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Insights into the bacterial communities of Nile tilapia – core members and intergenerational transfer

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Preface

This thesis is submitted in fulfillment of the requirements for the degree of Philosophiae Doctor (PhD) at the Faculty of Biosciences and Aquaculture (FBA), Nord University, Bodø, Norway. The studies included in this dissertation represent original research performed at the faculty. The studies were financially supported by Nord University and EPIFISH project (ERC grant agreement 683210).

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Yousri Abdelhafiz

Bodø, March 2022

DEDICATION

I lovingly dedicate this Thesis with gratitude to

My parents

My brothers and sisters

There is no doubt in my mind that without your patience, continued support and encouragements I could not have completed this project.

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Last, but not least, my sincere appreciation and gratitude to my family for their limitless love and support during my PhD journey.

THANK YOU!!!

Yousri

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List of abbreviations

FAO	-	Food And Agriculture Organization
GIFT	-	Genetically Improved Farmed Tilapia
HMP	-	Human Microbiome Project
EMP	-	Earth Microbiome Project
DNA	-	Deoxyribonucleic Acid
rRNA	-	Ribosomal Ribonucleic Acid
NGS	-	Next Generation Sequencing
OTUs	-	Operational Taxonomic Units
PCR	-	Polymerase Chain Reaction
ASVs	-	Amplicon Sequence Varients
PICRUSt	-	Phylogenetic Investigation Of Communities By Reconstruction Of Unobserved States
CPS	-	Capsular Polysaccharides
GBS	-	Group B *Streptococcus*
NKef	-	Natural Killer Cell Enhancing Factor Protein
TLR	-	Toll-Like Receptors
ATP	-	Adenosine Triphosphate
SCFAs	-	Short Chain Fatty Acids

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List of papers

Paper I Abdelhafiz Y, Fernandes JMO, Stefani E, Albanese D, Donati C, Kiron V. Power play of commensal bacteria in the buccal cavity of female Nile tilapia. Front Microbiol. 2021;12(3396); doi: 10.3389/fmicb.2021.773351.

Paper II Abdelhafiz Y, Fernandes JMO, Larger S, Albanese D, Donati C, Jafari O, Nedoluzhko AV and Kiron V. Breeding strategy shapes the composition of bacterial communities in female Nile tilapia reared in a recirculating aquaculture system. Front Microbiol. 2021;12(2563); doi: 10.3389/fmicb.2021.709611.

Paper III Abdelhafiz Y, Fernandes JMO, Donati C, Pindo M, Kiron V. Intergenerational transfer of persistent bacterial communities in female Nile tilapia. Front Microbiol. 2022;13; DOI 10.3389/fmicb.2022.879990

Summary

Nile tilapia (*Oreochromis niloticus*) is the second most farmed fish species around the globe and is known for its fast growth and ability to adapt to various environmental conditions. However, challenges such as disease outbreaks cause high mortality and economic losses. Hence, it is important to both produce tilapia strains that are disease resistant and deploy strategies such as microbial community control to ward off pathogenic organisms. It is well known that fish-associated microbes play a critical role in development and health. Exploring these microbes will reveal the importance of a subset in maintaining fish health.

This thesis is based on the hypothesis that gender, breeding strategies and parental microbial transfer may influence the buccal cavity and gut microbiome in Nile tilapia. To gain insights into the tilapia-associated bacterial communities, studies were conducted on fish that were produced from eggs of wild fish from River Nile. The bacterial assemblages of the fish were examined using a 16S amplicon sequencing technique. First, I investigated the bacterial compositions in the buccal cavity of male and female fish. Thereafter, I studied inbreeding or outbreeding-caused shaping of oral and gut microbiome. Lastly, the intergenerational transfer of bacteria associated with the gut and buccal cavity of Nile tilapia was investigated by unravelling the presence of bacteria in wild mothers and their offspring.

The first study revealed that female buccal cavity contains more beneficial microbes such as *Acinetobacter*, *Acidobacteria* and *Saccharibacteria* and less opportunistic pathogens. Some *Streptococcus* species or strains are opportunistic pathogens, and certain strains are linked to disease outbreaks in Nile tilapia was almost absent in the buccal cavity. A microbial profile that diverges away from opportunistic pathogens could be attributed to the conditions in the buccal cavity of this mouthbrooding fish.

The next study focused on host genetics that is also known to shape gut microbiota. The analysis revealed significant genetic differences between inbred and outbred tilapia groups. These differences were also reflected in the buccal cavity and gut microbiota of the breeding groups. Furthermore, inbreeding reduced the inter-individual bacterial variability and increased the abundance of known beneficial microbes such as *Cetobacterium*, *Sphingomonas*, and *Propionibacterium*.

The third study revealed that the core microbiome from the buccal cavity and gut of wild female fish can be transferred to offspring and eventually shape the microbial composition in the progeny, based on the presence of similar bacteria in different generations. *Nocardioides*, *Propionibacterium* and *Sphingomonas* were found to be core microbiome members in wild fish. In addition, they persisted in the subsequent generations of Nile tilapia.

Thus the significant finding from this research is that bacterial communities in Nile tilapia could be shaped through appropriate breeding strategies, allowing the establishment of favourable communities. Furthermore, the buccal cavity microenvironment of the female fish is less conducive to potential pathogenic bacteria. These findings could enable the tilapia farming industry to apply this knowledge to devise strategies for producing healthy fish with a microbial niche dominated by beneficial bacteria.

Sammendrag

Kunnskap om bakteriesamfunn i Nile tilapia – kjernemedlemmer og overføring mellom generasjoner

Nile tilapia, *Oreochromis niloticus,* er den nest mest oppdrettede fiskearten rundt om i verden og er kjent for sin raske vekst og evne til å tilpasse seg ulike miljøforhold. Utfordringer som sykdomsutbrudd forårsaker imidlertid høy dødelighet og økonomiske tap. En strategi for å forhindre sykdom og økonomiske tap kan derfor være å jobbe forebyggende både gjennom å avle fram stammer med bedre r sykdomsresistens og samtidig styre fiskens bakteriesamfunn mikrobiota i en gunstig retning for å forhindre opptak av patogene organismer. Det er velkjent at mikrobesammensetning spiller en avgjørende rolle for utvikling og god helse. Økt kunnskap om fiskens mikrobesammensetning er derfor viktig for å kunne styre den i en gunstig retning for å opprettholde god fiskehelse.

Denne oppgaven er basert på en hypotese om at kjønn, avlsstrategier og overføring av mikrober fra foreldre til avkom kan påvirke mikrobesammensetningen i både munnhulen og tarmmikrobiomet i Nile tilapia. For å få et innblikk i tilapia sitt bakteriesamfunn, ble det gjennomført studier på fisk produsert fra egg samlet inn fra villfisk i elven Nilen. Bakterieprofiler til fisken ble undersøkt ved bruk av en 16S amplikon sekvenseringsteknikk. Først undersøkte jeg bakteriesammensetningen i munnhulen til hann- og hunnfisk. Deretter studerte jeg innavl eller utavlsbetinget utforming av munn og tarmmikrobiom. Overføring av bakterier fra henholdsvis munn og tarm mellom generasjoner ble til slutt studert ved å sammenligne bakteriesammensetning hos ville mødre og deres avkom.

Den første studien viste at bakteriesammensetningen i munnhulen hos hunnfisk inneholdt mer gunstige stammer som *Acinetobacter, Acidobacteria* og *Saccharibacteria* og færre opportunistiske patogener. *Streptococcus* sp., en opportunistisk patogen som er knyttet til sykdomsutbrudd i Nile tilapia, var nesten fraværende i munnhulen. En mikrobiell

profil som divergerer bort fra opportunistiske patogener kan tilskrives forholdene i munnhulen til denne munnrugende fiskearten.

Den neste studien fokuserte på vertsgenetikk, som også er kjent for å forme tarmmikrobiota. Analysen avdekket betydelige genetiske forskjeller mellom innavlede og utavlede tilapiagrupper. Disse forskjellene ble også reflektert i munnhulen og tarmmikrobiotaen til avlsgruppene. Videre ble det observert at innavl reduserte bakterievariasjonen mellom fisk og økte mengden mikrober som er kjent for å være gunstige for å styrke fiskehelsen, som *Cetobacterium*, *Sphingomonas* og *Propionibacterium*.

Den tredje studien viste at kjernemikrobiomet fra munnhulen og tarmen til vill hunnfisk kan overføres til avkom og til slutt forme den mikrobielle sammensetningen i avkommet, basert på tilstedeværelsen av lignende bakterier i forskjellige generasjoner. *Nocardioides, Propionibacterium* og *Sphingomonas* ble funnet å være kjernemikrobiomer hos foreldregenerasjonen som stammet fra Nilen, og ble overført til de påfølgende generasjonene produsert under kontrollerte betingelser i laboratoriet.

Resultatene fra dette doktorgradsstudiet viser at bakterieprofiler i fiskearten Nile tilapia kan formes gjennom avl, og etablering av gunstige mikrobesamfunn kan dermed skje gjennom en systematisk avlsstrategi. Videre viser resultatene at munnhulemikromiljøet til hunnfisken er mindre gunstig for potensielle patogene bakterier. Kunnskapen fra denne forskningen kan direkte implementeres i tilapiaoppdrett til å produsere sunn fisk med en mikrobiell nisje dominert av nyttige bakterier.

1. Introduction

1.1. Nile tilapia aquaculture

The world population has been increasing continuously, and it is estimated that in 2050 there will be 9.7 billion people. Meeting the food demands of the future society is going to be a challenge since the consumption of our finite resources should not be high (Maja and Ayano, 2021). The United Nations has, among its 17 sustainable development goals, listed "no poverty" and "zero hunger" as the first and second items to make all the nations aware of the need for urgent appropriate actions to reduce both. To achieve these goals, the food industry is poised to increase the production, and in 2024 food fish supply is expected to increase by 19%. Fish and other aquatic animals are essential sources of protein and nutrients in human diets. World-wide fish production, from both capture and aquaculture, reached 179 million tons in 2018, the worth of which was USD 401 billion. Of this, 156 million tonnes were used for human consumption, equivalent to an estimated annual supply of 20.5 kg per capita. The remaining 22 million tonnes were destined for non-food uses, mainly to produce fishmeal and fish oil (FAO, 2020). To avoid the overexploitation of our depleting wild fish stocks, aquaculture can be considered as one of the best alternatives to meet the future demands of fish products (Melo-Bolívar et al., 2019, FAO, 2020).

Currently, aquaculture supplies 50 % of food fish for human consumption worldwide; in 2018, the production was 82.2 million tonnes (Miao and Wang, 2020, FAO, 2020). Asia is by far the dominant producer; this continent has produced 89 % of farmed aquatic food in the past 20 years. China is the major fish producer, holding a 46 % share of the global market. The other main players in the market are Norway, Bangladesh, Egypt, Chile, Indonesia, India and Vietnam (FAO, 2020). The most farmed aquatic animals are carp, tilapia and shrimp (Miao and Wang, 2020). Tilapia species can be bred easily, and they have high tolerance capacity and can adapt to different environments; they can even adapt to water pollution (Rahman et al., 2021), are resilient to climate changes and grow rapidly at warm temperatures. These qualities of tilapia make them one of the leading farmed fish

species of the world (Guyon et al., 2012, Moses et al., 2021). In fact, tilapia is the second most important farmed fish by quantity and has been produced in 145 countries around the world (FAO, 2020). The total production of tilapia increased to 7 million tons in 2020 (GLOBEFISH, 2020), which confirmed its position as the second most important farmed aquatic fish. In 2018, Nile tilapia (*Oreochromis niloticus*) and *Oreochromis* spp. accounted for 8.3 and 1.9 %, respectively of the global production, and the total economic value was USD 11.2 billion (Guyon et al., 2012, Omasaki et al., 2016, Miao and Wang, 2020). Thus, among the farmed tilapia, Nile tilapia is the dominant species and has the potential to become the most important farmed fish species in the world (Moses et al., 2021).

