0 g; Choline 0.3 mg, Excipient q.s. 2 g; Manganese, 10 g; Magnesium, 4.5 g; Zinc, 1.6 g; Iron, 0.2 g; Copper, 0.2 g; Iodine, 0.5 g; Selenium, 40 mg; Cobalt 60 mg. Excipient q.s. 1.5 g; [f]Pronat Ultra, Yucatán, México; [g]ROVIMIX® STAY-C® 35 –DSM, Guadalajara, México. NFE = Nitrogen-free extract:100-(% protein-% ether extract-% ash-%fiber).

2.4. Viability of *L. lactis* PH3-05 in the experimental diets

At the beginning and end of the experiment, the CFU/g of bacteria in each experimental diet were quantified to confirm the concentrations and viability. A representative sample was obtained from each diet using the quartering method. One g of the diet was weighed and suspended in 9 mL of saline (0.85%), and exponential dilutions were made from 10^{-1} to 10^{-10}. 100 µL of the different dilutions were inoculated in Agar (BHI) and added with an antifungal (natamycin) to reduce the fungi load in the formulated food. The inoculum was homogeneously dispersed with the help of a sterile Drigalsky loop; the plates were incubated for 24 h at 32 °C. Colonies were counted in dilution boxes with 10-30 colonies, and the CFU/g feed was calculated.

2.5. Experimental design

The experimental design was meticulously planned to ensure the validity of our results. We used 1,200 *A. tropicus* larvae 5 days after hatching (DAH), with an average weight of 0.02 ± 0.00 g and total length of 1.81 ± 0.18 cm, obtained from the aquaculture farm "Otot Ibam", Comalcalco, Tabasco. A total of 100 larvae per treatment were placed in 12 tanks of 70 L capacity, connected to a recirculation system driven by a 1HP water pump, connected to a 1500 L reservoir with a biological filter and an automated sand filter, and connected to an ultraviolet light lamp to minimize the possible bacterial discharge. Vat temperature (27.1 ± 0.08 °C), dissolved oxygen (5.5 ± 0.2 mg/L) and pH (7.3 ± 0.5) were monitored daily using an oximeter (YSI 85, USA) and a potentiometer (HANNA HI 991001, Romania).

Feeding was carried out with co-feeding during the first 5 DAH, providing *Artemia* sp. nauplii and microparticles of the experimental diets, including the control diet (approx. 350 µm); subsequently, the nauplius supply was eliminated and only the balanced diets were administered until the end of the bioassay (20 DAH) adjusting the size of the microparticles according to the larvae growth (500-800 µm). Larvae were fed six times daily (00:00, 3:00, 7:00, 11:00, 15:00, and 19:00) until apparent satiation. Cleaning of each tub were partial water replacement daily (30%) after each feeding by the siphoning method. All treatments were evaluated in triplicate.

2.6. Evaluation of growth and survival rate

At the beginning and end of the experiment, total weight was determined with an analytical balance (Ohaus HH120, precision 120 ± 0.01 g, Shenzhen, China) and total length using photographs analyzed in ImageJ 1.5 software. At the end of the bioassay, the following productive parameters were determined: specific growth rate (SGR): [(ln final weight − ln initial weight)/days] × 100; weight gain (WG): final weight (g) − initial weight (g) and survival rate (S): (number of final fish/number of initial fish) × 100.

2.7. Sample collection

At the end of the bioassay, the larvae were sacrificed by thermal shock. The head and tail were removed, and the larvae were washed with water. To ensure representativeness, the following samples were obtained per replicate. Three larvae were kept at −80 °C to determine enzyme activity. Three larvae were preserved in RNAse-free tubes for metagenomic analysis,

three larvae for gene expression analysis (RNAlater solution, Ambion) and three larvae were fixed in Davison solution for histological analysis. The study was carried out under the Helsinki Declaration and the protocol authorized by the Ministry of Agriculture, Livestock, Rural Development, Fisheries and Food (SAGARPA), Mexico, NOM-062-Z00-1999 (2001) [39].

2.8. Enzyme activities quantification

The multienzyme extract was obtained from the pool of three larvae per replicate in 50 mM Tris-HCl, pH 7.5, and centrifuged at 14,000 rpm for 15 min. The supernatant was stored at -80 °C. Soluble protein was quantified using [40].

Acid protease activity was quantified using 1% hemoglobin as a substrate in 0.1 M Glycine HCl buffer, pH 2. The absorbance was measured on a microplate reader (xMark, Bio-Rad, Hercules, CA, USA) at 280 nm, according to [41]. Alkaline protease activity was determined by the technique of [42] with 1% casein as a substrate and 100 mM Tris-HCl, CaCl2 10 mM, pH 9, and absorbance was measured at 280 nm. Trypsin activity was quantified with 1 mM BAPNA as a substrate (Nα-Benzoyl-DL-Arginine-P-nitroanilide) in 50 mM Tris-HCl, 10 mM CaCl2, pH 8.2, and absorbance was measured at 410 nm, according to the technique of [43]. Following technique of [44], chymotrypsin activity was determined, SAPNA 1.25 mM was used as a substrate (135 µL), pH 8.2, and absorbance was measured at 410 nm. Lipase activity was performed with the methodology of [45] using 4-nitrophenyl palmitate as a substrate, pH 7.4, 6 mM sodium taurocholate, and absorbance was measured at 540 nm. To quantify leucine aminopeptidase activity, 0.1 M leucine p-nitroanilide, pH 7.2 was used as a substrate and quantified at 405 nm absorbance, following the method of [46]. The activities of all enzymes were calculated with the following equations: (1) Units per mL (U mL^{-1}) = (Δabs × final volume of the reaction (mL)) × (MEC × time (min) × enzymatic extract volume $(mL)^{-1}$); (2) Units per mg of protein (U mg $protein^{-1}$) = Units per mL × mg of soluble $protein^{-1}$, where MEC is the molecular extinction coefficient.

2.9. Histological analysis

The samples were dehydrated in different concentrations of ethyl alcohol (50, 70, 80, 96, and 100%, OH 100%-Xylol). They were then embedded in paraffin. Cross sections of 7 µm thickness were obtained with a sliding microtome (Reichert-Jung, Hn40) and stained with hematoxylin and eosin (H-E). The slices were examined under a Zeiss optical microscope (Axio-star Plus), the photographs were taken with a digital camera (Zeiss, Axiocam MRc 5), and the morphometric measurements were taken with Zen 2.3 software. At the intestinal level, the height of the enterocytes (µm) was quantified.

2.10. RNA extraction, reverse transcription, and gene expression analysis

Total RNA was extracted using the Trizol technique (Invitrogen, Waltham, MA) according to the manufacturer's instructions. The concentration and purity of a single RNA were determined in a spectrophotometer (A260/280) (Jenway GenovaNano, Cole-Parmer,

Staffordshire, UK). One microgram of RNA was reverse transcribed into cDNA in a thermal cycler (Mastercycle nexus GSX1, Eppendorf, Hamburg, Germany) using the High-Capacity cDNA Reverse Transcription Kit (Maxima First Strand cDNA Synthesis Kit for RT-qPCR, Thermo Scientific, Waltham, MA) in a final volume of 20 µL, following the manufacturer's recommendations. The expression of two genes associated with intestinal barrier integrity, *muc-2* (intestinal mucus layer protein) and *zo-2* (tight junction protein), and two immune system genes, *il-8* (proinflammatory cytokine) and *il-10* (anti-inflammatory cytokine), were evaluated (Table 2). The qPCR reactions were carried out using 5 µL of Eva Green supermix (BioRad, Hercules, CA), 4.5 µL of cDNA, and 0.5 µL of primers mix (3mM) in a final volume of 10 µL per reaction. The β-actin gene was used as a reference gene [47]. The qPCR was performed on a CFX96TM real-time thermocycler (BioRad, Hercules, CA) using the following conditions: a 10 min denaturation cycle at 95 °C followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. Relative gene expression changes were calculated using the 2−ΔΔ CT method [48].

Table 2. Primers used for qPCR analysis.

Target Gene	Gene function	Primer Sequence (5´- 3')	Amplification Efficiency (%)	Amplicon Size (bp)	Reference
muc-2	mucus layer protein (mucin 2)	FW: GGCCTCCTCAAGAGCACGGTG RV:TCTGCACGCTGGAGCACTCAATG	90.94	100	[26]
zo-2	tight junction protein	W: TACCCATGGAAAATGTGCCTCA RV: CGGGGTCTCTTCACGGTAA	95.29	88	[28]
il-8	pro-inflammatory cytokine	FW: ATATTCACTGGTGGGCGGAG RV: GTGCGGCCTGAGATTGTTT	94.18	369	[28]
il-10	anti-inflammatory cytokine	FW: TTATAAAGCCATGGGGGAGCTG RV: CTGCACAGTCTGCCTCTAGT	94.47	91	This study
β-actin	cytoskeletic actin	FW:GAGCTATGAGCTGCCTGAGTGG RV:GTGGTCTCATGAATGCCACAGG	97.10	119	[47]

2.11. DNA isolation from gut microbiota and preparation of 16S rRNA gene libraries and sequencing

Genomic DNA extractions were performed using a commercial kit. DNA concentration was quantified using the Qubit 3.0 fluorometer and the

ds-DNA BR Assay kit (Invitrogen from Thermo Fisher Scientific). DNA integrity was verified by 1% agarose gel electrophoresis.

The V3 hypervariable region of the bacterial 16S rRNA gene was PCR amplified for library preparation using gene-specific primers V3-338f and 533r [49]. PCR amplification and second PCR were performed using the Nextera XT index (Illumina, San Diego, CA, USA). All PCR products were followed by an optimized clean-up step using Agencourt AMPure XP beads according to the protocol published by Illumina. Finally, V3 libraries were sequenced with a MiSeq v3 reagent kit (300 cycles) using the MiniSeq platform (Illumina, San Diego, CA, USA), and 2×150 cycles of paired-end sequencing were performed.

2.12. Data analysis and statistics

First, normality (Kolmogorov-Smirnov) and homoscedasticity (Bartlett) tests were performed on the growth data, digestive enzyme activities, and histological measurements, all of which met the required assumptions. Consequently, a one-way ANOVA followed by Tukey's post hoc test were performed. For gene expression analyses, the Kruskal-Wallis test and Nemenyi's post hoc test were used to identify significant differences. All data were statistically analyzed using GraphPad Prism 8 software (GraphPad Software, San Diego, CA, USA) with a significance value of 0.05.

3. Results

3.1. Growth indexes and survival rates

At the end of the bioassay (20 DAH), larvae fed with *L. lactis* PH3-05 10^8 CFU/g obtained the highest final weight (0.041± 0.00 g), followed by larvae with 10^6 CFU/g (0.040 ± 0.00 g) of *L. lactis* PH3-05. Both treatments showed significant differences ($p < 0.05$) with larvae fed with *L. lactis* PH3-05 10^4 CFU/g and CD (Table 3). The most significant total length was shown in larvae fed with all three *L. lactis* PH3-05 supplementations ($p < 0.05$); however, the 10^8 CFU/g treatment showed the greatest total length (2.38 ± 0.09 cm). SGR and WG were highest in larvae fed with the diets supplemented with 10^8 CFU/g of *L. lactis* PH3-05 (2.83 ± 0.003 and 52.14 ± 0.06, respectively), followed by those fed with 10^6 CFU/g (2.75 ± 0.15 and 50.33 ± 3.50, respectively) of *L. lactis*, showing significant differences ($p < 0.05$) with *L. lactis* 10^4 CFU/g and CD larvae. The highest survival rate was presented in the larvae of treatment 10^6 CFU/g of *L. lactis* PH3-05 with 46.36 ± 4.34%, showing significant differences ($p < 0.05$) with the rest of the treatments (Table 3).

Table 3. Indexes of growth performance and survival rate of *A. tropicus* larvae fed diets supplement different concentrations of *L. lactis* PH3-05 (10^4, 10^6 and 10^8 CFU/g) compared with the control diet.

	***Lactococcus lactis* PH3-05 (CFU/g)**			
	Control diet	**10^4**	**10^6**	**10^8**
Initial weight (g)	0.002 ± 0.007	0.002 ± 0.007	0.002 ± 0.007	0.002 ± 0.007
Final weight (g)	0.031 ± 0.002^b	0.034 ± 0.0004^b	0.040 ± 0.00^a	0.041 ± 0.00^a
Initial length (cm)	1.8 ± 0.18	1.8 ± 0.18	1.8 ± 0.18	1.8 ± 0.18
Final length (cm)	2.09 ± 0.05^b	2.13 ± 0. 12^a	2.18 ± 0.09^a	2.38 ± 0.09^a
SGR (% d^{-1})	1.53 ± 0.07^b	1.64 ± 0.07^b	2.75 ± 0. 15^a	2.83 ± 0.003^a
WG (%)	25.75 ± 1.35^b	27.74 ± 1.46^b	50.33 ± 3. 50^a	52.14 ± 0.06^a
S (%)	31.11 ± 1.92^b	33.75 ± 1.82^b	46.36 ± 4. 34^a	32.56 ± 6.72^b

SGR: specific growth rate; WG: weight gain; S: survival rate. Values are means ± SD. Significant differences are shown with different letters ($p < 0.05$).

3.2. Digestive enzyme activity

The specific activity of acid and alkaline protease was significantly higher in larvae fed with 10^6 CFU/g of *L. lactis* PH3-05 compared to the rest of the treatments ($p < 0.05$). Regarding CD, trypsin activity was higher ($p < 0.05$) in larvae fed 10^8 CFU/g of *L. lactis* PH3-05. On the other hand, larvae fed 10^4 and 10^6 CFU/g of *L. lactis* PH3-05 showed higher chymotrypsin activity than the rest of the treatments. Likewise, administering 10^6 CFU/g of *L. lactis* PH3-05 favored significantly ($p < 0.05$) higher lipase activity than the rest of the treatments. Finally, leucine aminopeptidase activity showed a significant increase for larvae fed with 10^4 CFU/g of *L. lactis* PH3-05 ($p < 0.05$) for those fed with CD (Table 4).

Table 4. Digestive enzymatic activities of *A. tropicus* larvae fed diets supplement of different concentrations of *L. lactis* PH3-05 (10^4, 10^6 and 10^8 CFU/g) compared with control diet.

Activities (U mg protein^{-1})	*Lactococcus lactis* PH3-05 (CFU/g)			
	Control diet	10^4	10^6	10^8
Acid protease	490.79 ± 36.69^b	490.27 ± 76.22^b	716.99 ± 5.57^a	235.30 ± 66.15^c
Alkaline protease	41.14 ± 4.68^b	44.45 ± 0.02^b	55.45 ± 1.82^a	37.46 ± 11.82^b
Trypsin	0.12 ± 0.02^b	0.22 ± 0.10^{ab}	0.28 ± 0.05^{ab}	0.35 ± 0.07^a
Chymotrypsin	79.57 ± 2.70^{ab}	87.45 ± 2.86^a	89.06 ± 0.78^a	65.86 ± 10.60^b
Lipase	17.52 ± 0.21^b	27.17 ± 1.21^b	37.75 ± 0.3^a	15.79 ± 8.64^b
Leucine aminopeptidase	60. 21 ± 11.18^b	83.66 ± 1.96^a	81. 21 ± 3.58^{ab}	71.72 ± 11.01^{ab}

*Values are means ± SD. Significant differences are shown with different letters ($p < 0.05$).