Nile tilapia, a member of the family Cichlidae, is an African freshwater cichlid. The farming of this fish, which might have started in ancient Egypt near the River Nile (Crespi and New, 2009), is now being practiced in many countries around the world. Egypt was the second largest producer until 2013 when Indonesia surpassed the production capacity of Egypt. In 2018, Indonesia produced 1.22 million tonnes of farmed tilapia which made up 20.3 % of the world production. However, China remains the first producer of tilapia around the globe. Among the African countries, only Egypt remains amongst the top-ten farmed tilapia producers (Miao and Wang, 2020). In addition to the essential role that Nile tilapia plays in food security and economy, it is an excellent experimental model for studying fish physiology. Nile tilapia, which is related to haplochromine cichlids or East African cichlids that are characterized by high speciation rates, can be considered as a good model to study their evolution and adaptation mechanisms (Guyon et al., 2012). These cichlids are good examples of adaptive radiations and recently mtDNA lineages belonging to the East African cichlid radiation was studied to understand the origin of the adaptive radiations of the Lakes Tanganyika, Malawi and Victoria (Danley et al., 2012, Schedel et al., 2019). Environmental and geological features might have helped in the extensive diversification of cichlids (Danley et al., 2012). For example, it was reported that the shape changes of lower jaw bone during the growth of Lake Victoria cichlid *Haplochromis chilotes* and *O. nicloticus* follow a similar pattern (Fujimura and Okada, 2008), probably to adapt to similar feeding habits and

environmental conditions. Moreover, Nile tilapia was used as a model in germ cell transplantation, and scientists have examined the spermatogenic process in this species (Vilela et al., 2003, Lacerda et al., 2010, Lacerda et al., 2018). Furthermore, Nile tilapia has been used in behavioural studies as well as in neuroscience; researchers have investigated their physiological and behavioural changes during stress (Delicio et al., 2006, Barreto et al., 2009, El-Khaldi, 2010).

1.1.1. Nile tilapia breeding, distribution, morphology and life cycle

Before mating, the male Nile tilapia prepares a simple nest at the bottom of a lake or pond and defends his territory. After the female lays her eggs in the nest, the males fertilizes them. The female then picks up the fertilized eggs and incubates them in her mouth (buccal cavity) until they hatch (approximately 14 days, depending on the temperature). Hence, Nile tilapia are maternal mouthbrooders. The free-swimming fry even returns to their mother's mouth, to seek protection (Popma and Masser, 1999, Gonçalves-de-Freitas et al., 2019). The breeding process of wild Nile tilapia is illustrated in Figure 1. The number of spawned eggs in wild mouthbrooders is less compared to those of fish that are captive-reared in ponds. The number of spawned eggs of Nile tilapia is proportional to the bodyweight of the female. For instance, a female that weighs 100 grams can produce 100 eggs per spawn, while a female weighing 600 – 1000 grams can yield 1000 – 1500 eggs (Njiru et al., 2006, Mashaii et al., 2016). The sexual maturity of Nile tilapia is influenced by age, size, host genetics and environmental conditions. In many East African lakes, this fish matures when it is 10 to 12 months old. Whereas, with the good growth conditions in ponds, Nile tilapia attains maturity faster, at 5 to 6 months (Popma and Masser, 1999).

Nile tilapia has a broad natural distribution; in coastal rivers, mainly Yarkon, of Israel, tropical and subtropical Africa. In Africa, we can find the fish in Nile and Niger river basins, smaller drainages and lakes in western and eastern Africa (Trewavas, 1983). Nile tilapia has a laterally compressed body, with brownish-gray color on the dorsal and lateral side and

white color on the ventral side. Its tail fin has black bands and 7-12 distinct stripes, and there are 16-18 dorsal spines. The fish has a small mouth, housing coarser and sharp teeth. While males are bluish pink, females are brownish, silvery/white. The color of head, lower body, dorsal and caudal fins of breeding males is pinkish-red and these fish will have dark margin on the dorsal fin. The males grow faster and are larger than the females. While males have a tapered shape below the anus and one urogenital opening, females have two openings (FishBase, 2015).

The interest in tilapia farming has been supported by several worldwide breeding programs to improve its productivity, quality, performance, sustainability and enhanced adaptation (Eknath et al., 1998, Rezk et al., 2009). The aim of selective breeding is to choose individuals with the best phenotypic and genotypic traits so that the next generation has a better gene set for faster growth rate, heritability and disease resistance (Puttaraksar and Center, 2004). For instance, selective breeding of tilapia is employed to obtain special phenotypic traits such as improved cold tolerance (Charo-Karisa et al., 2005, Nitzan et al., 2016, Kokou et al., 2018).

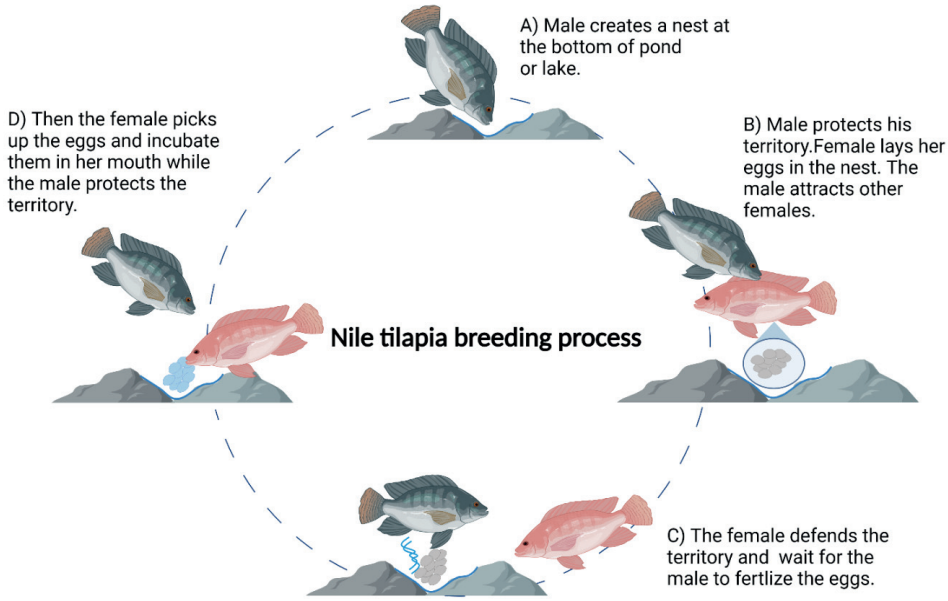

Figure 1. Illustration of the breeding process of wild and pond-dwelling Nile tilapia. *This figure was generated using biorender.com*

In 1988, a project to develop genetically improved farmed tilapia (GIFT) was initiated by WorldFish (Malaysia) and Akvaforsk (Norway). The first generation of GIFT was obtained via cross-breeding of four wild Nile tilapia from Africa and four farmed Nile tilapia from the Philippines. In the sixth-generation, GIFT strain showed an average genetic gain of 13% and achieved a body weight increment of 85%. The GIFT strain showed a significantly higher performance and therefore it was distributed to various countries around the world. In Asia, GIFT strain has had a remarkable impact on farmed Nile tilapia production in countries such as China, Malaysia, the Philippines and Sri Lanka (Eknath et al., 1998, WorldFish, 2016, Abwao et al., 2021). In Sri Lanka, the GIFT strain reached a genetic gain of 112% (Abwao et al., 2021). Selective breeding of GIFT for faster growth is still being undertaken in many countries, and by 2016, 20 generations of the GIFT strain were generated through selective breeding (WorldFish, 2016). Furthermore, selection of the host genotype through breeding

can shape the gut microbiota, which plays an important role in host health (Smith et al., 2015).

1.2. History of microbiota research

Microbiota that are found associated with different hosts has been a topic of scientific interest for centuries. The early discovery of microbiota as a part of the human system was in the 19th century. It was an Austrian pediatrician Theodor Escherich who discovered *Escherichia coli* in infant meconium, stool of breast-fed baby, and in faeces of children suffering from diarrhea (Escherich, 1882, Méric et al., 2016, Hayes and Sahu, 2020). This discovery paved the way for bacterial genetic research. In 1890, *Lactobacillus acidophilus* was discovered by Ernst Moro (Aimutis, 2014). After the discovery of *Veillonella parvula* in the oral, gut, urinary and respiratory tracts, *Bifidobacteria* were discovered in the gut microbiome in 1898. In 1907, a Russian zoologist Élie Metchnikoff proposed the concept "yogurt brings a long life" and brought to light the opportunistic pathogen inhibitory effect of *Lactobacillus*. This was the first theory that associated microorganisms to host health (Metchnikoff, 2004, Lu, 2020). Thereafter, Dubos et al. (1965) generated the first microscopic images of *Lactobacilli* and *Streptococci* in frozen tissues of rat intestine. Later in 1977, it was reported that bacteria and fungi are commonly found in the gut of humans and animals, and some of them can prevent diseases, but others can be the causative agents of diseases (Savage, 1977). In 1992, Bocci suggested that the intestine microbiota can perform metabolic functions and produce lipopolysaccharides to activate the immune system to positively affect the host health (Bocci, 1992). During the 20th century, many microbes were isolated from different body sites such as buccal cavity, nasal passages, skin, gut and vagina of humans (Hayes and Sahu, 2020).

The term microbiome was first coined by Whipps J et al. (1988), combining "micro" and "biome", to define a "characteristic microbial community" in a "reasonably well-defined habitat which has distinct physio-chemical properties". Later, Lederberg and McCray (2001) described the human microbiome as "the ecological community of commensal, symbiotic,

and pathogenic microorganisms that literally share our body space". When Relman and Falkow (2001) proposed that the human genome sequence will give insight into microbial pathogens and commensal microbes in the mouth, skin, gut and vagina. They also believed that the genome will provide a better understanding of host genes and their implications on microbial pathogenicity. Relman (2002) proposed several techniques to characterize the human microbiome and for assessment of intra-individual and inter-individual variation; the scientist recommended the use of random shotgun sequencing, targeted large-insert clone sequencing and high-density microarrays. Furthermore, he had the opinion that analysis of host genome-wide expression may provide knowledge about the endogenous microbiome (core microbiome) and its relation to health and diseases. Microbiome research has been advancing rapidly even after the Human Microbiome Project (HMP) that was launched in 2007 (Turnbaugh et al., 2007). The HMP was an international effort aimed to identify microbial communities in the human body and determine the role of each microbe in health and diseases (Turnbaugh et al., 2007). The strategies and techniques developed in HMP have been applied to study other species when the Earth Microbiome Project (EMP) was launched (Gilbert et al., 2014). The aim of EMP was to construct a global catalog of culturable and unculturable microbes of the earth. Samples were collected from various environments not exclusively from humans but also from marine, freshwater, sediments, plants, animals and many other ecosystems (Gilbert et al., 2014). In 2015, The European Tara Oceans initiative aimed to profile the diversity of the marine microbiome on earth (Sunagawa et al., 2015). In 2018, Pike and Forster (2018) highlighted the importance of studying and analyzing archaeome and its role in the human microbiome. On the other hand, the earliest study of microbes in the fish gut was in the late 1910s (Wang et al., 2018). After this, many studies were conducted to analyze the microbial composition in fish (Liston, 1957, Trust and Sparrow, 1974, Horsley, 1977, Lev Fishelson, 1985, Austin and Al-Zahrani, 1988, Cahill, 1990, Ringø and Strøm, 1994, Dhanasiri et al., 2011). These studies were different compared to those that employed advanced techniques such as Next Generation Sequencing (NGS); the techniques that the researchers employed in earlier years were not

good enough to identify unculturable bacteria. Recently advanced sequencing technology enabled researchers to identify massive microbial communities and various indigenous microbes in the fish gut (Wang et al., 2018). Figure 2 illustrates the timeline of microbiota research and the sequential method development.

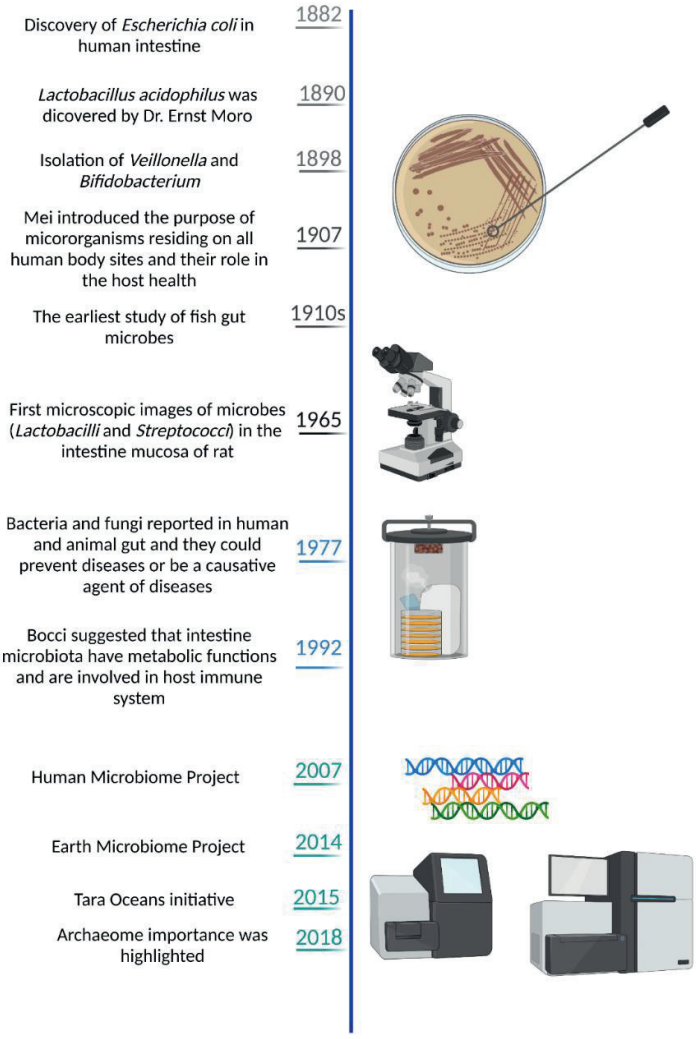

Figure 2. Timeline of the history of microbiota research. *Different font colors of years represent the introduction of various methodologies. This figure was generated using biorender.com, and is based on Lu (2020).*

1.3. Importance of microbiota in humans and animals

Human and other animals body houses complex and dynamic communities of various microbes. The microbiome plays a vital role in host development, immune system training, defence against invasive pathogens and building tolerance to commensal bacteria (Chai et al., 2014, Ferretti et al., 2018). At present, the belief is that bacteria are among the most abundant members of the microbial community, with an estimated 75 to 200 trillion cells though the reported numbers vary widely, while the human body entirely consists of about 50 to 100 trillion somatic cells (Rosner, 2014, Sender et al., 2016, Hayes and Sahu, 2020). This difference in the balance of microbial and host cell populations indicates that human body is a collection of human and microbial cells and genes (Hayes and Sahu, 2020). In hologenome theory, animals/plants with their associated microorganisms are regarded as a single unit of selection in evolution. Environmental stress may shift the microbial composition rapidly, and the microbes can help the host to acquire the necessary fitness. Furthermore, the symbiotic microbes are transmitted across generations. Therefore, microbial communities can play an essential role in the adaptation and evolution of humans and other organisms. In the event of a rapid microbial change, the diverse microbial symbionts can assist holobiont in surviving and allowing the host genome to evolve (Zilber-Rosenberg and Rosenberg, 2008). The human microbiome is involved in various metabolic and physiologic functions. Wikoff et al. (2009) conducted a comparative study on germ-free and normal mice and reported that most of the host blood metabolites were from the microbiome. The microbiome can positively or adversely affect the host metabolism, which in turn leads to either healthy or disease states. Several factors such as diet, environment, genetics, birth mode of infant delivery and age can affect the microbiota composition. For instance, carbamate insecticide aldicarb, which can be considered to produce an environmental effect, can alter the gut microbiome, induce the virulence of opportunistic pathogen during dysbiotic states and modify the brain metabolism (Gao et al., 2019). The imbalance of the microbiome (dysbiosis) is associated with various diseases of the skin (Byrd et al., 2018), buccal cavity (Su et al., 2020, Radaic and Kapila, 2021) and

gut (Martinez et al., 2021, Wei et al., 2021). In addition, dysbiosis can be the cause of anxiety and depression (Simpson et al., 2020). On the other hand, a healthy microbiome profile modulates the immune system and protects the host from infections. In the human skin microbiome, *Staphylococcus epidermidis* and *Propionibacterium acnes* is found to be predominant (Byrd et al., 2018); the former can produce antimicrobials whereas the latter produces short-chain fatty acids. These two molecules play an essential role in maintaining a healthy balanced skin microbiome (Christensen and Brüggemann, 2014). Furthermore, *Bifidobacterium longum* modulates host homeostasis and improve gut health (Wong et al., 2019).

In other animals, healthy host-microbe interactions also contribute to the homeostasis of the host. The associated bacteria, fungi, protozoa and archaea are involved in food conversion, nutrient uptake, removal of toxic materials, communication and behavior (Pope et al., 2010, Zhu et al., 2011, Shiffman et al., 2017, Malacrinò, 2018). Furthermore, as reported for human skin, microbes on amphibian skin play a key role in maintaining the immune system and warding off *Batrachochytrium dendrobatidis* infection (Ruthsatz et al., 2020). Marine animals are considered as key organisms of ocean ecosystems, and the microbiome that inhabits the body and mucosal surfaces of marine animals play an essential ecological role in ocean health (Estes et al., 2011) as well as a critical role in host nutrition and immune function development (Bik et al., 2016).

1.4. Fish microbiome and their importance

Fishes, similar to other animals, are exposed to several pathogens in their environment. Hence, their immune system is adapted to interact with these microorganisms. Furthermore, the skin, buccal cavity, gills and gut of fish are inhabited by complex microbial communities. These microbial communities that colonize the fish body have a significant role in the physiological functions of the host; for instance, in immune maturation, development, metabolism and digestion. Furthermore, various factors may influence the microbiome in fish including geographic region, feeding habits and host genetics (Egerton

et al., 2018, Johny et al., 2021). The fish microbiome is not extensively explored as the human microbiome. It was reported that lactic acid bacteria in the gut microbiome inhibit the adhesion of various pathogenic bacteria to the host intestinal mucosa (Balcázar et al., 2008). Moreover, the host-gut microbe association has an effect on fish growth and health (Balcázar et al., 2008).

In zebrafish (*Danio rerio*) and threespine stickleback (*Gasterosteus aculeatus*), the microbiome is mostly composed of the highly abundant phyla such as *Proteobacteria, Fusobacteria, Firmicutes, Bacteroidetes* and *Actinobacteria*. These phyla were found to be dominant among the microbial communities associated with other aquatic animals also (Legrand et al., 2020). A study on wild-type and fast-growing transgenic common carp (*Cyprinus carpio* L.) showed that the latter type grew faster due to massive energy intake, which was linked to the core microbiome; the analysis showed that *Proteobacteria, Bacteroidetes*, and *Firmicutes* contribute to host metabolism linked to growth (Li et al., 2013). Chapagain et al. (2019) reported that bacteria belonging to *Firmicutes* were abundant in fast-growing rainbow trout (*Oncorhynchus mykiss*) while pathogenic bacteria such as *Corynebacterium* and *Paeniclostridium* that produce toxins and cause diseases were more abundant in slow-growing fish. *Firmicutes* are involved in host lipid metabolism and fatty acid absorption which impact the body weight of the host (Chapagain et al., 2019, Balcázar et al., 2008).

1.5. Microbiota transfer across generations

Organisms are composed of complex and dynamic systems in which we find host-cell, host-microbe and microbe-microbe interactions. The microbiota plays a critical role in the development of hosts, either by exerting evolutionary pressure or via their effect on the immune system (Tlaskalová-Hogenová et al., 2011, Chai et al., 2014, Ferretti et al., 2018, Henry et al., 2021). Furthermore, gut microbes are involved in host energy balance and metabolism. The gut microbes associated with animals degrade plant polysaccharides such as starch and sucrose (Krajmalnik-Brown et al., 2012). At the early stage of host

development in humans, maternal microbiota is transferred to infants (Ferretti et al., 2018, Rackaityte and Lynch, 2020). Similar vertical transmission occurs in poultry (Lee et al., 2019), livestock animals (Estellé, 2019) and fish (Sylvain and Derome, 2017). Microbiota transfer can be via vertical, horizontal or environmental transmission (Leftwich et al., 2020); vertical transmission is necessary to transfer essential microbes for early development, horizontal transfer from diet or surrounding environment helps in enhancing the health of the host (Leftwich et al., 2020), and environmental transmission is required for the acquisition of symbionts which may shape the evolution of the host. In this transmission process, pathogens can also find their way into the host microbiota (Leftwich et al., 2020). The disturbance of maternal microbiota transmission to offspring is associated with various diseases in humans, as noted in the case of babies delivered via C-section (Funkhouser and Bordenstein, 2013, Mueller et al., 2015, Rackaityte and Lynch, 2020). In the livestock industry, producers aim to eliminate the risk of pathogenic exposure. For instance, dairy calves are separated from their mothers at birth and housed individually. Then they are fed milk substitutes for 8 weeks, after which calves of the same age are housed together. Whereas, in nature, calves are weaned for 10 months progressively (SchultzMarcolla et al., 2019). Similarly, in the poultry industry, laid eggs are collected from the nests and transferred to a specific hatchery facility, where they are disinfected and artificially incubated. After hatching, the chicks are reallocated into sanitized barns. This practice avoids the exposure of chicks to the microbiome from mature birds. However, this sort of management practices are not allowed in some countries such as Canada (SchultzMarcolla et al., 2019). The transmission source differs across species; for example in humans, skin and vagina microbiota are transferred to infants (Funkhouser and Bordenstein, 2013, Mueller et al., 2015, Ferretti et al., 2018), in livestock animals, the birth canal microbes colonize newborns (Estellé, 2019), and in chicken, microbiota from oviduct and cloaca are transferred to the embryo (Lee et al., 2019).

The microbial transmission mechanisms of aquatic animals differ from those of mammals. The fish egg is composed of thick inner (*zona radiata*) and thin outer (*chorion or zona*

pellucida) layers, but the structure differs across fish species. The chorion contains lectins that allow microbes to adhere to and colonize the eggs (Olafsen, 2001, de Bruijn et al., 2017). Since the majority of fish species lay fertilized eggs freely in the environment, the early stage microbiota is shaped by the surrounding environment and as the fish grows, the impact of the environment will be diminished by other factors (Olafsen, 2001, Llewellyn et al., 2014, Stephens et al., 2016, Lokesh et al., 2019) (Figure 3). In discus (*Symphysodon aequifasciata*), larvae acquire their microbial symbionts through horizontal transmission from the surrounding environment (Sylvain and Derome, 2017). Furthermore, during the fry stage of discus, vertical transmission of microbial symbionts shapes the gut microbiome when parents feed their skin mucus to their offspring (Sylvain and Derome, 2017). This combination of vertical and horizontal transfer was also observed in little skate (*Leucoraja erinacea*) (Mika et al., 2021) and pipefish (*Syngnathus typhle*) (Beemelmanns et al., 2019).