3.3. Histological analysis

The livers of the larvae fed with 10^6 and 10^8 CFU/g of *L. lactis* showed a significantly higher percentage of melanomacrophagic centers (MMC) compared to those fed with the CD ($p < 0.05$) (Table 5). In the intestine, the height of enterocytes was significantly higher in larvae fed with 10^6 CFU/g of *L. lactis* ($p < 0.05$) compared to all other treatments, especially those fed with CD, which exhibited the lowest enterocyte height (Table 5, Fig. 1a and b).

Table 5. Histological analysis of *A. tropicus larvae* fed diets supplement different concentrations of *L. lactis* PH3-05 (10^4, 10^6 and 10^8 CFU/g) compared with the control diet.

Morphological analysis	*Lactococcus lactis* PH3-05 (CFU/g)			
	Control diet	10^4	10^6	10^8
Area MMC (%/Area)	1.01 ± 0.43^b	1.45 ± 0.14^{ab}	1.91 ± 0.48^a	1.94 ± 0.31^a
Hepatocytes Area (μm^2)	5.48 ± 0.84^b	5.72 ± 0.81^{ab}	5.37 ± 0.66^b	7.36 ± 0.25^a
Enterocyte height (μm)	10.40 ± 0.40^d	12.61 ± 0.22^c	17.88 ± 0.40^a	14.23 ± 0.33^b

MMC: melanomacrophage centres. Values are means ± SD. Significant differences are shown with different letters ($p < 0.05$).

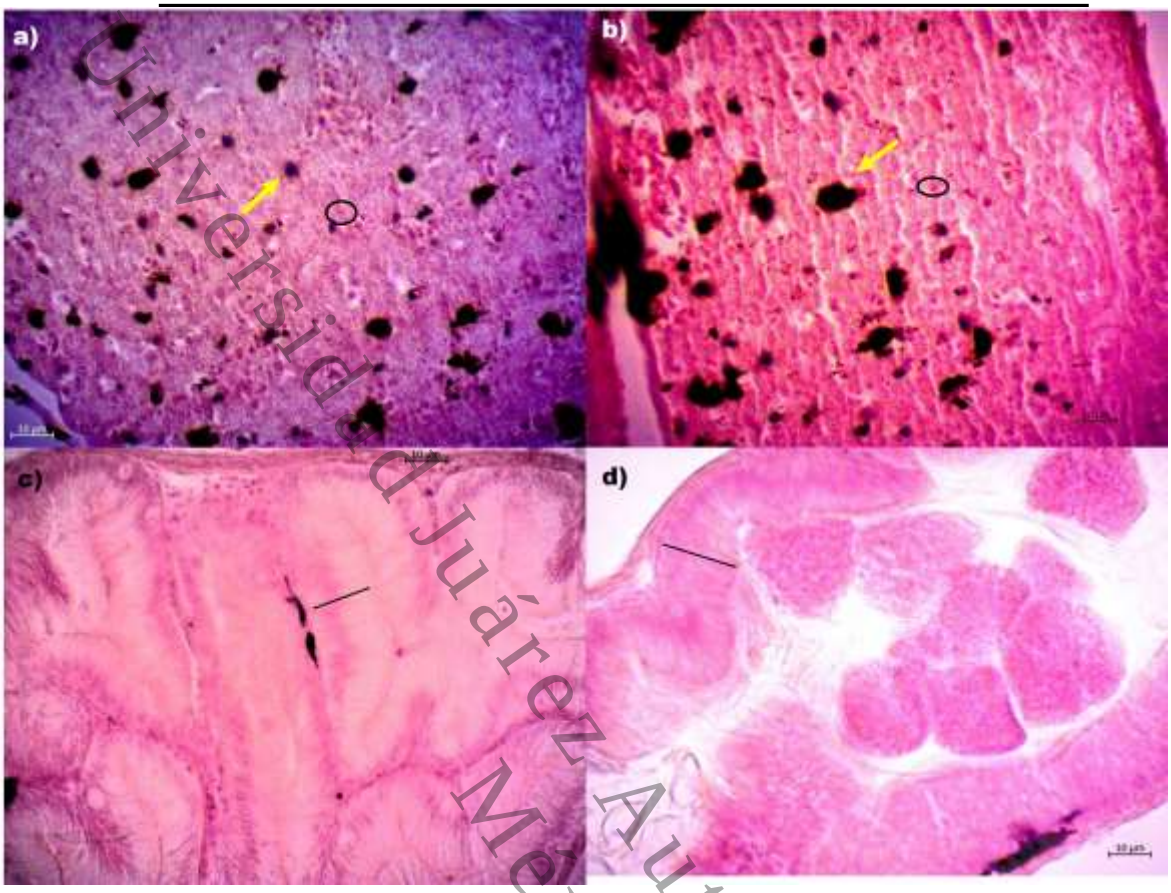

Figure 1. Representative images of the liver and digestive system of *A. tropicus* larvae treated with CD and 10^6 CFU/g of *L. lactis* PH3-05: a) CD, b) 10^6 CFU/g of *L. lactis* PH3-05. The liver images show melanomacrophage centers (yellow arrow) and hepatocytes (circle). Images of the intestine display the height of enterocytes (line) of *A. tropicus* larvae: c) CD, d) 10^6 CFU/g of *L. lactis* PH3-05.

3.4. Gene expression

The relative expression of *muc-2* a significantly increased ($p < 0.05$) in larvae fed with 10^4 and 10^6 CFU/g of *L. lactis* compared to the other treatments (Fig. 2a). In contrast, *zo-2* expression was significantly down-regulated ($p < 0.05$) in larvae across all three *L. lactis* supplementations groups (Fig. 2b). Additionally, all three *L. lactis* supplementations induced a significant increase in *il-8* expression ($p < 0.05$) compared to those fed with CD (Fig. 2c). However, the relative expression of *il-10* also showed a significant increase in larvae fed with 10^6 and 10^8 CFU/g of *L. lactis* ($p < 0.05$) compared to those fed with the other treatments (Fig. 2d).

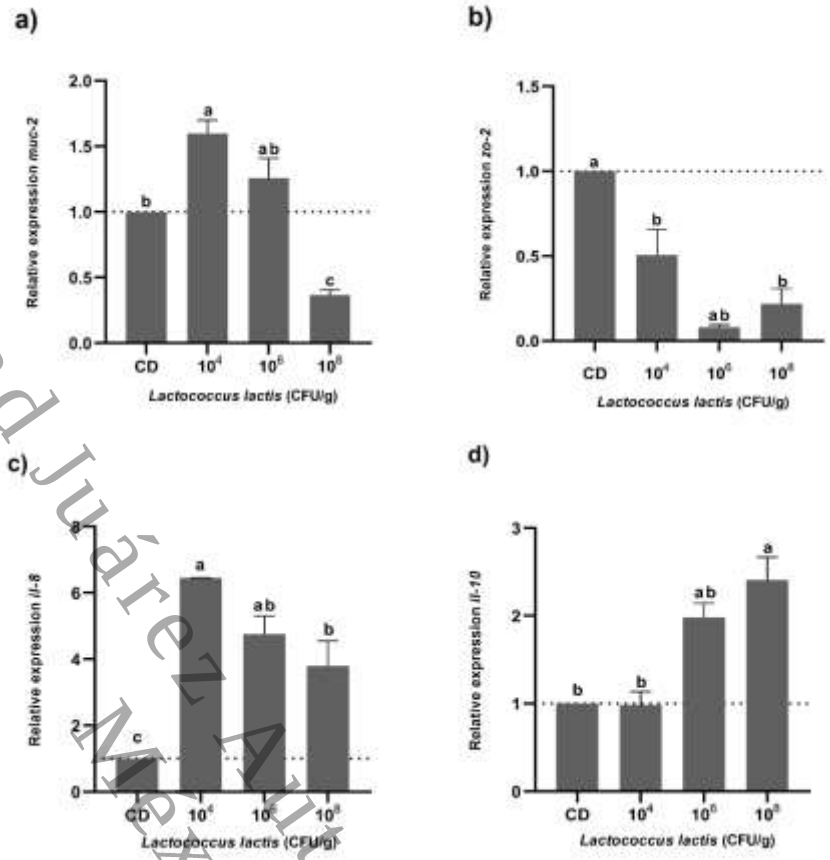

Figure 2. Relative expression levels of intestinal barrier function and immune system genes in *A. tropicus* larvae fed with *L. lactis* PH3-05 supplementation (10^4, 10^6 and 10^8 CFU/g) and control diet. Values are mean ± SD. Data are presented as fold-change relative to control diet samples (set to 1). Significant differences between treatments are indicated by letters ($p < 0.05$).

3.5. Classification and taxonomy of the bacterial taxonomic profile

The metagenomic analysis was conducted using 16S rRNA gene sequencing, yielding 765,724 sequences. After removing, chimeras 26,679 sequences were classified into 461 OTU (operational taxonomic units).

The alpha diversity indices are presented in Figure 3. According to Chao 1 ($p < 0.0897$), ACE ($p < 0.1331$), and Shannon-Weaver ($p < 0.2260$) estimators, there were no significant differences in alpha diversity larvae fed with the three *L. lactis* supplementations and the CD.

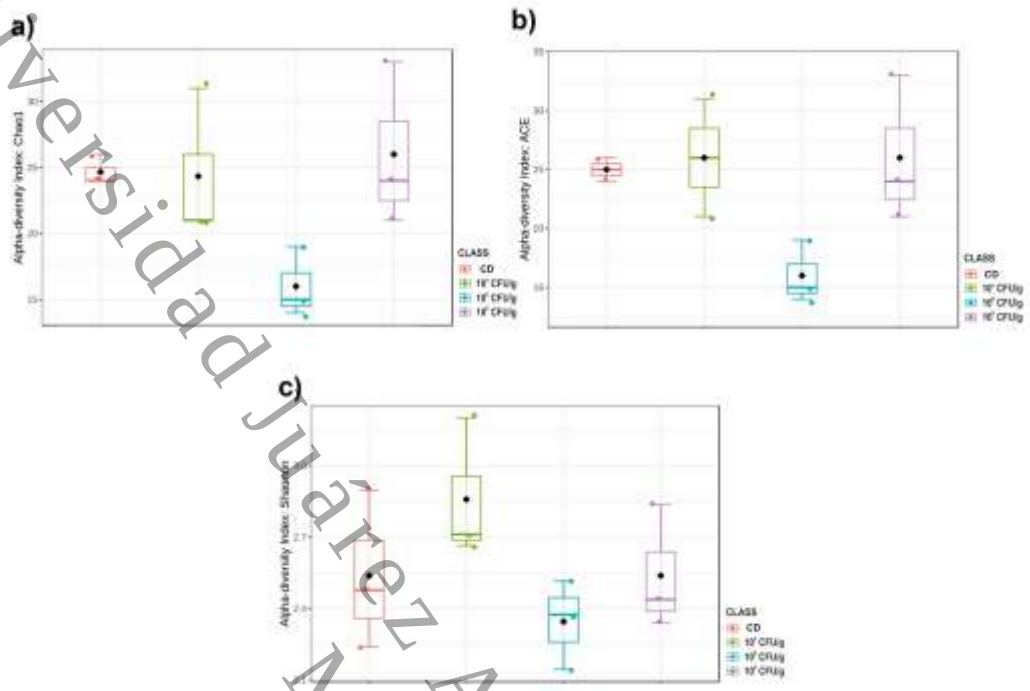

Figure 3. Alpha diversity of gut microbiota in *A. tropicus* larvae treated with *L. lactis* PH3-05 supplementations (10^4, 10^6 and 10^8 CFU/g*)* and control diet. Chao 1, ACE, and Shannon-Weaver indexes were calculated from the ASVs.

Figure 4 shows the composition of the intestinal microbiota of *A. tropicus* larvae, which

consists of the Phyla Proteobacteria, Bacteroidota, Firmicutes, Chloroflexi, and Desulfobacterota. Proteobacteria was the most dominant Phylum in all treatments. In this aspect, larvae fed with 10^8 CFU/g of *L. lactis* PH3-05 showed the highest relative abundance of Proteobacteria with 78.92%, followed by those fed with CD (71.35%), while in larvae fed with 10^6 CFU/g of *L. lactis* PH3-05 the abundance was 58.20% and finally, larvae fed with 10^4 CFU/g of *L. lactis* PH3-05 showed the lowest abundance of Proteobacteria with 49.85%.

The highest relative abundance of Bacteroidota was found in larvae fed with CD, with 13.53%, followed by larvae fed with 10^4 CFU/g of *L. lactis*, with 13.31%. Finally, the lowest relative abundances were presented in larvae fed with 10^6 CFU/g of *L. lactis* (8.14%) and 10^8 CFU/g of *L. lactis* (7.93%), respectively. The relative abundance of the Phylum Firmicutes was highest in larvae fed 10^4 CFU/g of *L. lactis* (12.66%) and 10^6 CFU/g of *L. lactis* PH3-05 (7.58%). In contrast, larvae fed CD and 10^8 CFU/g of *L. lactis* PH3-05 showed the lowest relative abundances (5.54% and 4.755%), respectively. The highest abundance of Phylum Chloroflexi occurred in larvae fed with 10^6 CFU/g of *L. lactis* PH3-05 (14.62%) and 10^4 CFU/g of *L. lactis* (9.36%), again the lowest relative abundances were obtained with larvae fed with CD and 10^8 CFU/g of *L. lactis* (3.82% and 3.54%), respectively.