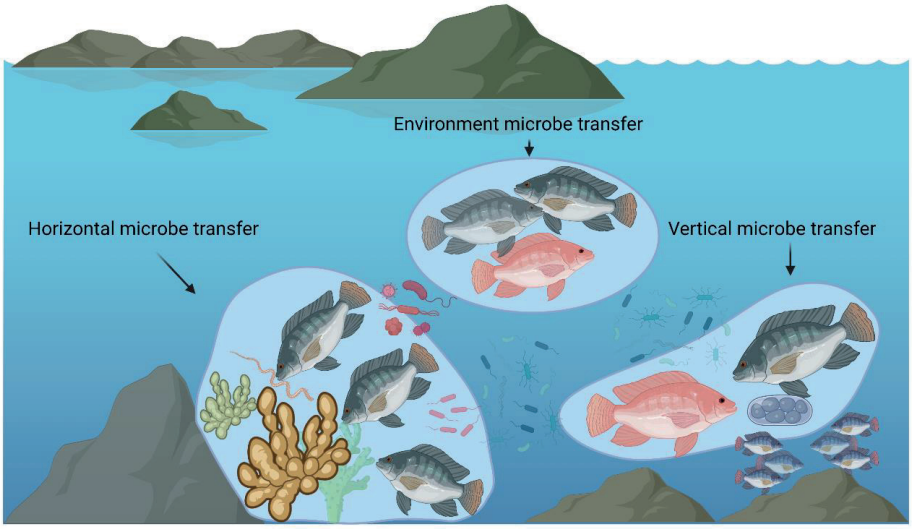

Figure 3. Microbial transmission in fish occurs via vertical, horizontal and environmental routes*. The idea is based on the concept described in Leftwich et al. (2020). This figure was generated using biorender.com*

1.6. Fish microbiome and its association with health

Microbiomes of fishes are complex and host-microbe and microbe-microbe interactions create a conducive environment for the commensal microbial communities. A state that favours a balanced healthy microbiota is known as normobiosis (Johny et al., 2021). A disturbance in this balance may harm the host, and such a state is known as dysbiosis (Wynne et al., 2020). Although niche-based theory and neutral theory exist, their joint influence leads to a well-established host microbial community (Liao et al., 2016). The host tolerates the cohabitants and it is believed that environmental changes can trigger an unfavourable or favourable antagonistic interaction (Zapién-Campos et al., 2015, Sibinelli-Sousa et al., 2022). Like the microbiome of mammals, the fish microbiome consists of commensal microbiota, including opportunistic pathogens (de Bruijn et al., 2017). For example, *Propionibacterium acnes* is an opportunistic pathogen that resists *Staphylococcus aureus* infection in human skin (Shu et al., 2013). The microbiome is involved in protection against pathogens, mucosal immunity, nutrition, metabolism and homeostasis. Several factors such as environment, diet, host genotype and health condition may alter the microbiome composition in fish (Kim et al., 2019) (Figure 4). Dysbiosis in fish may occur in various body sites such as the buccal cavity, skin and the gut and may result in the emergence of diseases and cause mortality (Xu et al., 2007, Llewellyn et al., 2017, Wynne et al., 2020, Bozzi et al., 2021). For example, Wynne et al. (2020) reported a dysbiosis caused by the dominance of *Tenacibaculum* sp. and the subsequent yellow mouth disease in Atlantic salmon (*Salmo salar*). In addition, bacteria belonging to the same genus caused skin infection, which in turn can cause dysbiosis in the gut microbiome of Atlantic salmon (Bozzi et al., 2021). In a freshwater fish, Nile tilapia, the genus *Streptococcus* was reported to cause streptococci outbreaks (Xu et al., 2007). On the other hand, antibiotics used in treating diseased fish may alter the microbial diversity and composition (Gupta et al., 2019), and may even increase the opportunistic pathogens such as *Vibrio scophthalmi* and *Photobacterium damselae*; such dysbiosis in the fish gut can affect the health of the host (de Bruijn et al., 2017, Kim et al., 2019).

The use of probiotics and prebiotics are suggested as alternative approaches for better health management in aquaculture, and are presumed to bring remarkable success in fish health improvement and disease resistance (Akhter et al., 2015, Xie et al., 2016, de Bruijn et al., 2017, Cavalcante et al., 2020). Probiotics are beneficial living microorganisms, while prebiotics are indigestible fibers that are utilized by host microbiota which produces metabolites to confer host health (Cani, 2018). For example, probiotics and prebiotics provided protection to Nile tilapia against *Aeromonas hydrophila* infection and enhanced the growth of the fish (Cavalcante et al., 2020). Furthermore, commensal bacteria, including lactic acid bacteria, can outcompete opportunistic pathogens such as *Aeromonas salmonicida* (Ringø and Holzapfel, 2000). Advanced DNA sequencing can be employed to understand the microbes that can be exploited for alternate health management strategies because the amount and depth of information can be employed to understand their association with diseases or health status in various hosts (Pękala et al., 2018, Wynne et al., 2020, Martinez et al., 2021).

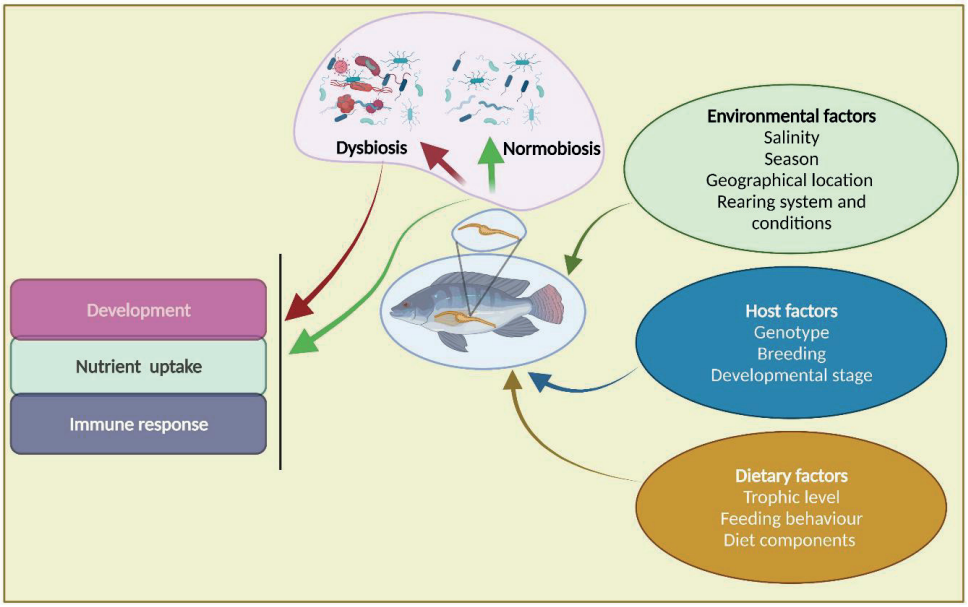

Figure 4. Factors that influence the gut microbiota in fish. *Environment, host and diet many influence the diversity and composition of gut microbiota to establish a healthy normobiosis or dysbiosis. The idea is adapted from Johny et al. (2021). This figure was generated using biorender.com*

1.7. Methods used in studying fish microbiomes

High-throughput sequencing is a technology that has rapidly evolved, and at present the low cost and speed has made it possible to profile whole community of microbes from various niches. In the past, the identification and characterization of microbes from any ecological niche was conducted using culture-dependent and conventional techniques such as Gram staining and biochemical characterization. These techniques can only detect 0.1% of the microbes in a complex community. On the other hand, many species that are unfeasible to cultivate under laboratory conditions can be detected using high-throughput sequencing technology (Kim et al., 2007, Tarnecki et al., 2017, Washburne et al., 2018, Nayfach et al., 2019). Advances in DNA sequencing revolve around three major technologies: first-generation sequencing (whole genome sequencing), second-generation sequencing, also known as NGS high throughput sequencing and third-generation sequencing (single-

molecule long-read sequencing) (Figure 5). These technologies have enabled us to understand how microbes function and interact with each other, and also with their host and their surroundings (Loman and Pallen, 2015). Low cost, high accuracy reads obtained from an illumina MiSeq sequencing platform, have provided new insights into fish microbiome research (Xie et al., 2016, Llewellyn et al., 2017, Kim et al., 2019, Wynne et al., 2020, Bozzi et al., 2021).

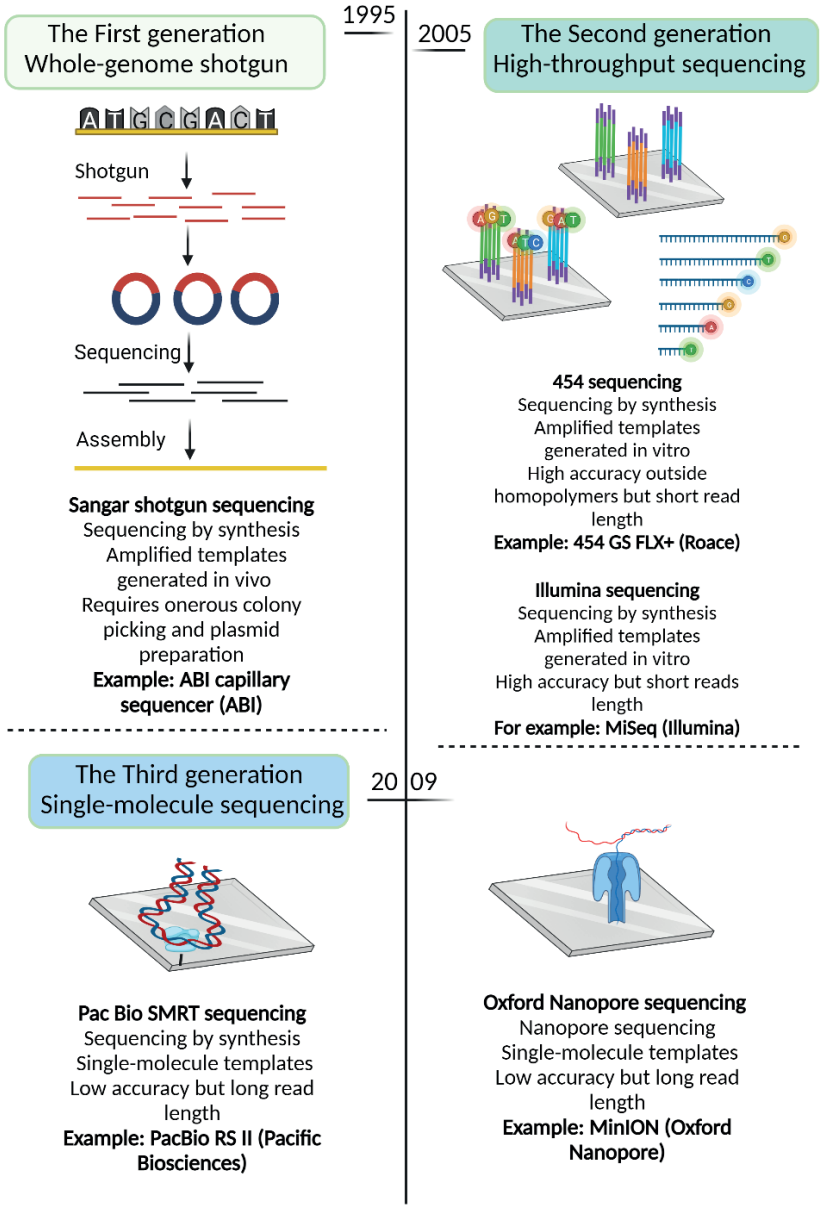

Figure 5. Timeline of the evolution of the sequencing revolution. *This figure was generated based on the idea from Loman and Pallen (2015). Recent advancements have increased the accuracy of PacBio sequencing to 99.5% (Hon et al., 2020). Reproduced with permission from Springer Nature License number 5260290472834. This figure was generated using biorender.com*