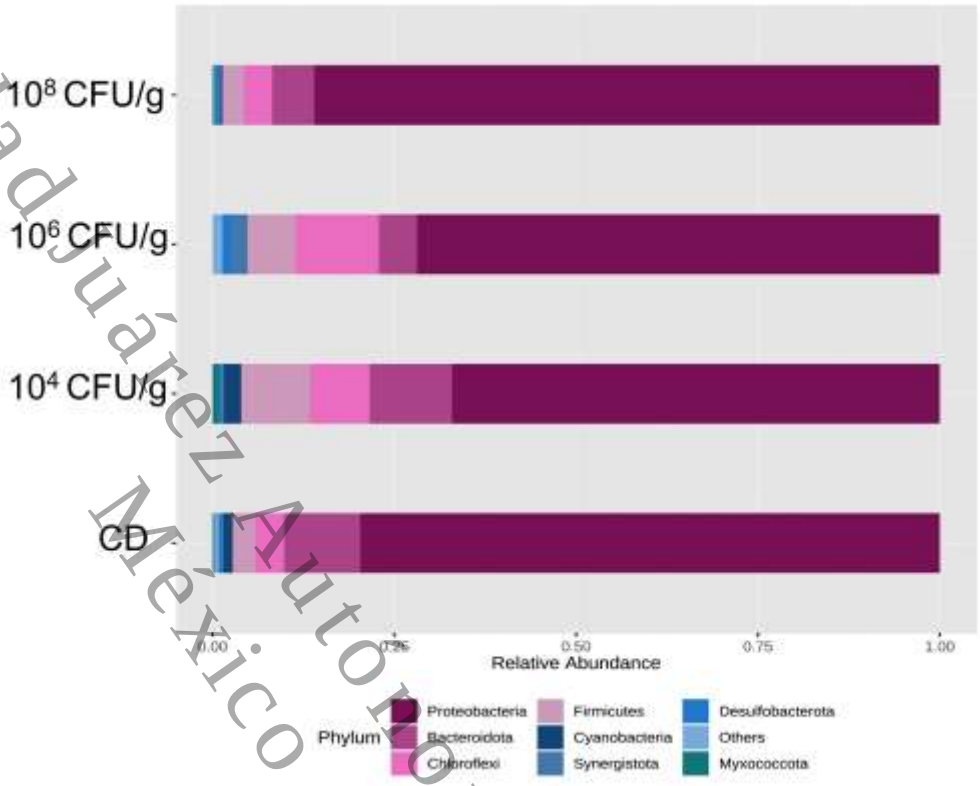

Figure 4. Relative abundance of bacterial phyla present in the intestinal microbiota of *A. tropicus* larvae fed with *L. lactis* PH3-05 (10^4, 10^6 and 10^8 CFU/g*)* and a control diet.

At the order level, Burkholderiales, Cytophagales, Bacteroidales, Sphinogomonadales, Pseudomonadales, and Lactobacilliales were the most representative in all treatments. The most representative families in all treatments were Oxalobacteraceae, Comamonadaceae, Sphinogomonadaceae, Anaerolineaceae, Pseudomonadaceae, Lactobacilliaceae, and Caulobacteraceae.

Figure 5 shows the relative abundance at the genus level of the intestinal microbiota of *A. tropicus* larvae, where the most representative genera are *Masillia*, *Sphaerotilus*, *Vibrio*, *Sphingomonas*, *Runella*, and, to a lesser extent, *Pseudomonas*, *Lactobacillus*, and *Brevundimonas*. *Massillia* has the highest relative abundance in larvae fed with 10^6 CFU/g of *L. lactis*, PH3-05 31.03%, followed by those fed with 10^8 CFU/g of *L. lactis* PH3-05 (26.70%). In larvae fed with CD, 21.23% of relative abundance was obtained, and the lowest proportion was for larvae fed with 10^4 CFU/g of *L. lactis* PH3-05 (12.94%). The

genus *Vibrio* was present only in larvae fed 10^8 CFU/g of *L. lactis* (18.36%) and those fed with CD (8.29%), respectively. The genus *Runella* presented 4.35% relative abundance in larvae fed with the CD and in smaller proportion in larvae fed with 10^4 CFU/g and 10^8 CFU/g of *L. lactis* (2.12% and 2.22%, respectively), while in larvae fed with 10^6 CFU/g of *L. lactis* PH3-05 the presence of this genus was not detected.

The highest relative abundance of the genus *Lactobacillus* was shown in larvae fed 10^6 CFU/g of *L. lactis* PH3-05 (4.29%), and the lowest relative abundance was observed in larvae fed 10^8 CFU/g of *L. lactis* PH3-05 (1.09%). Bacteria of the genus *Pseudomonas* showed a relative abundance of 6.38% in larvae fed with 10^6 CFU/g of *L. lactis* PH3-05 and the lowest abundance in larvae fed with 10^8 CFU/g of *L. lactis* PH3-05 (1.23%). The genus *Sphingomonas* recorded the highest relative abundance of the larvae fed with 10^4 CFU/g (3.82%), 10^6 CFU/g (6.33%), and 10^8 CFU/g (4.60%) of *L. lactis*, while the lowest relative abundance was detected in larvae fed with CD with 2.18%. Bacteria of the genus *Brevundimonas* presented the highest abundance in larvae fed with 10^6 CFU/g of *L. lactis* PH3-05 (3.44%), which decreased in larvae fed with 10^4 CFU/g (1.42%), CD (1.17%), and 10^8 CFU/g of *L. lactis* PH3-05 (1.02%).

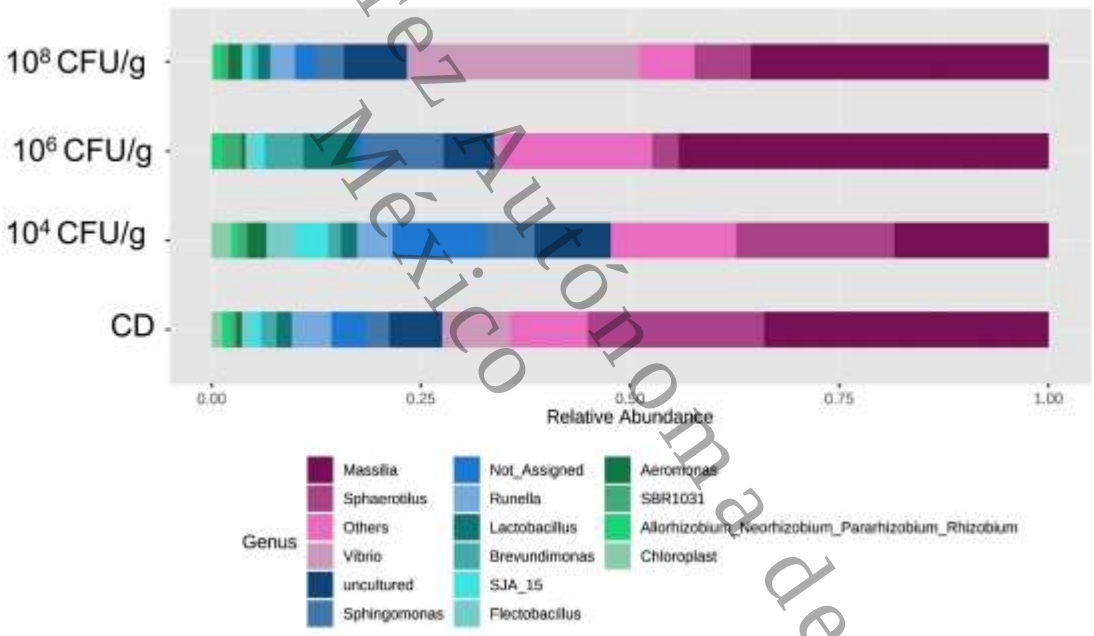

Figure 5. Relative abundance of bacterial genus present in the intestinal microbiota of *A. tropicus* larvae fed with *L. lactis* (10^4, 10^6 and 10^8 CFU/g) and a control diet.

3.6. Bacterial community structure through Beta diversity

Our study, based on taxonomic assignment and multivariate analysis of variance (PERMANOVA) with 4999 permutations, revealed a crucial finding, there were no significant differences in beta diversity indexes between the treatments with *L. lactis* PH3-05 and the CD. This finding underscores the importance of our study in understanding the bacterial community structure.

Figure 6 illustrates the precision of our research methods through principal coordinate analyses (PCoA) with beta diversity indices. The Bray-Curtis similarity (Fig. 6a) showed 51.94% clustering ($p < 0.105$), while the

cumulative variance by JACCARD (Fig. 6b) showed a value of 34.3% distance between treatments ($p < 0.12$). The metrics of phylogenetic distances through UNIFRAC presented values of 79.31% and 44.86% of the total variance in the weighted (WEIGHTED $p < 0.0212$) (Fig. 6c) and unweighted (UNWEITHED $p < 0.6596$) (Fig. 6d) analyses, respectively. These measurements confirm that none of the indices showed significant differences between the treatments with *L. lactis* and the CD.

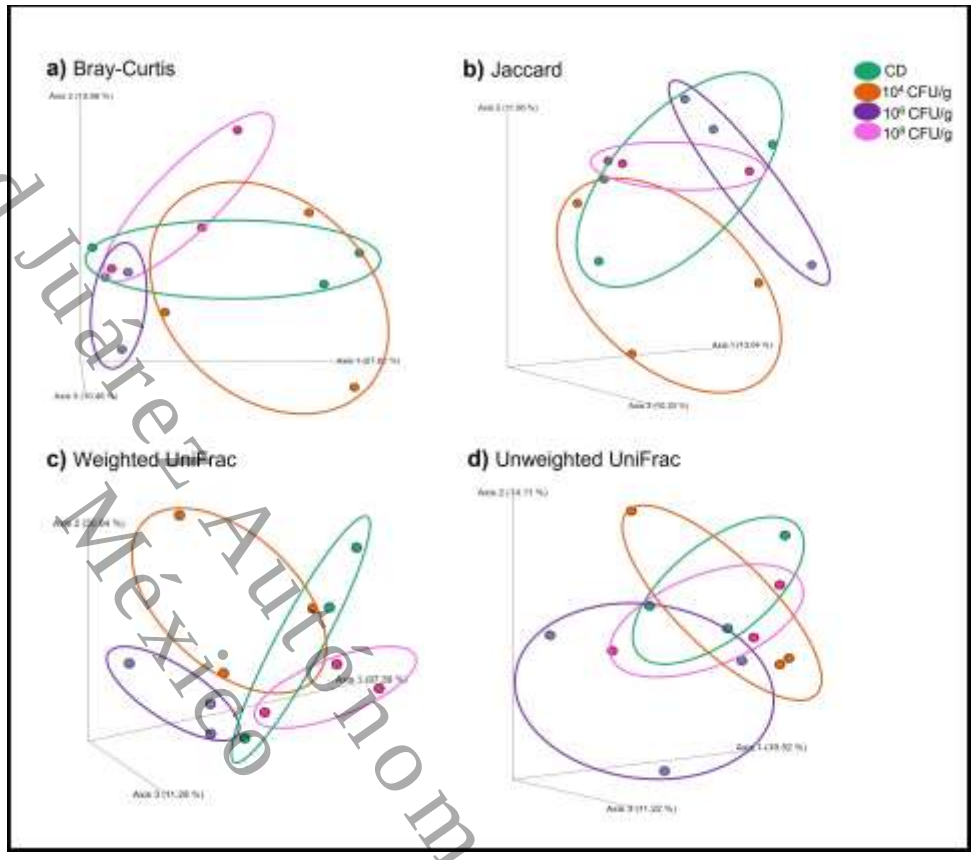

Figure 6. Principal coordinate analysis (PCoA) based on beta diversity analyses with Bray-Curtis (a), Jaccard (b), Weighted Unifrac (c), and Unweighted Unifrac (d) indexes of gut bacterial profiles of *A. tropicus* larvae treated fed *L. lactis* PH3-05 (10^4, 10^6 and 10^8 CFU/g*)* and a control diet.

3.7. Predicted metabolic functions of the intestinal microbiota from KEGG

An enhanced microbial function was detected in larvae fed with 10^8 CFU/g of *L. lactis* compared to those fed with the CD, as determined by Lefse analysis. Enrichment functions included bacterial chemotaxis, the biosynthesis of valine, leucine, and isoleucine, branched dibasic acid-C5 metabolism, flagellar assembly, fatty acid and lipopolysaccharide biosynthesis, pantothenate, and CoA synthesis. Additionally, functions related to D-glutamine and D-glutamate metabolism, biotin metabolism, lipid metabolism, carbon deposition via folate, peptidoglycan biosynthesis, and the sulfur relay system were also enriched. Conversely, larvae fed with

the CD exhibited higher microbial functions related to ansamycin synthesis and the degradation of ketone bodies (Fig. 7).

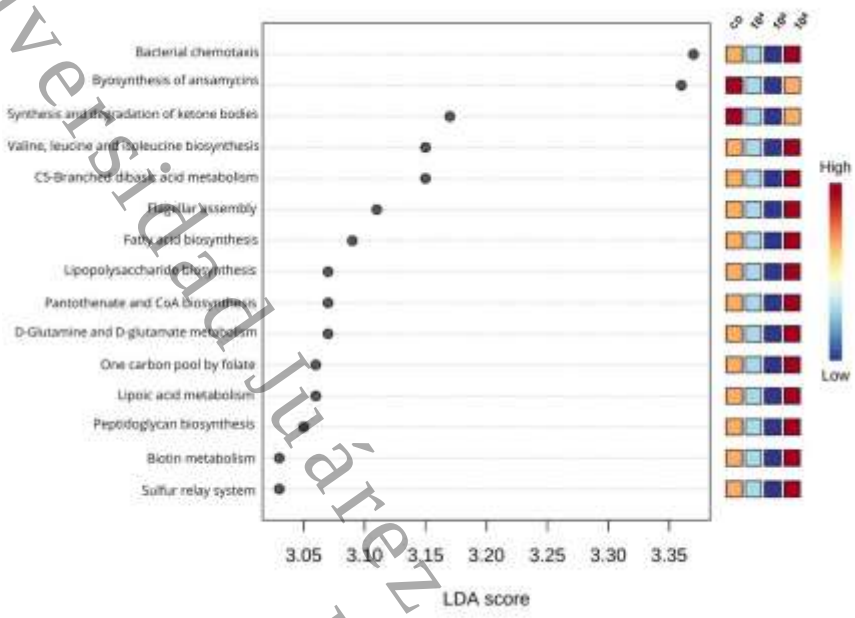

Figure 7. Heat map of microbial functions in the digestive tract of *A. tropicus* larvae fed with *L. lactis* PH3-05 (10^4, 10^6 and 10^8 CFU/g*)* and a control diet. Predictions are based on level 3 functional annotations using the KEGG database.

4. Discussion

4.1 Growth indexes and survival rate

Probiotics have successfully impacted aquaculture and have been considered a functional feed additive for cultured organisms [50]. Several studies have shown the beneficial effects of probiotic administration and have pointed out that they significantly improve the health and welfare of organisms, increasing survival rate and growth, enhancing and restoring the immune system, as well as making culture more profitable respectively [51-52]. However, probiotics must be administered adequately to confer a health benefit to the host [53]. In this regard, our results agree with what was reported since administering *L. lactis* PH3-05 (10^6 CFU/g) to *A. tropicus* larvae significantly improved productive values (final weight, final length, SGR, WG, and survival rate) compared to fish fed with CD.

Thus, the administration of *L. lactis* PH3-05 has provided positive results in different fish species, such as Nile tilapia (*Oreochromis niloticus*), where higher WG and higher survival were obtained [54]. In bastard halibut (*Paralichthys olivaceus*), including a concentration of 10^8 CFU/mL favored higher weight gain, feed efficiency, SGR, PER, and condition factor [55]. Likewise, administration of 10^8 CFU/g of *L. lactis* L19 improved growth, WG, feed efficiency index, SGR, and PER in snakehead fish (*Channa argus*) [56]. Similarly, 10^8 CFU/g concentration of *L. lactis* HNL12 in humpback grouper (*Cromileptes altivelis*) favored growth, total length, percentage weight gained,

and SGR [57]. The same effect was obtained when administering three different strains of *L. lactis* at high concentrations (5×10^8 CFU/g), which favours the final weight, WG, SGR, and survival of common carp (*Cyprinus carpio*) [23].

As demonstrated, proper administration of *L. lactis* improves productivity values and survival rates in various fish species, likely because these bacteria promote increased hydrolysis and absorption of nutrients, thereby enhancing the growth performance of the organisms [58-59]. Furthermore, lactic acid bacteria like, *L. lactis* efficiently metabolize various lactic acid fractions and release exogenous enzymes, short-chain acids, nucleic acids, and even functional peptides. This process improves the digestibility of nutrients in the balanced feeds given to fish [60-61]. Additionally, lactic acid bacteria, such as *L. lactis*, produce of short-chain fatty acids (SCFA), which are utilize by enterocytes as an energy substrate to maintain gut integrity, homeostasis, and digestive function. The release of these functional molecules enhances the hydrolytic capacity of digestive enzymes (proteases and lipases) and promotes increased selective permeability of the intestinal epithelium, leading to greater nutrient absorption. This, in turn, results in improved growth and development of the fish [62].

4.2. Digestive enzyme activity

Certain probiotics, such as *L. lactis*, have been reported to play a crucial role in the digestion of macronutrients in balanced diets fed to fish, which has been linked to increased hydrolysis and improved nutrient absorption in some fish species [21,63-64]. The presence of *L. lactis* has also been reported to increase the digestive enzyme activity of fish, in addition to the complementary hydrolysis due to the action of exogenous bacterial enzymes, which favor the hydrolysis of macromolecules (proteins, lipids, and carbohydrates). In this sense, *L. lactis* increases the digestive enzyme activity of fish, in addition to the complementary hydrolysis due to the action of exogenous bacterial hydrolytic enzymes, which promote the hydrolysis of macromolecules. Thus, bacterial pre-hydrolysis of feed nutrients and increased digestive enzyme activity in fish improve their growth and enhance their metabolic functions [65-66]. Our study observed that administering 10^6 CFU/g of *L. lactis* PH3-05 in *A. tropicus* larvae significantly increased digestive enzyme activity (acid protease, alkaline proteases, and lipase) compared to CD-fed larvae. In this regard, the addition of probiotics has been shown to significantly improve the regulation of amino acid, fatty acid, vitamin metabolism, and digestive enzyme synthesis in fish, offering a promising avenue for enhancing fish nutrition [67]. It has also been shown that the metabolic processes of probiotics can produce exogenous enzymes that benefit the host organism by supplementing digestive enzyme activity and pre-digesting the feed provided to the fish, thereby improving nutrient absorption. However, improved absorption will depend on the appropriate concentration of the probiotic [68]. For example, the use of a probiotic consortium (*Bacillus subtilis*, *Lactobacillus acidophilus*, *Clostridium butyricum*, and the yeast *Saccharomyces cerevisiae*) in *O. niloticus* led to an increase in trypsin-like and amylase activities [69].

Similarly, the application of a commercial probiotic (PrimaLac®: *L. acidophilus*, *Lactobacillus casei*, *Enterococcus faecium*, and *Bifidobacterium thermophilus*) resulted in a significant increase in amylase, protease, and alpha-glucosidase enzyme activities in Caspian white fish (*Rutilus frisii kutum*) [70]. In the case of *L. lactis*, when administered in rainbow trout (*Oncorhynchus mykiss*), trypsin, lipase, and alkaline digestive protease enzyme activities were highly detected [64]. The same is observed in *C. carpio*, where digestive enzyme activity increased (amylase, lipase, and protease), along with several production parameters, immune system enzymes, and antioxidant activity [71].

4.3. Histological analysis

Our research found that, the use of *L. lactis* PH3-05 significantly increases the height of the enterocytes in *A. tropicus* larvae, allowing fors a larger nutrient absorption area and, consequently, higher growth. These results align with findings in *O. niloticus*, where the administration of *L. lactis* ($10^{7\text{-}8}$ CFU/g diet), resulted in increased villi length and muscle layer thickness compared to a diet without probiotics [72]. Similarly, in the same species, *L. lactis* administration (10^8 CFU/g) increased the density and length of intestinal microvilli compared to the control treatment [54]. In gilthead sea bream (*Sparus aurata*), the administration of *L. lactis* (2 and 5 × 10^9 CFU/kg) reduced intestinal inflammatory processes and improved microbial composition [73].

Additionally, the hepatocyte area in *A. tropicus* larvae increased whit the use of *L. lactis* (10^8 CFU/g). These results are consistent with those reported by [74], who observed in vitro that *L. lactis* increases hepatocyte proliferation and promotes liver cell protection in snakehead (*Channa argus*). Similarly, in *O. niloticus*, an increase in lipid accumulation in hepatocytes was observed whit the administration of commercial probiotics (C.A. growth® and Tonolest®) [75]. Furthermore, our research detected an increase in the presence of MMC in the liver with *L. lactis* PH3-05 treatments. This finding coincides with observations in *O. niloticus* when a probiotic (Bacillus spp.) was administered, leading to an increase in MMC in the spleen. This response, is related to the organism's physiological reaction to the probiotic, which is perceived as a potential pathogen, thus activating the immune system to prevent possible infection [76].

Similarly, in *O. niloticus*, the number of MMC increased when a commercial symbiotic was supplied and the fish were challenging with *Pseudomonas fluorescens* [77]. A similar increase in MMC was observed when juveniles of the same species were supplemented with the probiotic *Pseudomonas putida* and challenged with *Aeromonas hydrophila* [78]. Thus, the increase in MMC is associated with the presence of the probiotic and is maximized when fish are challenged with pathogenic bacteria. However, *A. tropicus* larvae in our study were not challenged with any know pathogen. Therefore, further research is needed to explore the effect on immune capacity and its relationship with the increase in MMC in this species.

4.4. Gene expression

Probiotics, known for their potential to modulate the immune system and gut microbiota, and their promising antagonistic effect against pathogenic microorganisms [79], represent a significant area of interest in immune health research. The stimulation of the immune system by probiotics, particularly through components, such as peptidoglycan, lipopolysaccharides, and β-glucans in their cell walls, offers a promising avenue. These components can promote both innate and adaptive immune responses, modulate proinflammatory cytokines, activate natural killer cells, enhance mucosal, boost phagocytic activity, and increase lysozyme synthesis [80]. Additionally, certain bacteria (such as *Lactobacillus*, *Lactococcus*, *Enterococcus*, and *Carnobacterium*) produce bacteriocins, peptides that compromise the integrity of pathogenic organism's cell membranes [81-83]. Some bacteriocins can inhibit the adhesion or growth of pathogenic bacteria [84-86, 59].

In *A. tropicus* larvae, the administration of *L. lactis* PH3-05 (10^4 and 10^6 CFU/g) resulted in a significant increase in the relative expression of *muc-2*, suggesting an enhancement of the protective intestinal barrier. More importantly, this increase also indicates an activation of the immune system, as the mucus layer is the first line of defense against the translocation of toxic or pathogenic organisms. This finding is in line with the observations of [87], who also noted a similar increase in *muc-2* expression in response to a different stimuli. The mucus layer, secreted by goblet cells in the epidermis of fish, contains protective elements, such as glycoproteins, lysozymes, and immunoglobulins, among other antimicrobial compounds [88-89]. It serves multiple functions, including resistance and protection against infections, ionic and osmotic regulation, excretion, and nutrition absorption. This boost in mucus production can be interpreted as an active reinforcement of the immune system, highlighting the role of the mucus layer as primary physical defense against harmful pathogens in the intestinal barrier.

On the other hand, the underexpression of the *zo-2* gene in larvae fed with *L. lactis* PH3-05 is a significant finding. This underexpression may be promoting the synthesis of beneficial metabolites, such as bacteriocins, γ-aminobutyric acid, ornithine, exopolysaccharides, mannitol, among others [90], thereby eliminating the need for larvae to express *zo-2*. As a result, larvae fed with *L. lactis* PH3-05 experienced better growth. This finding is particularly noteworthy as a decrease in the expression of the *zo-2* gene has been reported to reduce intestinal function and can cause certain intestinal disorders in fish [91], which did not occur in *A. tropicus*. These results significantly enhance our understanding of the regulatory mechanisms of tight junction proteins, which are crucial in strengthening the intestinal barrier in *A. tropicus* in its larval stage.

Furthermore, the administration of *L. lactis* has been shown to directly improve the immune system [61]. In that sense, cytokines, the messenger proteins responsible for emitting the first warning signals of the immune system in response to harmful events, play a crucial role in the host organism's defense [92-93]. Different strains of *Lactobacillus* and *Bifidobacteria* are known to release or increase the expression of *il-8* or *il-10* during normal mucosal conditions or inflammatory processes, helping to neutralize or

prevent harmful stimuli [84]. In our study, *A. tropicus* larvae fed with *L. lactis* PH3-05 supplementation showed a higher expression of *il-8*, suggesting adaptation to the environment and formulated diet, as well as microbial colonization. In addition, it has been reported that certain strains of lactic acid bacteria can prevent an inflammatory response by activating the CD14 glycoprotein of epithelial cells [84]. Furthermore, the expression of the anti-inflammatory cytokine *il-10*, vital for maintaining mucosal immune homeostasis in the intestinal tract [94], was significantly enhanced by *L. lactis* PH3-05 in *A. tropicus* larvae. Our results show a substantial increase in the expression of *muc-2* (10^4 and 10^6 CFU/g) and *il-10* (10^6 and 10^8 CFU/g) in *A. tropicus* larvae fed *L. lactis*, which is consistent with the findings from crucian carp (*Carassius carassius*) fed with *L. lactis* PH3-05 supplements. These carp showed a significant immune system response after exposure to *Aeromonas hydrophila*, marked by increase expression of anti-inflammatory interleukin (*il-11*) and the gene related to the reinforcement of the intestinal barrier (*zo-1*). The overexpression of INF-γ, IL-1β, and TNF-α further indicates that *L. lactis* administration effectively reduces intestinal inflammation caused by exposure to pathogen bacteria [95]. The high resistance to pathogenic bacteria is attributed to the probiotic ability of *L. lactis* PH3-05 to modulate the immune system by releasing certain antimicrobial compounds. Also, i*l-11* expression suggests that the organism can protect and restore the gastrointestinal mucosa [96]. In Nile tilapia (*Oreochromis niloticus*), the inclusion of *L. lactis* strengthen the immune system, leading to increase expression of immune-related genes, specifically tumor necrosis factor (TNF-α) and interferon-gamma (IFN-γ), after exposure to the pathogen *Streptococcus agalactiae* [54]. This promising result opens potential applications of *L. lactis* PH3-05 in enhancing immune responses. However, the interaction between the gut microbiota and the immune system also involves specific metabolites secreted by the microbiota, which are absorbed by enterocytes and transported to the bloodstream and systemic lymphoid tissues. These metabolites can regulate host immune responses, play roles in inflammatory signaling, and interact directly or indirectly with host immune cells [97].

4.5. Gut microbiome

The microorganisms that compose the gut microbiome play essential roles in various metabolic, physiological, and immunological functions with the host organism [14]. The host microbiome t relationship can evolve over time, influenced by changes in diet, habitat and environmental adaptations. Maintaining a dynamic equilibrium between the microbiota and the host is crucial for optimal growth and development [98-99]. In larvae, this relationship is shaped by the initial bacterial community present at hatching or first mouth opening, microorganisms in the surrounding water, and the first food consumed [6,100]. The colonization and diversity of gut microbiota in fish are very complex and depend on various factors, including evolutionary and genetic processes [101-104]. Studies have shown that administering probiotics can alter the composition and abundance of gut bacteria. For example, in gilthead sea bream (*Sparus aurata*), administration of *L. lactis* (2 and 5×10^9 CFU/g) changed the gut bacterial composition,

increasing the abundance of *Pseudomonas*, *Sphyngomonas*, and *Lactobacillus* [73]. In *O. niloticus*, administering *L. lactis* (10^8 CFU/g) during the juvenile stage altered gut microbiota strengthened the immune system, resulting in reduced disease incidence [54]. Similarly, in red seabream (*Pagrus major*), probiotics (*L. rhamnosus* and *L. lactis*) also changed gut bacterial communities, enhancing the probiotics effect on the immune system [21].

Administering *L. lactis* PH3-05 (10^6 CFU/g) to *A. tropicus* larvae promotes an increase in the abundance of *Lactobacillus* in the intestinal microbiota. Previous studies [35] characterized the gut microbial composition of *A. tropicus* in both female and male juveniles and adults. The overall results showed that Fusobacteria is the most dominant phylum (42.26 %), followed by Proteobacteria (31.40 %), Firmicutes (12.96 %), and Bacteroides (11.79 %). Therefore, it can be considered a central gut microbial composition in *A. tropicus* at these stages. However, our results showed that the larval stage of *A. tropicus* is mainly composed of Proteobacteria and Bacteroides. The presence and function of Proteobacteria has been reported to increase the expression of genes related to RNA processing, degradation, outer membrane and lipopolysaccharide synthesis [105], which play roles in degrading Gram-negative (often pathogenic) microorganisms and enhancing the immune system. Bacteria belonging to the phyla Bacteroidetes and Proteobacteria could enhance metabolic and immune function and induce immune responses in the host, suggesting a possible relationship between these phyla and fish growth and immunity [106]. Additionally, the abundance of Proteobacteria may contribute to digestive functions in healthy fish [6].

In this context, our research on *A. tropicus* larvae has revealed intriguing findings that open new avenues for further exploration. The Proteobacteria genus, particularly *Massillia* and *Sphaerotilus*, showed the highest abundance, while potentially harmful genera like *Vibrio* and *Aeromona*s decreased. This finding suggests potential future studies to explore the role of these genera in the larvae's microbiome further. Similarly, Nile tilapia fed an experimental diet containing *L. lactis* PH3-05 (10^8 CFU/g) showed had the highest abundance of the Proteobacteria phylum [54]. It has also been documented that bacteria of the Bacteroidetes and Proteobacteria groups may originate from the aquatic environment [106]. The Bacteroidetes phylum of bacteria readily assimilates dietary carbohydrates, as members of this genus possess metabolic pathways to utilize them [107]. The genus *Sphingomonas* has been reported as an environmental microorganism, not as part of the gastrointestinal tract [108]. We consider that the presence of *Sphingomonas* in *A. tropicus* larvae results from the experiment's surrounding environment and that it is displaced by bacteria belonging to the genera *Massillia* and *Sphaerotilus*, which showed the highest abundance and could be considered part of the indigenous microbiota during colonization. However, we do not yet know whether the gut microbial composition of *A. tropicus* larvae can be classified as autochthonous or allochthonous. Nevertheless, we can consider that the administration of *L. lactis* PH3-05 (10^8 CFU/g) favored the abundance of proteobacteria in larvae fed with lower doses and the CD.