1.7.1. Amplicon sequencing method

The 16S rRNA in prokaryotes is approximately 1500 bp long, and is divided into nine hypervariable regions (V1-V9) flanked by highly conserved sequences (Cao et al., 2017, Ramazzotti and Bacci, 2018). However, due to the sequence length of the 16S rRNA gene, it is difficult to sequence the entire gene without requiring additional assembly steps (Di Bella et al., 2013, Cao et al., 2017). Therefore, a target region within the 16S rRNA is amplified and sequenced, and selection of the hypervariable regions for amplicon sequencing depends on the samples and the experimental design. Usually, samples from highly biodiverse habitats such as the gut or soil are treated differently compared to clinical specimens. It is recommended to select hypervariable region that is appropriate for a particular biome (Ramazzotti and Bacci, 2018). However, since the sequencing of the hypervariable region, the V4 enables the detection of most bacteria and archaea, it is chosen as the standard region in many amplicon sequencing protocols such as those in HMP (Turnbaugh et al., 2007, Faith et al., 2013) and EMP (Gilbert et al., 2014, Parada et al., 2016). Nevertheless, many scientists argue that sequencing one variable region can yield only low taxonomic resolution. As 16S rRNA variable regions are short and may share the same segment with many other microbes, combining two or more variable regions can enhance the taxonomic resolution and identification (Parada et al., 2016, Edgar, 2018, Fuks et al., 2018, Ramazzotti and Bacci, 2018, Bukin et al., 2019).

Based on the sequence similarity, the generated reads/sequences are clustered into Operational Taxonomic Units (OTUs). The clustered OTUs can be compared to sequences in different databases to identify microbes present in an ecological niche. Many studies have shown that OTU clustering is dependent on the variable regions; for example V1-V2 region-based analyses will produce OTUs that are different from those that employ V3-V4 region. Moreover, there will be differences in the abundance of microbes (Bukin et al., 2019, Schloss, 2010, Youssef et al., 2009). PCR bias is another issue that can also affect the OTU clustering because many genomes or fragments will be competing to recover their region

during the PCR amplification. On the other hand, amplicon sequence variants (ASVs) approach determines true biological sequences in a sample although there are amplification biases and sequencing errors, and the method differentiates sequence variants at a single nucleotide level. Moreover, ASV-based methods are regarded as highly sensitive and specific in distinguishing ecological patterns (Callahan et al., 2017). Furthermore, ASV approaches provide better accuracy and resolution compared to the OTU methods (Eren et al., 2015, Callahan et al., 2016, Callahan et al., 2017).

Various NGS technologies, especially the amplicon sequencing technique, made it possible to estimate the microbial community composition. Yet, these technologies have limitations caused mainly by the length of the read. Earlier amplicon sequencing studies employed the Roche 454 sequencer (Tamaki et al., 2011) which was able to generate reads of up to 800 bp long. However, Roche discontinued supporting the 454 platform in 2015. Currently, fish microbiome researchers also rely mainly on the illumina MiSeq platform, which can produce up to 350 bp single-end reads and up to 2 x 300 bp paired-end reads (Bukin et al., 2019, Johny et al., 2021). The short read lengths permit a microbial classification only up to the genus level (Bailén et al., 2020, Muhamad Rizal et al., 2020, Nygaard et al., 2020).

1.8. Knowledge gap

NGS methods have been widely used to study microbiome of humans, animals and fish. However, the human microbiome research has advanced considerably compared to those in fish; the microbes can be classified at the strain level (Ferretti et al., 2018). Moreover, using 16S rRNA data from human samples could be employed to predict the potential functions of a microbial community using PICRUSt software (Langille et al., 2013). The algorithms of this software are based on the prediction of an organism's genes without sequencing the whole genome. The 16S rRNA genes are mapped to homologous taxa with fully sequenced genomes. This approach is limited to available genomes in the databases which are mostly associated with microbes from humans (Sun et al., 2020). Functional prediction of human microbes is possible due to the availability of a well-established

database. However, PICRUSt-based function prediction will not be necessarily accurate in the case of fish and environmental studies (Sun et al., 2020). It is likely that aquatic environment may harbor novel microbes which may not exist in the database. However, the well-studied environments likely contain organisms that can be linked to information in the database. The frequently employed 16S rRNA method only allows profiling the taxonomic information. Furthermore, many microbes might not be detected by the 16S rRNA amplicon sequencing method; usually, these microbes are presented as unclassified meaning that similar sequences are not available in the 16S reference databases. Nevertheless, by acknowledging some caveats, functional information can be inferred in some cases. Although in recent years, metagenome shotgun sequencing has been used in fish microbiome studies, the lack of fish/marine microbiome information in the database makes it challenging to determine the functional profile of certain microbes (Tan et al., 2019b, Riiser et al., 2020). Shotgun metagenomics approach provides information of the potential functional capabilities of the organisms in the community. Multilayer omics, on the other hand, could give information on the actual metabolic activities of the communities and how they respond to the environmental inputs. Hence, to investigate the full potential of the fish microbiome in the future, we need to employ a multi-omics approach (Figure 6), which will enable microbial taxonomic profiling, functional annotation of microbial genes and metabolites (Frank et al., 2016, Kunath et al., 2017, Kunath et al., 2019).

Although there are over 50 000 articles in Google Scholar (accessed on 31 January 2022 with key words fish, microbiome, microbiota) about fish microbiota/microbiome, most of them focused on gut and skin bacterial communities. The buccal cavity microbiome in mouthbrooder species such as Nile tilapia has not been studied at length (Junior et al., 2016, Keller et al., 2018). Investigating the microbial communities in the buccal cavity of mothers will enable the identification of microbes that may be transferred across generations. Moreover, studying the microbiome can provide clues on the propensity of the fish towards certain diseases. The knowledge on the changes in the microbial community and its

association with fish breeding can help in taking advantage of a particular microbial community to devise health management strategies.

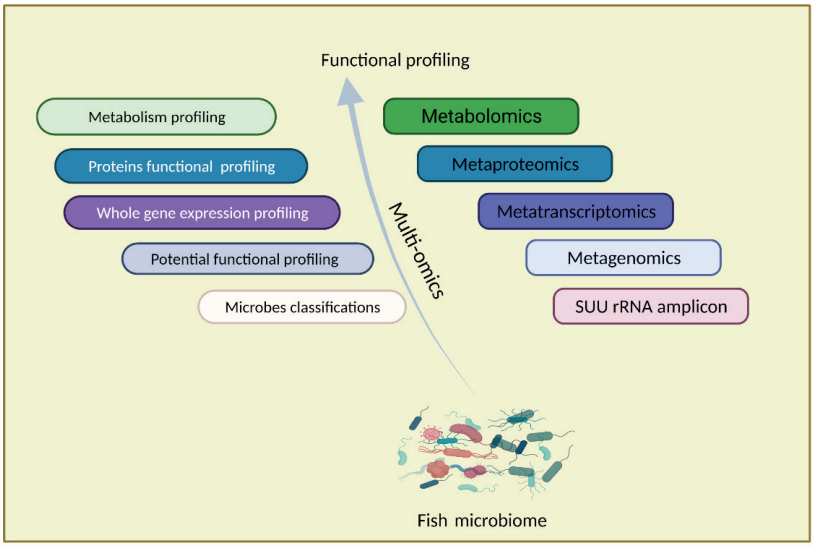

Figure 6. Multi-omics approach to reveal the full potential of microbial communities. *This figure is a modified version of an illustration in Ghanbari et al. (2015) and reproduced with permission from ELSEVIER with license no. 5261970527384. This figure was generated using biorender.com*

2. Objectives

For the present PhD project the hypothesis is that breeding, parental transfer and gender may affect the assembly of microbiota in Nile tilapia.

Accordingly, the specific objectives of the project are:

1) To profile the buccal cavity microbiome in male and female Nile tilapia (**Paper I**).
2) To determine the genetic differences in inbred and outbred female Nile tilapia as well as the associated inter-individual core microbiome variation (**Paper II**).
3) To compare maternal bacteria from wild female Nile tilapia and subsequent generations maintained under controlled conditions in order to identify the microbes that shape the buccal cavity and gut bacteria in the progeny (**Paper III**).

3. General discussion

Considering the increasing future food demands and limited natural resources, food industries have to increase productivity. However, they should deviate from an overexploitation route to a more sustainability-oriented direction. In this regard, aquaculture can provide high-quality proteins and other essential nutrients such as fatty acids, vitamins and minerals. When choosing to follow a sustainable path, among others, the industry should not adhere to antibiotic administration to reduce diseases. Good aquaculture practices include effective ways such as vaccination to reduce the use of antibiotics and their adverse effects on fish health and in turn consumers. Many studies on farmed aquatic animals have revealed the importance of microbiota in health and development, and scientists, farmers and policy makers are now keen to employ probiotics and prebiotics in aquaculture. Although studies on microbiomes of fish have gathered momentum during the last decade, there is much to be learned. For instance, more information on wild fish microbiota, intergenerational transfer of maternal microbiomes and microbial communities in body sites that can influence offspring microbiota can provide clues for improving farming practices. This is primarily because maintaining a balanced microbial community during the early life stages will decide the health throughout the life of farmed fish species (SchultzMarcolla et al., 2019).

Microbiota plays a critical role in development, nutrient acquisition, immunity and disease resistance of hosts. The microbiota is first established in newborns either through vertical, horizontal or environmental transfer. Disturbances in the transmission event from parents to offspring are associated with various health disorders in humans. Yet, only a few studies have investigated microbe transfer to progeny in mammals, let alone in fishes.

Nile tilapia, the second most farmed fish around the globe (Moses et al., 2021), is a mouthbrooder, and maternal to offspring transfer and later establishment of specific bacterial communities in different body sites are critical in maintaining homeostasis in specific host niches. In the PhD project, I investigated the microbial composition in the

buccal cavity of females and males (**Paper I**). Then I examined the breeding strategy-linked changes in bacterial communities in the buccal cavity and gut (**Paper II**) and lastly the maternal-microbe transfer across generations from wild mother to female offsprings (**Paper III**).

3.1 Nile tilapia buccal cavity microbiome

Nile tilapia female incubates eggs in her buccal cavity until hatching. The fry are also given shelter for the first 4-7 days after hatching and later when they return to the buccal cavity for security. Hence, the buccal cavity microbiome may have a role first in protection of the eggs from pathogenic bacteria and eventually in the immune system maturation of the fry. Nile tilapia buccal cavity contains complex microbial communities. The most abundant phyla in the fish are *Actinobacteria, Proteobacteria, Fusobacteria, Firmicutes* and *Bacteroidetes* (**Papers I** and **II**). These phyla are found to be dominant in fresh water and marine fish (Legrand et al., 2020), and also in feline and canine buccal cavities, as well as in humans (Davis, 2016). The buccal cavity is exposed to various microorganisms from the surrounding environment and diet, and the associated microbes may obtain an opportunity to colonize the host niches. Most of them may influence the oral microbiome and a shift in certain species can lead to dysbiosis, which could adversely affect the host health (Kim et al., 2019, Bozzi et al., 2021, Radaic and Kapila, 2021, Martinez et al., 2021).