The functions of these microorganisms are not yet fully understood, but they have a significant impact on the organism's physiology and the

interactions between the metabolic capacity and metabolism of the host gut microbiota. Our research findings on functional metabolic predictions, particularly through the pathways predicted by KEGG LefSe, provide a novel understanding of these functions. We can now comprehend specific functions related to carbohydrate metabolism through the lactic fermentation of hexoses via the Embden-Meyerhof-Parnas pathway [109] and pentoses, via the phosphogluconate and phosphoketolase pathway [110]. Disaccharide metabolism occurs via the Leloir pathway, particularly for galactose [111]. Likewise, the activation of the pathways for protein catalysis is known to occur through the activation of a proteolytic system by utilization of approximately 14 amino acids, with leucine, valine, and isoleucine being essential [112], via extracellular degradation by membrane-anchored proteinase, the peptide transport system across the membrane into the cytoplasm and degradation of peptides by intracellular endopeptidases such as proline peptidase and aminopeptidase [113-114]. Moreover, a di-, tri-, and oligopeptide transporter system has been detected, which, with the action of peptidases, releases amino acids for catabolism and energy production [115]. Finally, the ability to synthesize lipids from fatty acids such as octadecanoic acid that is converted into cyclopropane, oleic, and vaccenic acids, is achieved through the action of enzymes such as cyclopropane synthase, transforming into dihydrosterculic acid via methylation [116].

As can be seen, *L. lactis* PH3-05 can modify the microbiome and alter the abundance of bacteria from the phyla Bacterioides and Proteobacteria, particularly of the genera *Massillia*, *Sphaerotilus*, and S*phingomonas*, enabling various pathways to manifest. These, include: 1) bacterial chemotaxis, allowings bacteria to move in response to nutrients gradients and other environmental stimuli [117], 2) flagellar assembly, a transcriptional and post-transcriptional process enables bacteria to move towards tissue colonization sites and perform multiple functions in host communication [118], 3) fatty acid biosynthesis, revealing the diversity of the organization of the pfa genes, coding for a polyunsaturated fatty acid synthase complex [119], 4) the biosynthesis of valine, leucine and isoleucine, which are considered suitable targets for developing antibacterial agents [120], and 5) the metabolism of dibasic acid C5-triphosphate, where a complex pathway has been described in different bacteria [121]. These findings, reflected changes in the microbiome and enhanced growth of *A. tropicus* PH3-05 larvae when fed with *L. lactis* (10^{6-8} CFU/g).

5. Conclusions

Feeding *A. tropicus* larvae with live *L. lactis* PH3-05, isolated as part of the native microbiome, significantly improves production values, digestive morphology, digestive enzyme activity, and immune system gene expression. Additionally, this probiotic modulates and strengthens various metabolic pathways of the microbiome, showing highly significant results when using doses of 10^{6-8} CFU/g in balanced feeds during weaning in the larval period. Ultimately, it is demonstrated that *L. lactis* PH3-05 can be considered a highly efficient probiotic that improves larval culture.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table S1: Antagonistic activity of *Lactococcus lactis* PH3-05 against pathogenic strains of fish.

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Data Availability Statement: The data presented in this study are available upon request from the corresponding authors.

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References

1. FAO (Food and Agriculture Organization of the United Nations). *The state of world fisheries and aquaculture 2020*. FAO. Rome, Italy, **2020**. https://doi.org/10.4060/ca9229en
2. Reinoso, S.; Gutiérrez, M.S.; Domínguez-Borbor, C.; Argüello-Guevara, W.; Bohórquez-Cruz, M.; Sonnenholzner, S.; Nova-Baza, D.; Mardones, C.; Navarrete, P. Selection of Autochthonous Yeasts Isolated from the Intestinal Tracts of Cobia Fish (*Rachycentron canadum*) with Probiotic Potential. *J. Fungi*. **2023,** *9*, 274. https://doi.org/10.3390/jof9020274
3. Reda, R.M.; Selim, K.; El-Sayed, H.M.; El-Hady, M.A. In Vitro Selection and Identification of Potential Probiotics Isolated from the Gastrointestinal Tract of Nile Tilapia, *Oreochromis niloticus*. *Probiotics & Antimicro. Prot*. **2017**, *10*, 692-703. https://doi.org/10.1007/s12602-017-9314-6
4. Rohani, M.F.; Islam, S.M.; Hossain, M.K.; Ferdous, Z.; Siddik, A.; Nuruzzaman, M.; Padeniya, U.; Brown, C.L.; Shahjahan, M. Probiotics, prebiotics and synbiotics improved the functionality of aquafeed: Upgrading growth, reproduction, immunity and disease resistance in fish. *Fish Shellfish Immunol*. **2022**, *120*, 569-589. https://doi.org/10.1016/j.fsi.2021.12.037
5. Galdeano, C.M.; Cazorla, S.I.; Dumit, M.L.; Del Mar Vélez, M.; Perdigón, G. Beneficial Effects of Probiotic Consumption on the Immune System. *Ann Nutr Metab*. **2019**, *74*, 115-124. https://doi.org/10.1159/000496426
6. Nayak, S. Probiotics and immunity: A fish perspective. *Fish Shellfish Immunol*. **2010**, *29*, 2-14. https://doi.org/10.1016/j.fsi.2010.02.017
7. Pérez-Sánchez, T.; Ruiz-Zarzuela, I.; De Blas, I.; Balcázar, J.L. Probiotics in aquaculture: a current assessment. *Rev Aquac*. **2013**, *6*, 133-146. https://doi.org/10.1111/raq.12033
8. Ringø, E.; Harikrishnan, R.; Soltani, M.; Ghosh, K. The effect of gut microbiota and probiotics on metabolism in fish and shrimp. *Animals*. **2022**, *12*, 3016. https://doi.org/10.3390/ani12213016
9. Valipour, A.; Nedaei, S.; Noori, A.; Khanipour, A.; Hoseinifar, S.H. Dietary *Lactobacillus plantarum* affected on some immune parameters, air-exposure stress response, intestinal microbiota, digestive enzyme activity and performance of narrow clawed crayfish (*Astacus leptodactylus*, Eschscholtz). *Aquaculture*. **2019**, *504*, 121-130. https://doi.org/10.1016/j.aquaculture.2019.01.064
10. Yi, Y.; Zhen-Hua, Z.; Zhao, F.; Liu, H.; Yu, L.; Zha, J.; Wang, G. Probiotic potential of *Bacillus velezensis* JW: Antimicrobial activity against fish pathogenic bacteria and immune enhancement effects on *Carassius auratus*. *Fish Shellfish Immunol*. **2018**, *78*, 322-330. https://doi.org/10.1016/j.fsi.2018.04.055
11. Srirengaraj, V.; Razafindralambo, H.; Rabetafika, H.; Nguyen, H.T.; Sun, Y. Synbiotic Agents and Their Active Components for Sustainable Aquaculture: Concepts, Action Mechanisms, and Applications. *Biology*. **2023**, *12*, 1498. https://doi.org/10.3390/biology12121498
12. Bolotin, A.; Wincker, P.; Mauger, S.; Jaillon, O.; Malarme, K.; Weissenbach, J.; Ehrlich, S.D.; Sorokin, A. The Complete Genome Sequence of the Lactic Acid Bacterium *Lactococcus lactis* ssp. *lactis* IL1403. *Genome Res*. **2001**, *11*, 731-753. https://doi.org/10.1101/gr.gr-1697r
13. Lee, S. H.; Beck, B. R.; Hwang, S.; Song, S. K. Feeding olive flounder (*Paralichthys olivaceus*) with *Lactococcus lactis* BFE920 expressing the fusion antigen of *Vibrio* OmpK and FlaB provides protection against multiple *Vibrio* pathogens: A universal vaccine effect. *Fish Shellfish Immunol*. **2021**, *114*, 253-262. https://doi.org/10.1016/j.fsi.2021.05.007
14. Ray, A. K.; Ghosh, K. S.; Ringø, E. Enzyme-producing bacteria isolated from fish gut: a review. *Aqua. Nutr*. **2012**, *18*, 465-492. https://doi.org/10.1111/j.1365- 2095.2012.00943.x
15. Zhu, C.; Liu, D.; Chen, W.; Ban, S.; Liu, T.; Huang, W.; Jiang, M. Effects of dietary host-associated *Lactococcus lactis* on growth performance, disease resistance, intestinal morphology and intestinal microbiota of mandarin fish (*Siniperca chuatsi*). *Aquaculture*. **2021**, *540*, 736702. https://doi.org/10.1016/j.aquaculture.2021.736702
16. Goupil-Feuillerat, N.; Cocaign-Bousquet, M.; Godon, J.; Ehrlich, S. D.; Renault, P. Dual role of alpha-acetolactate decarboxylase in *Lactococcus lactis* subsp. *lactis*. *J. Bacteriol*. **1997**, *179*, 6285-6293. https://doi.org/10.1128/jb.179.20.6285-6293.1997

17. Kaktcham, P. M.; Piame, L. T.; Sileu, M. S.; Kouam, M. F.; Temgoua, J.; Ngoufack, F. Z.; de Lourdes Pérez-Chabela, M. Bacteriocinogenic *Lactococcus lactis* subsp. *lactis 3MT* isolated from freshwater Nile Tilapia: isolation, safety traits, bacteriocin characterisation, and application for biopreservation in fish pâté. *Arch. Microbiol*. **2019**, *201*, 1249-1258. https://doi.org/10.1007/s00203-019-01690-4
18. Sperandio, B.; Polard, P.; Ehrlich, D.; Renault, P.; Guédon, É. Sulfur Amino Acid Metabolism and Its Control in *Lactococcus lactis* IL1403. *J. Bacteriol*. **2005**, *187*, 3762-3778. https://doi.org/10.1128/jb.187.11.3762-3778.2005
19. Sun, Y.; Yang, H.; Ma, R.; Zhai, S. Does Dietary Administration of *Lactococcus lactis* Modulate the Gut Microbiota of Grouper, *Epinephelus coioides*. *J. World Aquac. Soc*. **2012**, *43*, 198-207. https://doi.org/10.1111/j.1749-7345.2012.00555.x
20. Beck, B. R.; Kim, D.; Jeon, J.; Lee, S. M.; Kim, H. K.; Kim, O.; Lee, J. I.; Suh, B. S.; Ki, H.; Lee, K. H.; Holzapfel, W. H.; Hwang, J. Y.; Kwon, M.; Song, S. K. The effects of combined dietary probiotics *Lactococcus lactis* BFE920 and *Lactobacillus plantarum* FGL0001 on innate immunity and disease resistance in olive flounder (*Paralichthys olivaceus*). *Fish Shellfish Immunol*. **2015,** *42*, 177-183. https://doi.org/10.1016/j.fsi.2014.10.035
21. Dawood, M. A.; Koshio, S.; Ishikawa, M.; Yokoyama, S.; Basuini, E.; Hossain, M. S.; Nhu, T. H.; Dossou, S.; Moss, A. S. Effects of dietary supplementation of *Lactobacillus rhamnosus* or/and *Lactococcus lactis* on the growth, gut microbiota and immune responses of red sea bream, *Pagrus major*. *Fish Shellfish Immunol*. **2016**, *49*, 275-285. https://doi.org/10.1016/j.fsi.2015.12.047
22. Linh, T. H.; Nagai, S.; Nagasaka, N.; Okane, S.; Taoka, Y. Effect of *Lactococcus lactis* K-C2 on the growth performance, amino acid content and gut microflora of amberjack *Seriola dumerili*. *Fish. Sci*. **2018**, *84*, 1051-1062. https://doi.org/10.1007/s12562-018-1243-0
23. Feng, J.; Chang, X.; Zhang, Y.; Yan, X.; Zhang, J.; Nie, G. Effects of *Lactococcus lactis* from *Cyprinus carpio* L. as probiotics on growth performance, innate immune response and disease resistance against *Aeromonas hydrophila*. *Fish Shellfish Immunol*. **2019**, *93*, 73–81. https://doi.org/10.1016/j.fsi.2019.07.028
24. Márquez-Couturier, G.; Álvarez-González, C.A.; Contreras-Sánchez, W.M.; Hernández-Vidal, U.; Hernández-Franyutti, A.; Mendoza-Alfaro, R.; Goytortúa-Bores, E. Avances en la Alimentación y Nutrición del Pejelagarto *Atractosteus tropicus*; Avances En Nutrición Acuícola VIII; Universidad Autónoma de Nuevo León: Monterrey, Nuevo León, México, **2006**. pp. 446–523.
25. Nájera-Arzola, I. C.; Álvarez-González, C. A.; Frías-Quintana, C. A.; Peña, E.; Martínez-García, R.; Camarillo-Coop, S.; Méndez-Marín, O.; Gisbert, E. Evaluation of Mannan oligosaccharides (MOS) in balanced diets for tropical gar juveniles (*Atractosteus tropicus*). *Hidrobiológica*. **2018**, *28*, 239-246. https://doi.org/10.24275/uam/izt/dcbs/hidro/2018v28n3/pena
26. Nieves-Rodríguez, K. N.; Álvarez-González, C. A.; Peña-Marín, E. S.; Vega-Villasante, F.; Martínez-García, R.; Camarillo-Coop, S.; Tovar-Ramírez, D.; Guzmán-Villanueva, L. T.; Andrée, K. B.; Gisbert, E. Effect of β-Glucans in Diets on Growth, Survival, Digestive Enzyme Activity, and Immune System and Intestinal Barrier Gene Expression for Tropical Gar (*Atractosteus tropicus*) Juveniles. *Fishes*. **2018**, *3*, 27. https://doi.org/10.3390/fishes3030027
27. Sepúlveda-Quiroz, C. A.; Peña-Marín, E. S.; Pérez-Morales, A.; Martínez-García, R.; Álvarez Villagómez, C. S.; Maytorena-Verdugo, C. I.; Camarillo-Coop, S.; Vissio, P. G.; Sirkin, D. I. P.; Tovar-Ramírez, D.; Galavíz, M. A.; Álvarez-González, C. A. Fructooligosaccharide supplementation in diets for tropical gar (*Atractosteus tropicus*) juvenile: Effects on morphophysiology and intestinal barrier function. *Aquac. Res*. **2020**, *52*, 37-50. https://doi.org/10.1111/are.14867
28. Pérez-Jiménez, G. M.; Peña-Marín, E. S.; Maytorena-Verdugo, C. I.; Sepúlveda-Quiroz, C. A.; Jiménez-Martínez, L. D.; De la Rosa-García, S.; Asencio-Alcudia, G. G.; Martínez, R.; Tovar-Ramírez, D.; Galavíz, M. A.; Martínez-Burguete, T.; Álvarez-González, C. A.; Álvarez-Villagómez, C. S. Incorporation of Fructooligosaccharides in Diets Influence Growth Performance, Digestive Enzyme Activity, and Expression of Intestinal Barrier Function Genes in Tropical Gar (*Atractosteus tropicus*) Larvae. *Fishes*. **2022**, *7*,137. https://doi.org/10.3390/fishes7030137
29. De la Cruz-Marín, E.; Martínez-García, R.; López-Hernández, J. F.; Méndez-Marín, O.; De la Rosa-García, S.; Peña-Marín, E. S.; Tovar-Ramírez, D.; Sepúlveda-Quiroz, C. A.; Pérez-Jiménez, G. M.; Jiménez-Martínez, L. D.; Asencio-Alcudia, G. G.; Álvarez-González, C. A. Inulin Supplementation in Diets for Tropical Gar (*Atractosteus tropicus*) Larvae: Effects on Growth, Survival, and Digestive and Antioxidant Enzyme Activities. *Aquac. J.* **2023**, *3*, 43-55. https://doi.org/10.3390/aquacj3010006

30. Cigarroa-Ruiz, L.; Toledo-Solís, F. J.; Frías-Gómez, S. A.; Guerrero-Zárate, R.; Camarillo-Coop, S.; Álvarez-Villagómez, C. S.; Peña-Marín, E. S.; Galavíz, M. A.; Martínez-García, R.; Álvarez-González, C. A. Addition of β-glucans in diets for tropical gar (*Atractosteus tropicus*) larvae: effects on growth, digestive enzymes and gene expression of intestinal epithelial integrity and immune system. *Fish Physiol. Biochem*. **2023**, *49*, 613-626. https://doi.org/10.1007/s10695-023-01207-3
31. Hernández-López, I. A.; Tovar-Ramírez, D.; De la Rosa-García, S.; Álvarez-Villagómez, C. S.; Asencio-Alcudia, G. G.; Martínez-Burguete, T.; Galavíz, M. A.; Guerrero-Zárate, R.; Martínez-García, R.; Peña-Marín, E. S.; Álvarez-González, C. A. Dietary live yeast (*Debaryomyces hansenii*) provides no advantages in tropical gar, *Atractosteus tropicus* (Actinopterygii: Lepisosteiformes: Lepisosteidae), juvenile aquaculture. *Acta Ichthyol. Piscat*. **2021**, *51*, 311-320. https://doi.org/10.3897/aiep.51.67095
32. Palma-Cancino, D. J.; Martínez-García, R.; Álvarez-González, C. A.; Camarillo-Coop, S.; Peña-Ma, E. S. Evaluation of feeding strategies in tropical gar (*Atractosteus tropicus* Gill) larvae: growth, survival and cannibalism. *Ecosist. Recur. Agropecu*. **2019**, *6*, 273-281. https://www.cabdirect.org/cabdirect/abstract/20203438791
33. Jiménez-Martínez, L. D.; Tovar-Ramírez, D.; Álvarez-González, C. A.; Peña-Marín, E. S.; Camarillo-Coop, S.; Martínez-García, R.; Elena, P.; Martínez-Yáñez, R.; Concha-Frías, B. Assessment of dietary lipid sources in tropical gar, *Atractosteus tropicus* larvae: Growth parameters and intermediary lipogenic gene expression. *Aquac. Res.* **2020**, *51*, 2629-2640. https://doi.org/10.1111/are.14603
34. Sepúlveda-Quiroz, C. A.; Pérez-Jiménez, G. M.; Asencio-Alcudia, G. G.; Mendoza-Porras, O.; Jiménez-Martínez, L. D.; Galaviz-Espinoza, M. A.; Tovar-Ramírez, D.; Martínez, R.; Álvarez-Villagómez, C. S.; Álvarez-González, C. A. Tryptophan Reduces Intracohort Cannibalism Behavior in Tropical Gar (*Atractosteus tropicus*) Larvae. *Fishes*. **2024**, *9*, 40. https://doi.org/10.3390/fishes9010040
35. Méndez-Pérez, R.; García-López, R.; Bautista-López, J. S. B.; Vázquez-Castellanos, J. F.; Álvarez-González, C. A.; Peña-Marín, E. S.; Baltierra-Trejo, E.; Adams-Schroeder, R.; Domínguez-Rodríguez, V. I.; Melgar-Valdés, C. E.; Martínez-García, R.; Moyá, A.; Gómez-Cruz, R. High-throughput sequencing of the 16S rRNA gene to analyze the gut microbiome in juvenile and adult tropical gar (*Atractosteus tropicus*). *Lat. Am. J. Aquat.* **2020**, *48*, 456-479. https://doi.org/10.3856/vol48-issue3-fulltext-2419
36. Hoben, H. J.; Somasegaran, P. Comparison of the pour, spread, and drop plate methods for enumeration of Rhizobium spp. In inoculants made from presterilized peatt. *Appl. Environ. Microbiol.* **1982**, *44*, 1246-1247.
37. Álvarez-González, C. A.; Civera-Cerecedo, R.; Galindo, J. L. O.; Dumas, S.; Moreno Legorreta, M.; Álamo, T. G. Effect of dietary protein level on growth and body composition of juvenile spotted sand bass, *Paralabrax maculatofasciatus*, fed practical diets. *Aquaculture*. **2001**, *194*, 151-159. https://doi.org/10.1016/s0044-8486(00)00512-3
38. AOAC (Association of Official Analytical Chemists). Official Methods of Analysis, 17th ed.; Association of Official Analytical Chemists. **2000**, Arlington, VA, USA.
39. NOM-062-ZOO-1999. Norma Oficial Mexicana: Especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio, **2001**. Retrieved from https://www.gob.mx/senasica/documentos/nom-062-zoo-1999
40. Bradford, M. M. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal. Biochem.* **1976**, *72*, 248-254. https://doi.org/10.1006/abio.1976.9999
41. Anson, M. L. The estimation of pepsin, trypsin, papain, and cathepsin with hemoglobin. *J. Gen. Physiol*. **1938**, *22*, 79–89. https://doi.org/10.1085/jgp.22.1.79
42. Walter, H. Proteinases: Methods with hemoglobin, casein and azocoll as substrates. In *Methods of Enzymatic Analysis*; Verlag Chemie: Weinheim, Germany, **1984**. pp. 270–277.
43. Erlanger, B. F.; Kokowsky, N.; Cohen, W. W. The preparation and properties of two new chromogenic substrates of trypsin. *Arch. Biochem. Biophys*. **1961**, *95*, 271-278. https://doi.org/10.1016/0003-9861(61)90145-x
44. Del Mar, E. G.; Largman, C.; Brodrick, J.; Geokas, M. C. A sensitive new substrate for chymotrypsin. *Anal. Biochem.* **1979**, *99*, 316-320. https://doi.org/10.1016/s0003-2697(79)80013-5
45. Versaw, W. K.; Cuppett, S. L.; Winters, D.; Williams, L. E. An Improved Colorimetric Assay for Bacterial Lipase in Nonfat Dry Milk. *J. Food Sci*. **1989**, *54*, 1557-1558. https://doi.org/10.1111/j.1365-2621.1989.tb05159.x
46. Maroux, S.; Louvard, D.; Barath, J. The aminopeptidase from hog intestinal brush border. *Biochim. Biophys. Acta–Enzymology*. **1973**, *321*, 282–295. https://doi.org/10.1016/0005- 2744(73)90083-1

47. Jiménez-Martínez, L.; Morales, V.; Frías-Quintana, C.; Castillo, A.; Ascencio-Alcudia, G.; Alvarez-Villagomez, C.; Peña-Marín, E.; Concha-Frías, B.; Alvarez-González, C.A. Quality Evaluation of Reference Gene Expression on Different Tissues in Adults of Tropical Gar *Atractosteus tropicus*. *Pak. J. Zool*. **2021**, *54*,363-372. https://doi.org/10.17582/journal.pjz/20200913180928
48. Livak, K. J.; Schmittgen, T. D. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. *Methods*. **2001**, *25*, 402-408. https://doi.org/10.1006/meth.2001.1262
49. Huse, S. M.; Dethlefsen, L.; Huber, J. A.; Welch, D. B. M.; Relman, D. A.; Sogin, M. L. Exploring Microbial Diversity and Taxonomy Using SSU rRNA Hypervariable Tag Sequencing. *PLOS Genet.* **2008**, *4*, e1000255. https://doi.org/10.1371/journal.pgen.1000255
50. Ibrahem, M. D. Evolution of probiotics in aquatic world: potential effects, the current status in Egypt and recent prospectives. *J. Adv. Res*. **2015**, *6*, 765-791. https://doi.org/10.1016/j.jare.2013.12.004
51. Hoseinifar, S. H.; Ringø, E.; Masouleh, A. S.; Esteban, M. Á. Probiotic, prebiotic and synbiotic supplements in sturgeon aquaculture: a review. *Rev. Aquac*. **2014**, *8*, 89-102. https://doi.org/10.1111/raq.12082
52. Gibson, G. R.; Hutkins, R.; Sanders, M. E.; Prescott, S. L.; Reimer, R. A.; Salminen, S. J.; Reid, G. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat. Rev. Gastroenterol. Hepatol.* **2017**,*14*, 491–502. https://doi.org/10.1038/nrgastro.2017.75
53. Gatesoupe, F. The use of probiotics in aquaculture. *Aquaculture*. **1999**, *180*,147-165. https://doi.org/10.1016/s0044-8486(99)00187-8
54. Xia, Y.; Lu, M.; Chen, G.; Cao, J.; Gao, F.; Wang, M.; Liu, Z.; Zhang, D.; Zhu, H.; Yi, M. Effects of dietary *Lactobacillus rhamnosus* JCM1136 and *Lactococcus lactis* subsp. *lactis* JCM5805 on the growth, intestinal microbiota, morphology, immune response and disease resistance of juvenile Nile tilapia, *Oreochromis niloticus*. *Fish Shellfish Immunol.* **2018**, *76*, 368-379. https://doi.org/10.1016/j.fsi.2018.03.020
55. Heo, W.; Kim, Y.; Kim, E. Y.; Bai, S. C.; Kong, I. Effects of dietary probiotic, *Lactococcus lactis* subsp. *lactis I2*, supplementation on the growth and immune response of olive flounder (*Paralichthys olivaceus*). *Aquaculture*. **2013**, *376-379*, 20-24. https://doi.org/10.1016/j.aquaculture.2012.11.009
56. Kong, Y.; Gao, C.; X.; Zhao, J.; Li, M.; Shan, X.; Wang, G. Effects of single or conjoint administration of lactic acid bacteria as potential probiotics on growth, immune response and disease resistance of snakehead fish (*Channa argus*). *Fish Shellfish Immunol.* **2020**, *102*, 412-421. https://doi.org/10.1016/j.fsi.2020.05.003
57. Sun, Y.; He, M.; Cao, Z.; Xie, Z.; Liu, C.; Wang, S.; Guo, W.; Zhang, X.; Zhou, Y. Effects of dietary administration of *Lactococcus lactis* HNL12 on growth, innate immune response, and disease resistance of humpback grouper (*Cromileptes altivelis*). *Fish Shellfish Immunol.* **2018**, *82*, 296-303. https://doi.org/10.1016/j.fsi.2018.08.039
58. Ghosh, K.; Ray, A. K.; Ringø, E. Applications of plant ingredients for tropical and subtropical freshwater FINFISH: Possibilities and challenges. *Rev. Aquac*. **2018**, *11*, 793-815. https://doi.org/10.1111/raq.12258
59. Li, X.; Ringø, E.; Hoseinifar, S. H.; Lauzon, H. L.; Birkbeck, H.; Yang, D. The adherence and colonization of microorganisms in fish gastrointestinal tract. *Rev. Aquac*. **2018**, *11*, 603-618. https://doi.org/10.1111/raq.12248
60. Ringø, E.; Hoseinifar, S. H.; Ghosh, K.; Van Doan, H.; Beck, B. R.; Song, S. K. Lactic Acid Bacteria in Finfish—An Update. *Front. Microbiol*. **2018**, *9*. https://doi.org/10.3389/fmicb.2018.01818
61. Dawood, M. A.; Koshio, S. Recent advances in the role of probiotics and prebiotics in carp aquaculture: A review. *Aquaculture*. **2016**, *454*, 243-251. https://doi.org/10.1016/j.aquaculture.2015.12.033
62. Fu, J.; Zheng, Y.; Gao, Y.; Wang, X. Dietary fiber intake and gut microbiota in human health. *Microorganisms.* **2022**, *10*, 2507. https://doi.org/10.3390/microorganisms10122507
63. Sun, Y.; Yang, H.; Ma, R.; Song, K.; Li, J. Effect of *Lactococcus lactis* and *Enterococcus faecium* on growth performance, digestive enzymes and immune response of grouper *Epinephelus coioides*. *Aquac. Nutr.* **2011**, *18*, 281-289. https://doi.org/10.1111/j.1365-2095.2011.00894.x
64. Yeganeh, S.; Adel, M.; Nosratimovafagh, A.; Dawood, M. A. O. The Effect of *Lactococcus lactis* subsp. *lactis* PTCC 1403 on the Growth Performance, Digestive Enzymes Activity, Antioxidative Status, Immune Response, and Disease Resistance of Rainbow Trout (*Oncorhynchus mykiss*). *Probiotics Antimicro. Proteins.* **2021**, *13*, 1723-1733. https://doi.org/10.1007/s12602-021-09787-3