The exposure of tilapia eggs and fry to the buccal cavity microbiome can facilitate the microbes in mothers to colonize the egg surfaces and body sites. We can assume that the assembly occurs via a complex process that is governed by environmental and host genetic factors as well as host-microbe and microbe-microbe interactions. In **paper I**, it was found that the female tilapia buccal cavity contained more beneficial bacteria than opportunistic pathogens. For instance, *Streptococcus* spp. was about 0.01% in females compared to 3.02% in males. Moreover, the host genetic factors also have a role in maintaining the low abundance of these microbes in the buccal cavity of female Nile tilapia (**Paper II**), possibly

pointing to host-microbe selection. However, *Streptococcus* spp. were not profiled in the buccal cavity of wild female Nile tilapia that were incubating eggs (**Paper III**).

Streptococcus spp. may play a different role in mammals because these bacteria are core members in mammals but not in Nile tilapia. For example, *Streptococcus* spp. were found dominant in healthy human (Sangwan et al., 2016) and mice (Abusleme et al., 2020) buccal cavities. In mice, *Streptococcus* spp. are vertically transferred from mother to offspring and these bacteria have a role in oral microbiome assemblage in the offspring (Abusleme et al., 2020). However, under certain circumstances, *Streptococcus* spp. are associated with many diseases in humans (Almeida et al., 2020). Similarly, in Nile tilapia, *Streptococcus* spp. is a causative agent of streptococcal infections (Wang et al., 2020). Bacteria belonging to *Streptococcus* have many virulence factors, which include capsular polysaccharide (CPS). However, *Streptococcus* spp. tend to lose their virulence when they lack the capability to produce CPS that play an essential role in their pathogenicity (Zhang, 2021). CPS is known to inhibit the phagocytic activity to prevent host immune cell recognition (Zhang et al., 2019, Zhang, 2021). However, absence of CPS in some phenotypes gives the bacteria the ability to adhere to intestinal epithelial tissue of Nile tilapia (Barato et al., 2016). Temperature is an important factor that triggers streptococcal infections. Hence, the outbreak of this disease is mainly observed in tropical countries, in particular during the summer season. *Streptococcus* spp. are known to regulate the production of CPS in response to environment, i.e. to alter the adherence capability in order to enter the host cells (Zhang, 2021). It should be noted that streptococcal infections induced by offering contaminated feed with *Streptococcus agalactiae* did not reveal any clinical signs and caused only late mortality in Nile tilapia (Owatari et al., 2022). *S. agalactiae* stimulation was reported to increase the expression of natural killer enhancing factor-A (Nkef-A) in the head kidney, spleen, gills and skin of Nile tilapia (Huang et al., 2021). Furthermore, during parental care, the expression of natural killer cell enhancing factor protein-B (Nkef-B belonging to peroxiredoxin family of antioxidant enzymes) increased in the buccal cavity of female Nile tilapia (Iq and Shu-Chien, 2011). These observations are likely indicating that the buccal cavity microbiome play a role

in disease resistance. *Streptococcus* spp. in the buccal cavity of Nile tilapia reared in captivity was low (**Papers I** and **II**) and because *Streptococcus* spp. was not found as a core microbiome in Nile tilapia buccal cavity (**Papers II** and **III**), these bacteria might have colonized via horizontal or environmental transfer. Interestingly, the bacteriocins produced by *Staphylococcus* can limit the growth of *Streptococcus* spp. (Janek et al., 2016), while the latter can produce hydrogen peroxide to kill the former (Wu et al., 2019). In **paper I**, microbe-microbe interactions were observed between *Staphylococcus* and *Streptococcus* spp. which indicated a competition for resources.

On the other hand, bacteria belonging to *Propionibacterium* and *Sphingomonas* were found dominant and were members of the core microbiome in wild female Nile tilapia (**Paper III**) and laboratory-reared fish (**Paper II**), and these microbes are likely to be vertically (present in different generations) transmitted across generations. Hence, *Propionibacterium* and *Sphingomonas* are possibly commensal microbes in the buccal cavity of female Nile tilapia (**Paper III**). *Sphingomonas* was detected in the buccal cavity of both male and female Nile tilapia (**Paper I**). Furthermore, these microbes were members of core microbiome in human milk (Mueller et al., 2015). It was reported that human milk oligosaccharides provide protection against pathogens such as *Streptococcus agalactiae* (Group B *Streptococcus*, GBS) via their antimicrobial and biofilm formation-preventing abilities (Craft et al., 2018). *Propionibacterium* and *Sphingomonas* are oligosaccharide producers (Li et al., 2016, Sabater et al., 2019), and oligosaccharides in human milk is known to play a key role in promoting the growth of commensal bacteria over pathogens (Craft et al., 2018). Hence, bacteria belonging to these two genera could prevent biofilm formation by pathogenic bacteria. It should be noted that *Propionibacterium acnes* which is a commensal bacteria in mammals are recognized by Toll-like receptors, namely TLR-2 and TLR-4 (extracellullar) as well as TLR-9 (intracellular) to evoke appropriate immune responses (Achermann et al., 2014).

The low abundant *Streptococcus* spp. was not among the persistent communities in the parents and offspring (**Papers I, II** and **III**). On the contrary, the dominant *Propionibacterium* and *Sphingomonas* were the core members of Nile tilapia (**Papers II** and **III**). These results may indicate host-microbe selection to maintain a healthy microbiome in the buccal cavity of female Nile tilapia. For instance, it was reported that selection of cold-tolerance genetic components such as mitochondrial genes *ATP6* and *ATP8* in blue tilapia are maternally transferred to offspring (Nitzan et al., 2016) and these cold resistant fish possesses a microbiome composition that favours the adaptation to cold conditions compared to cold-sensitive fish (Kokou et al., 2018). I speculate that a healthy balanced microbiome with *Propionibacterium* and *Sphingomonas* is likely to be selected by female Nile tilapia for egg and fry protection. This supports the hypothesis that the buccal cavity microbiome of the female mouthbrooders of Nile tilapia is likely to play an essential role in development and health of the fry of the fish.

Gender differences may have a bearing on the host microbial composition. In humans, it was reported that the saliva microbiome profiles of men and women, both during fed and fasted conditions, were different and specific bacteria (*Porphyromonas* and *Capnocytophaga*) were abundant in males (Minty et al., 2021). To my knowledge, gender-based differences in the gut microbiota of fish were investigated but not those associated with the buccal cavity (Bolnick et al., 2014). Comparison of the buccal cavity microbiome in female and male Nile tilapia did not reveal any statistically significant differences in either alpha or beta diversities. This finding is similar to a study on dog oral microbiome (Isaiah et al., 2017). On the other hand, a study on Finnish children reported that the diversity and composition of the saliva microbiota of males and females were significantly different (Raju et al., 2019). In the aforementioned study, girls were found to have more *Neisseria*, *Streptococcus, Haemophilus, Oribacterium,* and less *Brachymonas, Sphingomonas, Lactococcus*, *Mesorhizobium*, *Paludibacter*. These results may indicate that the male and female buccal cavities are colonized by similar microbial communities only in some organisms. However, even in these organisms the abundance of the microbes may

differ between sexes (**Paper I**). Furthermore, the microbial profiles of human male and female repoductive system were different and this disparity was associated with the fertility rate and pregnancy (Rowe et al., 2020). The differences between male and female tilapia microbial profiles could be linked to egg incubation and development (**Papers I, II** and **III**). For example, as mentioned before, the female tilapia buccal cavity contains less opportunistic pathogens compared to males, and under certain circumstances where the microbial balance is toppled the abundance of pathogenic bacteria such as biofilm-producing *Streptoccous* spp. may have an adverse effect during egg incubation. On the other hand, the higher abundance of the beneficial microbes may help in maintaining the low abundance of pathogenic microbes and a healthy buccal cavity microbiome (**Paper I**).

3.2 Nile tilapia gut microbiome

It is well known that microorganisms residing in the fish gut can impact the physiological status of the host. However, many factors including host genotype and environmental conditions can influence the gut microbiome. Wild fish migrate to different locations, and studies have revealed the preponderant effect of geographic location and diet on the gut microbiome composition (Le and Wang, 2020). Nevertheless, host genetics have an important role in shaping the gut microbiome, as observed in wild mullet, *Mugil cephalus,* that migrates to reproduce (Le and Wang, 2020). However, Kim et al. (2021) argued that host habitat is key in shaping the gut microbiome of wild fish compared to host genetics and feeding habits. Furthermore, Li et al. (2017) suggested that genetically homogeneous fishes may have different structured gut microbial communities and this may also impact the host metabolic profiles. Moreover, it was reported that when environmental variables are controlled, host-microbe selection influences gut microbiota composition (Nikouli et al., 2021). In this thesis too an apparent host genetic effect was observed in the gut microbial composition as well as for the core microbiome abundance **(Paper II**), based on the comparison of the bacterial composition of the inbred and outbred groups of Nile tilapia. In addition, host genetic effect was observed across generations of the fish (**Paper III**). Wild

Nile tilapia from Egypt had a core gut microbiome (**Paper III)** that is distinct from those of tilapia that thrive in Lake Awassa and Lake Chamo in Ethiopia (Bereded et al., 2020). It was reported that core microbiome members have lower interspecies competition which in turn allows ecological stability and distribution across hosts (Kokou et al., 2019). Huang et al. (2020) proposed that gut microbiome in fish is shaped via interaction between host-related and environmental factors. Hence, the assembly of the core gut microbiome is likely determined by host genetics and habitat (Elsaied et al., 2019).

In Nile tilapia, commensal bacteria contribute to gut normobiosis. For instance, various dietary probiotics such as *Lactobacillus* are reported to have a positive impact on growth, immune response and disease resistance in Nile tilapia (Xia et al., 2018, Tan et al., 2019a, Xia et al., 2020). Similar functions can be evoked by maternal microbes such as *Propionibacterium* and *Sphingomonas* that are likely vertically transmitted to offspring (**Paper III**) and as stated earlier these bacteria have the ability to produce prebiotic compounds such as oligosaccharides (Li et al., 2016, Sabater et al., 2019). In humans, these microbes contribute to the development and shaping of infant gut microbiota (Craft et al., 2018). Furthermore, infants require vitamin B12 during early life development, for the production of healthy red blood cells as well as to support brain development (CDC, 2022, Golding et al., 2021). In **paper II**, it was reported that inbreeding of Nile tilapia increased the abundance of beneficial bacteria such as *Cetobacterium*, *Sphingomonas*, and *Propionibacterium* in the gut of the fish. These microbes are crucial for vitamin B12 production (Tsuchiya et al., 2008) and secretion of antimicrobial metabolites (Gesheva and Vasileva-Tonkova, 2012, Shu et al., 2013). Furthermore, *Propionibacterium, Nocardioides* (**Paper III**) and *Cetobacterium* belonged to the core microbiome of the wild (Negash et al., 2020) and reared (**Paper II**) Nile tilapia. Fish that harbour *Cetobacterium* do not require dietary vitamin B12 (Tsuchiya et al., 2008); *Nocardioides* are known for their ability to produce a spectrum of antimicrobial compounds against both Gram-positive and Gram-negative bacteria (Gesheva and Vasileva-Tonkova, 2012). Hence, the core microbiome in

wild Nile tilapia is likely to have critical roles in host development and in preventing colonization by pathogenic bacteria.