65. LeBlanc, J. G.; Chain, F.; Martín, R.; Bermúdez-Humarán, L. G.; Courau, S.; Langella, P. Beneficial effects on host energy metabolism of short-chain fatty acids and vitamins produced by commensal and probiotic bacteria. *Microb. Cell Fact.* **2017**, *16*. https://doi.org/10.1186/s12934-017-0691-z
66. Hardy, R. W.; Kaushik, S.; Mai, K.; Bai, S. C. Fish nutrition history and perspectives. Elsevier eBooks, **2022**. pp. 1-16. https://doi.org/10.1016/b978-0-12-819587-1.00006-9
67. Misra, S.; Pandey, P.; Mishra, H. N. Novel approaches for co-encapsulation of probiotic bacteria with bioactive compounds, their health benefits and functional food product development: A review. *Trends Food Sci. Technol*. **2021**, *109*, 340-351. https://doi.org/10.1016/j.tifs.2021.01.039
68. Assan, D.; Kuebutornye, F. K. A.; Hlordzi, V.; Chen, H.; Mraz, J.; Mustapha, U. F.; Abarike, E. D. Effects of probiotics on digestive enzymes of fish (finfish and shellfish); status and prospects: a mini review. *Comp. Biochem. Physiol. Part B, Biochem. Mol. Biol.* **2022**, *257*, 110653. https://doi.org/10.1016/j.cbpb.2021.110653
69. Taoka, Y.; Maeda, H.; Jo, J.; Sakata, T. Influence of commercial probiotics on the digestive enzyme activities of tilapia, *Oreochromis niloticus*. *Aquac. Sci.* **2007**, *55*, 183-189. https://doi.org/10.11233/aquaculturesci1953.55.183
70. Mirghaed, A. T.; Yarahmadi, P.; Hosseinifar, S. H.; Tahmasebi, D.; Gheisvandi, N.; Ghaedi, A. The effects singular or combined administration of fermentable fiber and probiotic on mucosal immune parameters, digestive enzyme activity, gut microbiota and growth performance of Caspian white fish (*Rutilus frisii kutum*) fingerlings. *Fish Shellfish Immunol*. **2018**, *77*, 194-199. https://doi.org/10.1016/j.fsi.2018.02.007
71. Wang, J.; Feng, J.; Liu, S.; Cai, Z.; Song, D.; Yang, L.; Nie, G. The probiotic properties of different preparations using *Lactococcus lactis* Z-2 on intestinal tract, blood and hepatopancreas in *Cyprinus carpio*. *Aquaculture*. **2021**, *543*, 736911. https://doi.org/10.1016/j.aquaculture.2021.736911
72. Won, S.; Hamidoghli, A.; Choi, W.; Park, Y.; Jang, W. J.; Kong, I.; Bai, S. C. Effects of *Bacillus subtilis* WB60 and *Lactococcus lactis* on Growth, Immune Responses, Histology and Gene Expression in Nile Tilapia, *Oreochromis niloticus*. *Microorganisms*. **2020**, *8*, 67. https://doi.org/10.3390/microorganisms8010067
73. Moroni, F.; Naya-Català, F.; Piazzon, M. C.; Rimoldi, S.; Calduch-Giner, J.; Giardini, A.; Martínez, I.; Brambilla, F.; Pérez-Sánchez, J.; Terova, G. The Effects of Nisin-Producing *Lactococcus lactis* Strain Used as Probiotic on Gilthead Sea Bream (*Sparus aurata*) Growth, Gut Microbiota, and Transcriptional Response. *Front. Mar. Sci*. **2021**, *8*. https://doi.org/10.3389/fmars.2021.659519
74. Kong, Y.; Kong, N.; Liu, H.; Han, M.; Peng, S.; Fang, Q.; Chen, X.; Wang, G.; Li, M. Molecular mechanism of homologous lactic acid bacteria regulating liver cell injury of snakehead fish. *Aquac. Rep*. **2024**, *34*, 101905. https://doi.org/10.1016/j.aqrep.2023.101905
75. Tanekhy, M.; Khalil, R.; Hofi, H.; Hashish, E. The Biochemical, Pathological and Immunological Effectiveness of Commercial Probiotics in Nile Tilapia, *Oreochromis niloticus*. *Pakistan J. Zool*. **2016**, *48*,1269-1282.
76. Dighiesh, H. S.; Alharbi, N. A.; Awlya, O. F.; Alhassani, W. E.; Hassoubah, S. A.; Albaqami, N. M.; Aljahdali, N.; El-Aziz, Y. M. A.; Eissa, E. H.; Munir, M. B.; Sakr, S. E. Dietary multi-strains *Bacillus* spp. enhanced growth performance, blood metabolites, digestive tissues histology, gene expression of *Oreochromis niloticus*, and resistance to *Aspergillus flavus* infection. *Aquac. Int*. **2024**, https://doi.org/10.1007/s10499-024-01502-7
77. Ismail, M.; Wahdan, A.; Mohamed, S. Y.; Metwally, E.; Mabrok, M. Effect of dietary supplementation with a synbiotic (Lacto Forte) on growth performance, haematological and histological profiles, the innate immune response and resistance to bacterial disease in *Oreochromis niloticus*. *Aquac. Res.* **2019**, *50*, 2545-2562. https://doi.org/10.1111/are.14212
78. Abomughaid, M. M. Isolation and Identification of Some Probiotic Bacteria and Their Potential Role in Improving Immune Response and Resistance of Nile Tilapia (*Oreochromis niloticus*) in Comparison with a Commercial Product. *Int. J. Microbiol*. **2020**,1-9. https://doi.org/10.1155/2020/8865456
79. Arsène, M. M. J.; Davares, A. K. L.; Andreevna, S. L.; Vladimirovich, E. A.; Carime, B. Z.; Marouf, R.; Khelifi, I. The use of probiotics in animal feeding for safe production and as potential alternatives to antibiotics. *Vet. World*. **2021**, *14*, 319-328. https://doi.org/10.14202/vetworld.2021.319-328
80. Harikrishnan, R.; Balasundaram, C.; Heo, M. Effect of probiotics enriched diet on *Paralichthys olivaceus* infected with lymphocystis disease virus (LCDV). *Fish Shellfish Immunol*. **2010**, *29*, 868-874. https://doi.org/10.1016/j.fsi.2010.07.031
81. Ringø, E.; Gatesoupe, F. Lactic acid bacteria in fish: a review. *Aquaculture*. **1998**, *160*,177-203. https://doi.org/10.1016/s0044-8486(97)00299-8
82. Gatesoupe, F. Live yeasts in the gut: natural occurrence, dietary introduction, and their effects on fish health and development. *Aquaculture*. **2007**, *267*, 20-30. https://doi.org/10.1016/j.aquaculture.2007.01.005
83. Teplitski, M.; Wright, A. C.; Lorca, G. L. Biological approaches for controlling shellfish-associated pathogens. *Curr. Opin. Biotechnol*. **2009**, *20*, 185-190. https://doi.org/10.1016/j.copbio.2009.03.001

84. Servin, A. L. Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens. *FEMS Microbiol. Rev.* **2004**, 28, 405-440. https://doi.org/10.1016/j.femsre.2004.01.003
85. Berger, M.; Neumann, A.; Schulz, S.; Simon, M.; Brinkhoff, T. Tropodithietic acid production in *Phaeobacter gallaeciensis* is regulated by N-Acyl homoserine Lactone-Mediated quorum sensing. *J. Bacteriol.* **2011**, *193*, 6576-6585. https://doi.org/10.1128/jb.05818-11
86. Alakomi, H.; Skytta, E.; Saarela, M.; Mattila-Sandholm, T.; Latva-Kala, K.; Helander, I. M. Lactic Acid Permeabilizes Gram-Negative Bacteria by Disrupting the Outer Membrane. *Appl. Environ. Microbiol*. **2000**, *66*, 2001-2005. https://doi.org/10.1128/aem.66.5.2001-2005.2000
87. Merrifield, D. L.; Rodiles, A. The fish microbiome and its interactions with mucosal tissues. Elsevier eBooks, **2015**. (pp. 273-295). https://doi.org/10.1016/b978-0-12-417186-2.00010-8
88. Shephard, K. L. Mucus on the epidermis of fish and its influence on drug delivery. *Adv. Drug Deliv. Rev.* **1993**, *11*, 403-417. https://doi.org/10.1016/0169-409x(93)90018-y
89. Dash, S.; Das, S.; Samal, J.; Thatoi, H. Epidermal mucus, a major determinant in fish health: a review. *Iran J Vet Res*. **2018**, *19*,72-81. PMCID: PMC6056142
90. Lee, S.J.; Jeon, H.S.; Yoo, J.Y.; Kim, J.H. Some Important Metabolites Produced by Lactic Acid Bacteria Originated from Kimchi. *Foods*. **2021**, *10*,2148. https://doi.org/10.3390/foods10092148
91. García-Hernández, V.; Quiros, M.; Nusrat, A. Intestinal epithelial claudins: expression and regulation in homeostasis and inflammation. *Ann. NY. Acad. Sci.* **2017**, *1397*, 66-79. https://doi.org/10.1111/nyas.13360
92. Abbas, A. K.; Lichtman, A. H.; Pillai, S. Cellular and molecular immunology. Elsevier Brasil, **2014**.
93. Beck, B. R.; Song, J. H.; Park, B.; Kim, D.; Kwak, J-H.; Ki, H.; Ki Do, H.; Kim, A.; Kim-W-J.; Song, S.K. Distinct immune tones are established by *Lactococcus lactis* BFE920 and *Lactobacillus plantarum* FGL0001 in the gut of olive flounder (*Paralichthys olivaceus*). *Fish Shellfish Immunol.* **2017**, *55*, 434-443. https://doi.org/10.1016/j.fsi.2016.06.022
94. Gómez, G. D.; Balcázar, J. L. A review on the interactions between gut microbiota and innate immunity of fish. *FEMS Immunol. Med. Microbiol*. **2008**, *52*, 145-154. https://doi.org/10.1111/j.1574-695x.2007.00343.x
95. Dong, Y.; Yang, Y.; Liu, J.; Awan, F.; Lu, C.; Liu, Y. Inhibition of *Aeromonas hydrophila*-induced intestinal inflammation and mucosal barrier function damage in crucian carp by oral administration of *Lactococcus lactis*. *Fish Shellfish Immunol*. **2018**, *83*, 359-367. https://doi.org/10.1016/j.fsi.2018.09.041
96. Wang, X.; Jiang, W.; Feng, L.; Wu, P.; Liu, Y.; Zeng, Y.; Jiang, J.; Kuang, S.; Tang, L.; Tang, W.; Zhou, X. Low or excess levels of dietary cholesterol impaired immunity and aggravated inflammation response in young grass carp (*Ctenopharyngodon idella*). *Fish Shellfish Immunol*. **2018**, *78*, 202-221. https://doi.org/10.1016/j.fsi.2018.04.030.
97. Wang, G.; Huang, S.; Wang, Y.; Cai, S.; Yu, H.; Liu, H.; Zeng, X.; Zhang, G.; Qiao, S. Bridging intestinal immunity and gut microbiota by metabolites. *Cell. Mol. Life Sci*. **2019**, *76*, 3917-3937. https://doi.org/10.1007/s00018-019-03190-6
98. Banerjee, G.; Ray, A. K. Bacterial symbiosis in the fish gut and its role in health and metabolism. *Symbiosis*. **2016**, *72*, 1-11. https://doi.org/10.1007/s13199-016-0441-8
99. Larsen, A. M.; Mohammed, H. H.; Arias, C. R. Characterization of the gut microbiota of three commercially valuable warmwater fish species. *J. Appl. Microbiol*. **2014**, *116*, 1396-1404. https://doi.org/10.1111/jam.12475
100. Balcázar, J. L.; Decamp, O.; Vendrell, D.; De Blas, I.; Ruiz-Zarzuela, I. Health and nutritional properties of probiotics in fish and shellfish. *Microb. Ecol. Health Dis*. **2006**, *18*, 65-70. https://doi.org/10.1080/08910600600799497
101. Sullam, K. E.; Essinger, S. D.; Lozupone, C.; O'Connor, M.; Rosen, G.; Knight, R.; Kilham, S. S.; Russell, J. A. (2012). Environmental and ecological factors that shape the gut bacterial communities of fish: a meta-analysis. *Mol. Ecol*. **2012**, *21*, 3363-3378. https://doi.org/10.1111/j.1365-294x.2012.05552.x
102. Xia, J.; Lin, G.; Fu, G.; Wan, Z. Y.; Lee, M. M.; Wang, L.; Liu, X. J.; Yue, G. H. The intestinal microbiome of fish under starvation. *BMC Genomics*. **2014**, *15*. https://doi.org/10.1186/1471-2164-15-266
103. Ye, L.; Amberg, J.; Chapman, D.; Gaikowski, M.; Liu, W. Fish gut microbiota analysis differentiates physiology and behavior of invasive Asian carp and indigenous American fish. *ISME J.* **2014**, *8*, 541-551. https://doi.org/10.1038/ismej.2013.181

104. Zhang, C.; Derrien, M.; Levenez, F.; Brazeilles, R.; Ballal, S. A.; Kim, J.; Degivry, M.; Quéré, G.; Garault, P.; Van Hylckama Vlieg, J. E. T.; Garrett, W. S.; Doré, J.; Veiga, P. Ecological robustness of the gut microbiota in response to ingestion of transient food-borne microbes. *ISME J.* **2016**, *10*, 2235-2245. https://doi.org/10.1038/ismej.2016.13
105. Fang, G.; Rocha, E.; Danchin, A. How Essential Are Nonessential Genes? *Mol. Biol. Evol*. **2005**, *22*, 2147-2156. https://doi.org/10.1093/molbev/msi211
106. Sylvain, F.; Derome, N. Vertically and horizontally transmitted microbial symbionts shape the gut microbiota ontogenesis of a skin-mucus feeding discus fish progeny. *Sci. Rep*. **2017**, *7*. https://doi.org/10.1038/s41598-017-05662-w
107. Sonnenburg, E.D.; Zheng, H.; Joglekar, P.; Higginbottom, S. K.; Firbank, S. J.; Bolam, D. N.; Sonnenburg, J. L. Specificity of Polysaccharide Use in Intestinal Bacteroides Species Determines Diet-Induced Microbiota Alterations. *Cell*. **2010**, *141*,1241-1252. https://doi.org/10.1016/j.cell.2010.05.005
108. Ringø, E.; Zhou, Z.; Vecino, J.; Wadsworth, S.; Romero, J.; Krogdahl, Å.; Olsen, R.; Dimitroglou, A.; Foey, A.; Davies, S.; Owen, M.; Lauzon, H.; Martinsen, L.; De Schryver, P.; Bossier, P.; Sperstad, S.; Merrifield, D. Effect of dietary components on the gut microbiota of aquatic animals. A never-ending story? *Aquac. Nutr*. **2015**, *22*, 219-282. https://doi.org/10.1111/anu.12346
109. Pessione, E. Lactic acid bacteria contribution to gut microbiota complexity: lights and shadows. *Front. Cell. Infect. Microbiol*. **2012**, *22*, 86. https://doi.org/10.3389/fcimb.2012.00086
110. Abdel-Rahman, M.A.; Tashiro, Y.; Sonomoto, K. Recent advances in lactic acid production by microbial fermentation processes. *Biotechnol. Adv*. **2013**, *31*, 877-902. https://doi.org/10.1016/j.biotechadv.2013.04.002
111. Widyastuti, Y.; Rohmatussolihat, R.; Febrisiantosa, A. The Role of Lactic Acid Bacteria in Milk Fermentation. *Food Nutr. Sci*. **2014**, *5*, 435-442. https://doi.org/10.4236/fns.2014.54051
112. Savijoki, K.; Ingmer, H.; Varmanen, P. Proteolytic systems of lactic acid bacteria. *Appl. Microbiol. Biotechnol.* **2006**, *71*, 394–406. https://doi.org/10.1007/s00253-006-0427-1.
113. Mayo, B. The proteolytic system of lactic acid bacteria. *Microbiologia*. **1993**, *9*, 90-106. PMID: 8172695.
114. Vinderola, G.; Ouwehand, A.; Salminen, S.; von Wright, A. Lactic Acid Bacteria. Microbiological and Functional Aspects. 5th edition. CRC Press. Boca Raton, USA. **2019**. 740 p. https://doi.org/10.1201/9780429057465
115. Liu, M.; Bayjanov, J.R.; Renckens, B.; Siezen, R.J. The proteolytic system of lactic acid bacteria revisited: a genomic comparison. *BMC Genomics*. **2010**, *11*, 36. https://doi.org/10.1186/1471-2164-11-36
116. Broadbent, J.R.; Larsen, R.L.; Deibel, V.; Steele, J.L. Physiological and transcriptional response of *Lactobacillus casei* ATCC 334 to acid stress. *J. Bacteriol*. **2010**, *192*, 2445-58. https://doi.org/10.1128/JB.01618-09
117. Sourjik, V.; Wingreen, N.S. Responding to chemical gradients: bacterial chemotaxis. *Curr. Opin. Cell. Biol*. **2012**, *24*, 262-8. https://doi.org/10.1016/j.ceb.2011.11.008
118. Aldridge, P.; Hughes, K.T. Regulation of flagellar assembly. *Curr. Opin. Microbiol*. **2002**, *5*, 160-165. https://doi.org/10.1016/S1369-5274(02)00302-8
119. Yoshida, K.; Hashimoto, M.; Hori, R.; Adachi, T.; Okuyama, H.; Orikasa, Y.; Nagamine, T.; Shimizu, S.; Ueno, A.; Morita, N. Bacterial Long-Chain Polyunsaturated Fatty Acids: Their Biosynthetic Genes, Functions, and Practical Use. *Mar Drugs*. **2016,** *14*, 94. https://doi.org/10.3390/md14050094
120. Amorim-Franco, T.M.; Blanchard, J.S. Bacterial Branched-Chain Amino Acid Biosynthesis: Structures, Mechanisms, and Drugability. *Biochemistry*. **2017**, *56*,5849-5865. https://doi.org/10.1021/acs.biochem.7b00849
121. National Center for Biotechnology Information. PubChem Pathway Summary for Pathway SMP0012440, C5-Branched Dibasic Acid Metabolism, Source: PathBank. Retrieved July 20, **2024** from https://pubchem.ncbi.nlm.nih.gov/pathway/PathBank:SMP0012440.