Colonization of microbes during the early developmental stages of Nile tilapia is a dynamic and complex process, and the microbial composition stabilizes during the later stages (Deng et al., 2021). Nikouli et al. (2019) have reported core microbiome members that were detected in all early developmental stages (egg to larvae) of sea bream (*Sparus aurata*). Moreover, these microbes were not associated with the rearing system. Some members of the core microbiome remain in the later developmental stages though their abundance differs between individuals (Deng et al., 2021). Human gut microbiome associated inter-individual variation is mainly caused by diet and lifestyle. However, the factors that affect the inter-individual variation in fishes cannot be deciphered easily. Furthermore, inter-individual variation in abundances of microbes may occur even in same fish species that are farmed in a common garden (Nikouli et al., 2018, Nikouli et al., 2021). Inter-individual variation in the abundance of fish gut bacteria is well established and the same is true in the present study too (**Papers II** and **III**). Findings in **paper II** regarding microbial inter-individual variation is in agreement with Nikouli et al. (2021). However, it was found that inbreeding reduces this variability in individuals (**Paper II**). Kokou et al. (2019) have reported that the core bacterial members in sea bass (*Dicentrarchus labrax*) follow a uniform distribution; probably the fish maintains almost similar abundance of the core microbiome. It is likely that these microbes with low metabolic competition have a significant role in fish health and development. For instance, *Undibacterium* was found in gut core microbiome in wild, inbred and outbred Nile tilapia (**Papers II** and **III**). It was also found as a core microbe in the gut of the GIFT strain (Wu et al., 2021). *Undibacterium* spp. is involved in lipid metabolism in Nile tilapia and these bacteria are capable of producing fatty acids and polar lipids (Wu et al., 2021). If the microbe-microbe interactions between the members of core microbiome and other microbial communities is positive then competition will be less (Kokou et al., 2019). This may allow the host genetics/selection or ecological pressure to decide the microbial compositional variation (Gatesoupe et al., 2016, Le and Wang, 2020,

Nikouli et al., 2021). These findings indicate that many factors decide the persistence of core microbiome in the gut microbial communities.

3.3 Nile tilapia microbiome- a host health perspective

Emerging diseases cause high mortality among farmed fishes like Nile tilapia and the industry still relies on antibiotics to stem such losses. Antibiotics reduce the microbial diversity, shift their composition and increase the abundance of opportunistic pathogens and may eventually lead to a disease outbreak (Kim et al., 2019). Furthermore, pathogenic microbes in the buccal cavity may translocate to the gut and cause diseases such as streptococcal infection, as observed in humans (Olsen and Yamazaki, 2019). During streptococcal infection in tilapia, the microbial community in the gut is mostly dominated by *Streptococcus* spp. (Silva et al., 2020).

Disease-resistant fish for aquaculture can be obtained by adopting appropriate breeding strategies (Wiens et al., 2018, Li et al., 2019). There are efforts to produce *Streptococcus resistance* Nile tilapia through selective breeding approaches (Joshi et al., 2021b, Joshi et al., 2021a). Furthermore, inbreeding can conserve commensal microbes such as *Enhydrobacter, Cetobacterium* and *Propionibacterium* in the Nile tilapia gut (**Paper II**). Commensal microbes have a direct effect on pathogenic bacteria and could prevent diseases, using various mechanisms such as niche exclusion, competition for resources or producing antimicrobial compounds known as antibiosis (de Bruijn et al., 2017). For example, *Enhydrobacter* can produce antitoxin peptide known as entericidin which can inhibit the growth of pathogens such as *Flavobacterium* (Legrand et al., 2020). In addition, motile Aeromonas septicemia is one of the emerging diseases that affect Nile tilapia farming and is caused *Aeromonas hydrophila* (El-Gohary et al., 2020). A combination of probiotics (DBA®; *Bifidobacterium* sp., *Lactobacillus acidophilus* and *Enterococcus faecium*) and prebiotics (mannan oligosaccharides) was found to enhance the immunity of Nile tilapia and was effective in protecting the host from *A. hydrophila* infection (Cavalcante et al., 2020), further indicating the ability of specific microbes in disease prevention. *Bifidobacterium* sp.

can produce bacteriocins which can act as an antimicrobial against pathogens (Choyam et al., 2019). In **paper II**, *Bifidobacterium* sp. was found to have higher abundance in inbred Nile tilapia. However, the present study did not identify species belonging to lactobacilli. Nevertheless, the presence of *Sphingomonas* and *Propionibacterium* that belong to the core microbiome may indicate the production of prebiotics like oligosaccharides. Interestingly, these core microbiome members can be found across generations (**Paper III**). Because fish eggs mostly rely on maternally transferred components for their protection (de Bruijn et al., 2017), microbes such as *Sphingomonas* and *Propionibacterium* that are likely to be vertically transmitted might have important roles in development and in building defence against potential pathogenic microbes. The oligosaccharides produced by the bacteria belonging to these two genera can prevent the adherence of pathogenic phenotypes of *Streptococcus* (Craft et al., 2018), indicating the ability of core microbiome in maintaining a healthy microbial composition (**Paper II** and **III**).

Microbiota plays a key role in feed conversion and overall gut health in animals. For instance, microbes in animal gut can ferment or convert feeds to produce macro- and micro-nutrients such as proteins, short chain fatty acids (SCFAs) and vitamins for the host. Moreover, livestock animals with higher feed efficiency require less feed for maintenance, growth, milk or egg production (BaseClear, 2022). Microbial features including their metabolic characteristics are associated with host feed efficiency, and these features that could be transferred from parents to offspring are influenced by host genetics (Li et al., 2018, Li et al., 2019, Aliakbari et al., 2021). In recent years, breeding strategies were employed to obtain breeds with gut microbes linked to high feed efficiency; for example in livestock animals including ruminants (Li et al., 2019), pigs (Aliakbari et al., 2021) and poultry (BaseClear, 2022). Furthermore, host genetics affects the microbial assemblage of fish with different feeding habits (Li et al., 2018). However, until now selective breeding has considered only host genetics, but not feed conversion efficiency-linked gut microbes (de Verdal et al., 2018, Fry et al., 2018, Dvergedal et al., 2019, Besson et al., 2020, Nofima, 2022).

Commensal microbes in fish gut play a vital role in nutrient uptake and fish health. Notably, in germ-free zebrafish, only few goblet cells and enteroendocrine cells were observed in the gut of larvae, suggesting defective protein uptake and impaired behavior (de Bruijn et al., 2017). In cyprinid fishes, it was reported that there is a significant correlation between gut bacteria (e.g. *Cetobacterium*) and fish metabolic profiles (Li et al., 2017). The commensal microbe *Propionibacterium* can produce SCFAs such as acetate and propionate. *Cetobacterium* and *Propionibacterium* are core microbiome members (**Paper II** and **III)**, suggesting their importance in nutrient uptake and maintenance of gut health in Nile tilapia. Furthermore, the phylum *Firmicutes* that are associated with better feed conversion in ruminants (BaseClear, 2022) was found to be dominant in bred Nile tilapia (**Paper II**) and wild Nile tilapia (**Paper III**). However, it should be noted that farmed Nile tilapia grow faster than their wild counterparts. Hence, the species-level information and the correlation with feed conversion efficiency have to be established through future studies. The knowledge about feed efficiency-associated microbes and their inheritance in livestock animals, and the findings from my studies can be used to manipulate gut microbes for enhanced feed conversion in Nile tilapia. Because host genetics influence gut microbes, breeding strategies such as inbreeding may be used to increase the abundance or conserve microbes associated with high feed conversion. In other words, Nile tilapia strain with high feed conversion capability can be produced by creating specific microbes in the gut of the fish.

4. Conclusion

The current thesis provides novel information about Nile tilapia buccal cavity and gut microbial communities. In this PhD project, I found that the most abundant phyla in the fish buccal cavity and gut were *Actinobacteria, Proteobacteria, Fusobacteria, Firmicutes* and *Bacteroidetes.* These phyla are known to dominate in fresh water, providing an opportunity for the bacteria belonging to these phyla to colonize the body sites of the fish. The core microbiome that is essential for fish development can be transferred vertically or horizontally to offspring and some of them persist even in the later life stages. However, there will be inter-individual variation in their abundances. Another significant finding is that inbreeding could reduce the inter-individual variation in abundances of core microbiome. Furthermore, the core microbiome was affected by host genetics, as observed in the gut and buccal cavity of the inbred and outbred Nile tilapia. Inbred Nile tilapia possessed core bacteria such as *Cetobacterium, Propionibacterium*, *Sphingomonas* and *Enhydrobacter,* that are known to produce vitamin B12 and antimicrobials in the gut. *Propionibacterium*, *Sphingomonas* and *Nocardioides* belonged to the core microbiome of wild Nile tilapia and their offspring. *Nocardioides* are known for their ability to produce a spectrum of antimicrobial compounds against both Gram-positive and Gram-negative bacteria. *Propionibacterium* can produce both acetate and propionate which are SCFAs. *Cetobacterium* and *Propionibacterium* were core microbiome members, this result is likely indicating the important role of these bacteria in nutrient uptake and maintaining the gut health in Nile tilapia.

Female tilapia buccal cavity is the site of egg incubation and the mucus here contained less opportunistic pathogens such as *Streptococcus* spp. that have special virulence factors. Even in the buccal cavity of wild female fish that were incubating eggs these pathogenic bacteria were not identified, probably due to immune components like natural killer cell enhancing factor protein that are known to increase during parental care. The buccal cavity and gut microbiomes of female Nile tilapia contained other commensal microbes, including

Sphingomonas and *Propionibacterium* that are known to produce pathogen adhesion preventing oligosaccharides. Microbe interactions observed between *Staphylococcus* and *Streptococcus* spp. could be a strategy to maintain a healthy microbiome in the buccal cavity of female Nile tilapia. Because fish eggs mostly rely on maternally transferred components, microbes such as *Sphingomonas* and *Propionibacterium* that occur in different generations might have important roles in development and defence against pathogenic microbes.

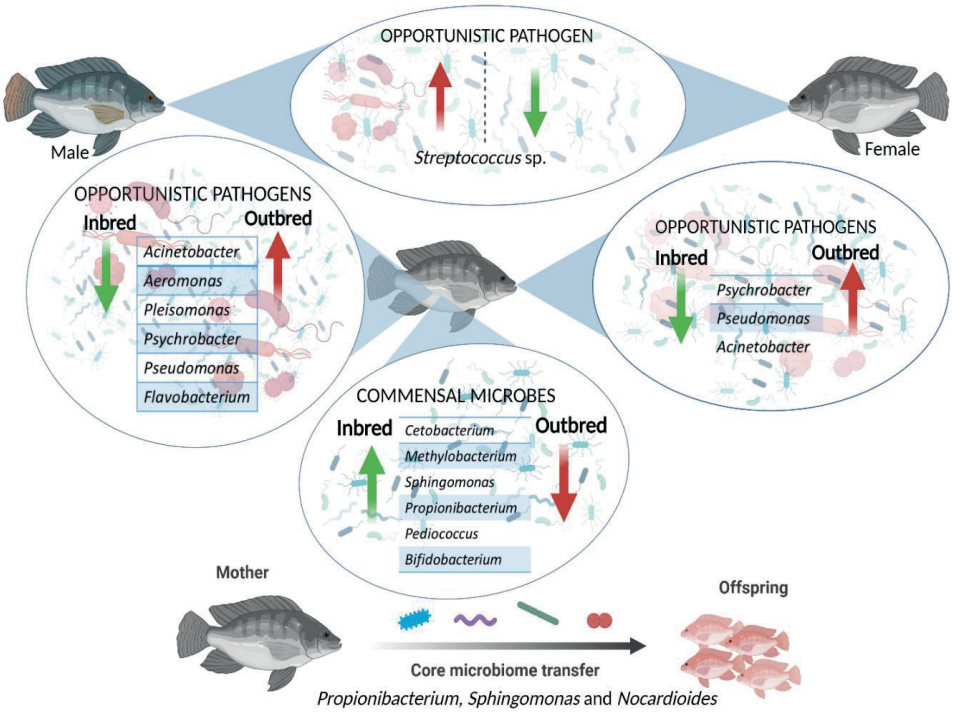

Figure 7. Graphical abstract of the key findings in this thesis. *Created with biorender.com*

5. Contribution to the field

Nile tilapia has a significant role in food security of the future population. However, various disease outbreaks affect this farmed fish and the profitability of the industry. It is well known that the fish microbiome is essential for host development and health. The findings in this thesis will aid in understanding the presence of specific commensal bacteria and their importance in fish health.