12. Capítulo III

Conclusiones y perspectivas

CONCLUSIONES

En la presente investigación se evaluó el efecto de la inclusión de la levadura autóctona *Candida tropicalis*, la levadura probiótica *Debaryomyces hansenii* y la bacteria autóctona *Lactococcus lactis* PH3-05 sobre el crecimiento, supervivencia, actividad enzimática digestiva, morfología intestinal, expresión de genes asociados a la función de barrera intestinal y el sistema inmunológico, así como los cambios en la composición de la microbiota intestinal de juveniles y larvas de pejelagarto (*A. tropicus*).

Los resultados obtenidos en este estudio indican que la incorporación de bajas dosis de *D. hansenii* (10^3 UFC/g) en la dieta mejoran significativamente el crecimiento y la fisiología digestiva de juveniles de pejelagarto. Asi mismo, favorece la función de barrera intestinal y la respuesta inmunológica debido al incremento de la expresión de los genes *muc-2* e *il-10* a la par que disminuye *il-8*. La inclusión de *D. hansenii* (10^3 UFC/g) promueve la síntesis de la capa de mucosa (*muc-2*), la primera línea de defensa y, el refuerzo de las uniones entre las células epiteliales que mejoran la función de barrera intestinal y la respuesta inmunológica. Adicionalmente, la administración de *D. hansenii* (10^3 UFC/g) promovió la abundancia de bacterias pertenecientes al filo Tenericutes y del género Mycoplasma. Estos microorganismos, que habitan de forma simbiótica en el intestino, desempeñan un papel en la digestión de los alimentos y en la síntesis de ácidos grasos, elementos esenciales para el metabolismo energético de otras bacterias presentes en el intestino del huésped. Debido a nuestros resultados existe una posibilidad que al utilizar esta levadura (*D. hansenii* 10^3 UFC/g) provoquen beneficios en la microbiota intestinal en organismos acuáticos, lo que puede beneficiar a la acuicultura.

En larvas, la suplementación de 10^4 UFC/g de *L. lactis* PH3-05 mejora significativamente los valores productivos, la morfología digestiva, la actividad de las enzimas digestivas y la expresión de genes del sistema inmunológico. Además, *L. lactis* (10^6 y 10^8 UFC/g) modula la composición de la microbiota intestinal, en donde se destaca un aumento en la abundancia de bacterias ácido lácticas, lo que sugiere un mecanismo de acción a favor del equilibrio microbiano y de la salud de las larvas. Este estudio demostró por primera vez la composición microbiana intestinal de las larvas del pejelagarto está compuesta principalmente por los filos Bacteroidetes y Proteobacteria, ambos

presentan bacterias que mejoran la función metabólica e inmunológica, lo que favorece el crecimiento de los peces. Nuestra investigación aporta nuevos conocimientos sobre las funciones metabólicas de los microorganismos intestinales de *A. tropicus*, donde se mostró que las bacterias intestinales presentes están relacionadas con el metabolismo de carbohidratos y proteínas, así como la síntesis de lípidos, lo cual, esta información contribuye a la comprensión de la interacción entre los microorganismos intestinales y la fisiología del pejelagarto a través de la inclusión de *L. lactis* (10^6 y 10^8 UFC/g). Estos hallazgos tienen importantes implicaciones para la acuicultura del pejelagarto, al ofrecer una alternativa sostenible para mejorar la producción y principalmente, el bienestar de esta especie. Nuestros resultados respaldan el potencial de los probióticos para mejorar la eficiencia productiva en la acuicultura, mejorando la digestión y absorción de nutrientes en pejelagarto. La inclusión de *D. hansenii* y *L. lactis*, se muestran prometedoras como suplementos dietéticos eficaces para aplicaciones en la acuicultura del pejelagarto. Adicionalmente, los resultados de este estudio aportan nuevas perspectivas para el desarrollo de estrategias alimenticias con la inclusión de probióticos autóctonos específicos para otras especies acuáticas. De acuerdo con la fisiología del pejelagarto en ambas etapas de crecimiento, el presente estudio contribuyó al conocimiento de la composición microbiana intestinal y la comprensión de las vías metabólicas entre huésped-hospedero. En este sentido, se propone continuar la investigación para evaluar la adición de simbióticos u otras especies de probióticos y evaluar los mecanismos moleculares subyacentes a estos beneficios evaluados. Así como incluir otras herramientas metagenómicas para continuar con la línea de investigación de la microbiota intestinal del pejelagarto.

PERSPECTIVAS

La presente tesis doctoral ha proporcionado una comprensión detallada de la dinámica de la fisiología digestiva y de la microbiota intestinal en larvas y juveniles de pejelagarto (*Atractosteus tropicus*) en respuesta a la administración de *C. tropicalis*, *D. hansenii* y *L. lactis*. A través de un enfoque multidisciplinario, integrado por técnicas bioquímicas, moleculares, histológicas y metagenómicas. Los hallazgos obtenidos abren diversas perspectivas y líneas de investigación para futuras investigaciones y aplicaciones en la larvicultura y el cultivo de juveniles de esta importante especie en la región.

En primer lugar, la identificación de cepas probióticas que ejercieron efectos beneficiosos significativos sobre los parámetros de crecimiento, la actividad enzimática digestiva y la composición de la microbiota intestinal en la primera etapa de vida del pejelagarto, sienta las bases para el desarrollo de protocolos de alimentación optimizado. Las futuras investigaciones podrían enfocarse en la evaluación a largo plazo, particularmente de *D. hansenii* y *L. lactis*, considerando su persistencia en el tracto digestivo y su impacto en la resistencia a enfermedades y el rendimiento productivo en las etapas de pre y engorda. Asi mismo, la exploración de otro consorcio de probióticos, que combinen diferentes cepas con mecanismos de acción complementarios, así como la mezcla con prebióticos anteriormente reportados con efectos benéficos en muchos niveles fisiológicos puede ser prometedor para favorecer la fisiología de esta especie. La implementación de simbióticos permitirá a los productores optimizar y mejorar el cultivo. Lo cual podrá contribuir a la reducción de pérdidas económicas.

Desde una perspectiva fisiológica, la elucidación de los mecanismos por los cuales los probióticos influyen en la actividad enzimática digestiva y la morfología intestinal, siguiere la posibilidad de desarrollar estrategias nutricionales sinérgicas. Se puede considerar, en futuras investigaciones, investigar la interacción entre la suplementación probiótica y la composición de la dieta a nivel larval y juvenil, buscar una dieta formulada que maximicen los beneficios de los microorganismos exógenos. Además, evaluar el impacto de los probióticos a través de la expresión de genes relacionados con la digestión y absorción de nutrientes, para comprender los mecanismos

moleculares involucrados en la digestibilidad. Además, se considera buscar levaduras probióticas alternativas que ejerzan múltiples efectos benéficos para esta especie, con el fin de contribuir a estrategias innovadoras para mejorar la sostenibilidad y rentabilidad del cultivo del pejelagarto.

A nivel larvicultura, donde la alta mortalidad en la edad temprana representa un desafío significativo, la aplicación de probióticos específicos podría mejorar y convertirse en una herramienta clave y asertiva para mejorar la supervivencia y el establecimiento de una microbiota intestinal saludable. Investigaciones futuras deberían explorar la ventana de tiempo óptima para la administración de probióticos en larvas y evaluar diferentes métodos de administración, como la encapsulación en el alimento vivo, adición directa al agua de cultivo o por aspersión en el alimento.

Adicionalmente, la caracterización de la microbiota nativa del pejelagarto en sus diferentes etapas de desarrollo proporciona una valiosa línea base para futuras comparaciones y para la identificación de probióticos endógenos con gran potencial, mayor adoptación y eficacia. El aislamiento y la evaluación de cepas probióticas autóctonas del pejelagarto representa una línea de investigación prometedora para el desarrollo de estrategias de manejo de la microbiota específica para esta especie tan importante en la región.

Finalmente, la metodología utilizada en esta tesis combinando análisis metagenómicos con evaluaciones fisiológicas e histológicas, podrían aplicarse al estudio de la respuesta a probióticos en otras especies acuáticas de interés comercial o especies nativas de la región Sureste de México. La comprensión de la interacción entre la microbiota y la fisiología digestiva en las etapas tempranas es fundamental para el desarrollo de prácticas de cultivo más sostenibles y eficientes.

Los resultados de esta tesis doctoral no solo amplían el conocimiento fundamental sobre la fisiología del pejelagarto, sino que también ofrecen perspectivas claras para el desarrollo de estrategias de manejo de la microbiota intestinal basadas en probióticos, con el potencial de mejorar significativamente la larvicultura y el cultivo juvenil en la acuicultura regional del pejelagarto.

Alojamiento de la Tesis en el Repositorio Institucional	
Título de Tesis:	"MICROBIOTA Y FISIOLOGÍA DIGESTIVA DE LARVAS Y JUVENILES DE PEJELAGARTO (*Atractosteus tropicus*) DURANTE LA ADMINISTRACIÓN DE PROBIÓTICOS"
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Resumen de la Tesis:	El uso de probióticos en la acuicultura representa una herramienta eficaz, ya que estos contribuyen a mejorar el crecimiento, supervivencia y la salud de los organismos mediante la estimulación de la actividad digestiva, respuesta inmunológica y la modulación de la microbiota intestinal, lo que deriva en un mayor rendimiento del cultivo. En la presente investigación se evaluó el efecto de la administración de alimento enriquecido con la bacteria autóctona *Lactococcus lactis* (10^4, 10^6, y 10^8 UFC/g), así como con la levadura

	autóctona *Candida tropicalis* (10^4, 10^5, y 10^6 UFC/g) y la levadura probiótica *Debaryomyces hansenii* (10^3, 10^5, y 10^7 UFC/g), sobre los indicadores de productividad acuícola, la actividad enzimática digestiva, morfología digestiva, expresión de genes asociados a la función de barrera intestinal y el sistema inmunológico, y composición de la microbiota intestinal de larvas y juveniles de pejelagarto.
Palabras claves de la Tesis:	pejelagarto; probióticos; *Lactococcus lactis; Debaryomyces hansenii;* microbiota
Referencias citadas:	Angulo, M., Reyes-Becerril, M., Medina-Córdova, N., Tovar-Ramírez, D. & Angulo, C. (2020). Probiotic and nutritional effects of *Debaryomyces hansenii* on animals. *Applied Microbiology and Biotechnology*, 104: 7689-7699. https://doi.org/10.1007/s00253-020-10780-z Bolotin, A. Wincker, P., Mauger, S., Jaillon, O., Malarme, K., Weissenbach, J., Ehrlich, S.D. & Sorokin, A. (2001). The Complete Genome Sequence of the Lactic Acid Bacterium *Lactococcus lactis* ssp. *lactis* IL1403. *Genome Research*, *11*, 731–753. https://doi.org/10.1101/gr.gr-1967r

Bolyen, E., Rideout, J. R., Dillon, M. et al. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, *37*(8), 852-857. https://doi.org/10.1038/s41587-019-0209-9

Bradford, M. (1976). A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle of protein-dye binding. *The Journal Analytical Biochemistry*, 72: 248-254.

Campos, M.M. & Campos, C.R. (2017). Applications of quartering method in soils and foods. *International Journal of Engineering Research and Applications*, *7*: 35-39. https://doi.org/10.9790/9622-0701023539

Caporaso, J., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F., Costello, E., Fierer, N., Peña, A., Goodrich, J., Gordon, J., Huttley, G., Kelley, S., Knights, D., Koenig, E., Ley, E., Lozupone, C., McDonald, D., Muegge, B., Pirrung, M. Reeder, J., Sevinsky, J., Turnbaugh, P., Walters, W., Widmann, J., Yatsunenko, T., Zaneveld, J. & Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, 7(5), 335-336. https://doi.org/10.1038/nmeth.f.303

Carrias, A., Ran, C., Terhune, J. & Liles, M. (2012). Bacteria and bacteriophages as biological agents for disease control in aquaculture. In: Austin B (ed) Infectious Disease in Aquaculture, pp. 353–393. Elsevier, Cambridge, UK.

FAO/WHO. (2001). *Report of a Join FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria: Córdoba, Argentina, 1-4 October 2001*. Food and Agriculture Organization of the United Nations and World Health Organization. Retrieved from: https://openknowledge.fao.org/server/api/core/bitstreams/382476b3-4d54-4175-803f-2f26f3526256/content

Janda, J. M. & Abbott, S. L. (2007). 16S RRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. *Journal of Clinical Microbiology*, 45(9), 2761-2764. https://doi.org/10.1128/jcm.01228-07

Jahan, N., Islam, S.M., Rohani, Md., Hossain, M. & Shahjahan, Md. (2021). Probiotic yeast enhances growth performance of rohu (*Labeo rohita*) through upgrading hematology, and intestinal microbiota and morphology. *Aquaculture*, 545: 737243.

https://doi.org/10.1016/j.aquaculture.2021.737243

Kim, D., Brunt, J. & Austin, B. (2007). Microbial diversity of intestinal contents and mucus in rainbow trout (*Oncorhynchus mykiss*). *Journal of Applied Microbiology,* 102: 1654–1664. https://doi.org/10.1111/j.1365-2672.2006.03185.x.

PRINCIPIOS BIOÉTICOS

Definir cuáles son las leyes, reglamentos, normas u otras disposiciones legales aplicables a nivel nacional e internacional que fueron consideradas en la investigación que involucre el uso de seres vivos con fines observacionales y experimentales y así como también al uso de compuestos químicos, biológicos y/o innovaciones biotecnológica, a fin de reducir a medida de lo posible la ya marcada huella ecológica del ser humano de acuerdo a lo establecido en el Código Institucional de Ética para la Investigación publicado en el 2019.

El presente estudio se realizó bajo el acuerdo de la Declaración de Helsinki. Y de acuerdo al protocolo autorizado por la Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (SAGARPA), México, NOM-062-ZOO-1999.2001.