I have reported the impact of inbreeding and outbreeding in Nile tilapia because breeding can shape the buccal cavity and gut microbiome in fish, affecting the health of the host. The buccal cavity microbiome of mouthbrooder species is not widely explored. The information obtained from this thesis regarding buccal cavity microbiome will be an add-on to understanding the biology of mouthbrooder species and the role of the buccal microbiome in both protection during egg incubation and shaping of fry microbiome when they find shelter at times of danger. Furthermore, the buccal microbiome profile can be regarded as a bellwether for disease outbreaks.

The reported maternal core bacteria of Nile tilapia will aid in devising strategies for proper development and protection of eggs of this mouthbrooder species. Furthermore, the generated information may be used to understand host-microbe and microbe-microbe interactions as well as host adaptation to various conditions.

6. Future perspectives

The results from this thesis can form a baseline knowledge for future research. For instance (**Papers II,** and **III**), commensal bacteria such as *Propionibacterium* and *Sphingomonas* that belong to human milk core microbiome and produce oligosaccharides were found to be dominant in Nile tilapia buccal cavity and gut. These bacteria are widely used in industries and can be candidate probiotics in Nile tilapia feeds.

The importance of the buccal cavity microbiome in mouthbrooder species was highlighted in **papers I** and **II**. However, only 16S rRNA was used to profile the microbial composition. Future studies should exploit the potential of metagenomics and metatranscriptomics to provide insight into the functional profiles and host-microbes interaction.

Investigating maternal microbes (**Paper III**) further using meta-omics can provide valuable information about the offspring development and disease at later stages. For instance, understanding host genetics and microbial interaction to identify microbial signatures that can be associated with disease or mortality in fish. Also gene expression profile of microbes and in host tissues during egg incubation could provide information about the role of the microbiome in egg colonization.

Fish microbiome research is not well established compared to human microbiome research. More standard protocols for microbial DNA extraction from fish for metagenome shotgun sequencing need to be developed by researchers. Such fine-tuning is also important to generate accurate results using bioinformatics tools, especially because of the lack of fish related microbiome databases.

This thesis has highlighted the importance of the buccal cavity microbiome, and the results are expected to pave way for future indepth studies. In addition, the influence of breeding strategies on the gut microbiome is also an interesting topic that should be explored further for the benefit of the aquaculture industry.

7. References

Abusleme, L., O'gorman, H., Dutzan, N., Greenwell-Wild, T. & Moutsopoulos, N.M. (2020). Establishment and Stability of the Murine Oral Microbiome. *J Dent Res,* 99**:** 721-729. DOI 10.1177/0022034520915485

Abwao, J., Jung'a, J., Barasa, J.E., Kyule, D., Opiyo, M., Awuor, J.F., Ogello, E., Munguti, J.M. & Keya, G.A. (2021). Selective breeding of Nile tilapia, *Oreochromis niloticus*: A strategy for increased genetic diversity and sustainable development of aquaculture in Kenya. *J Appl Aquacult***:** 1-20. DOI 10.1080/10454438.2021.1958728

Achermann, Y., Goldstein, E.J.C., Coenye, T. & Shirtliff, M.E. (2014). *Propionibacterium acnes*: from commensal to opportunistic biofilm-associated implant pathogen. *Clin Microbiol Rev,* 27**:** 419-440. DOI 10.1128/CMR.00092-13

Aimutis, W.R. (2014). Microflora of the intestine | Biology of *Lactobacillus acidophilus*. IN Batt, C.A. & Tortorello, M.L. (Eds.) *Encyclopedia of Food Microbiology (Second Edition).* Oxford: Academic Press.

Akhter, N., Wu, B., Memon, A.M. & Mohsin, M. (2015). Probiotics and prebiotics associated with aquaculture: A review. *Fish Shellfish Immunol,* 45**:** 733-741. DOI https://doi.org/10.1016/j.fsi.2015.05.038

Aliakbari, A., Zemb, O., Billon, Y., Barilly, C., Ahn, I., Riquet, J. & Gilbert, H. (2021). Genetic relationships between feed efficiency and gut microbiome in pig lines selected for residual feed intake. *J Anim Breed Genet,* 138**:** 491-507. DOI https://doi.org/10.1111/jbg.12539

Almeida, V.D.S.M., Azevedo, J., Leal, H.F., Queiroz, A.T.L.D., Da Silva Filho, H.P. & Reis, J.N. (2020). Bacterial diversity and prevalence of antibiotic resistance genes in the oral microbiome. *PLoS One,* 15**:** e0239664. DOI 10.1371/journal.pone.0239664

Austin, B. & Al-Zahrani, A.M.J. (1988). The effect of antimicrobial compounds on the gastrointestinal microflora of rainbow trout, *Salmo gairdneri Richardson*. *J Fish Biol,* 33**:** 1-14. DOI https://doi.org/10.1111/j.1095-8649.1988.tb05444.x

Bailén, M., Bressa, C., Larrosa, M. & González-Soltero, R. (2020). Bioinformatic strategies to address limitations of 16rRNA short-read amplicons from different sequencing platforms. *J Microbiol Methods,* 169**:** 105811. DOI https://doi.org/10.1016/j.mimet.2019.105811

Balcázar, J.L., Vendrell, D., De Blas, I., Ruiz-Zarzuela, I., Muzquiz, J.L. & Girones, O. (2008). Characterization of probiotic properties of lactic acid bacteria isolated from intestinal microbiota of fish. *Aquaculture,* 278**:** 188-191. DOI https://doi.org/10.1016/j.aquaculture.2008.03.014

Barato, P., Martins, E.R., Vasquez, G.M., Ramirez, M., Melo-Cristino, J., Martínez, N. & Iregui, C. (2016). Capsule impairs efficient adherence of *Streptococcus agalactiae* to intestinal epithelium in tilapias *Oreochromis sp*. *Microb Pathog,* 100**:** 30-36. DOI https://doi.org/10.1016/j.micpath.2016.08.040

Barreto, R.E., Volpato, G.L., De Brito Faturi, C., Giaquinto, P.C., Gonçalves De Freitas, E. & Fernandes De Castilho, M. (2009). Aggressive behaviour traits predict physiological stress responses in Nile tilapia (*Oreochromis niloticus*). *Mar Freshwat Behav Physiol,* 42**:** 109-118. DOI 10.1080/10236240902850392

Baseclear (2022). Harnessing the microbiome to increase animal feed efficiency. BASECLEAR. https://www.baseclear.com/blog/animal-performance-health/harnessing-the-microbiome-to-increase-animal-feed-efficiency/. Access date.12.01.2022

Beemelmanns, A., Poirier, M., Bayer, T., Kuenzel, S. & Roth, O. (2019). Microbial embryonal colonization during pipefish male pregnancy. *Sci Rep,* 9**:** 3. DOI 10.1038/s41598-018-37026-3

Bereded, N.K., Curto, M., Domig, K.J., Abebe, G.B., Fanta, S.W., Waidbacher, H. & Meimberg, H. (2020). Metabarcoding analyses of gut microbiota of Nile tilapia (*Oreochromis niloticus*) from lake Awassa and lake Chamo, Ethiopia. *Microorganisms,* 8**:** 1040.

Besson, M., Komen, H., Rose, G. & Vandeputte, M. (2020). The genetic correlation between feed conversion ratio and growth rate affects the design of a breeding program for more sustainable fish production. *Genet Sel Evol,* 52**:** 5. DOI 10.1186/s12711-020-0524-0

Bik, E.M., Costello, E.K., Switzer, A.D., Callahan, B.J., Holmes, S.P., Wells, R.S., Carlin, K.P., Jensen, E.D., Venn-Watson, S. & Relman, D.A. (2016). Marine mammals harbor unique microbiotas shaped by and yet distinct from the sea. *Nat Commun,* 7**:** 10516. DOI 10.1038/ncomms10516

Bocci, V. (1992). The neglected organ: bacterial flora has a crucial immunostimulatory role. *Perspect Biol Med,* 35**:** 251-260.

Bolnick, D.I., Snowberg, L.K., Hirsch, P.E., Lauber, C.L., Org, E., Parks, B., Lusis, A.J., Knight, R., Caporaso, J.G. & Svanbäck, R. (2014). Individual diet has sex-dependent effects on vertebrate gut microbiota. *Nat Commun,* 5**:** 4500. DOI 10.1038/ncomms5500

Bozzi, D., Rasmussen, J.A., Carøe, C., Sveier, H., Nordøy, K., Gilbert, M.T.P. & Limborg, M.T. (2021). Salmon gut microbiota correlates with disease infection status: potential for monitoring health in farmed animals. *Anim Microbiome,* 3**:** 30. DOI 10.1186/s42523-021-00096-2

Bukin, Y.S., Galachyants, Y.P., Morozov, I.V., Bukin, S.V., Zakharenko, A.S. & Zemskaya, T.I. (2019). The effect of 16S rRNA region choice on bacterial community metabarcoding results. *Scientific Data,* 6**:** 190007. DOI 10.1038/sdata.2019.7

Byrd, A.L., Belkaid, Y. & Segre, J.A. (2018). The human skin microbiome. *Nat Rev Microbiol,* 16**:** 143-155. DOI 10.1038/nrmicro.2017.157

Cahill, M.M. (1990). Bacterial flora of fishes: A review. *Microb Ecol,* 19**:** 21-41. DOI 10.1007/bf02015051

Callahan, B.J., Mcmurdie, P.J. & Holmes, S.P. (2017). Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *ISME J,* 11**:** 2639-2643. DOI 10.1038/ismej.2017.119

Callahan, B.J., Mcmurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A. & Holmes, S.P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods,* 13**:** 581-583. DOI 10.1038/nmeth.3869

Cani, P.D. (2018). Human gut microbiome: hopes, threats and promises. *Gut,* 67**:** 1716-1725. DOI 10.1136/gutjnl-2018-316723

Cao, Y., Fanning, S., Proos, S., Jordan, K. & Srikumar, S. (2017). A review on the applications of next generation sequencing technologies as applied to food-related microbiome studies. *Front Microbiol,* 8. DOI 10.3389/fmicb.2017.01829

Cavalcante, R.B., Telli, G.S., Tachibana, L., Dias, D.D.C., Oshiro, E., Natori, M.M., Silva, W.F.D. & Ranzani-Paiva, M.J. (2020). Probiotics, prebiotics and synbiotics for Nile tilapia: Growth performance and protection against *Aeromonas hydrophila* infection. *Aquac Rep,* 17**:** 100343. DOI https://doi.org/10.1016/j.aqrep.2020.100343

CDC (2022). Breastfeeding and special circumstances. Center for Disease Control and Prevention. https://www.cdc.gov/breastfeeding/breastfeeding-special-circumstances/diet-and-micronutrients/vitamin-b12.html. Access date.17.02.2022

Chai, J.N., Zhou, Y.W. & Hsieh, C.-S. (2014). T cells and intestinal commensal bacteria-ignorance, rejection, and acceptance. *FEBS Lett,* 588**:** 4167-4175. DOI https://doi.org/10.1016/j.febslet.2014.06.040

Chapagain, P., Arivett, B., Cleveland, B.M., Walker, D.M. & Salem, M. (2019). Analysis of the fecal microbiota of fast- and slow-growing rainbow trout (*Oncorhynchus mykiss*). *BMC Genomics,* 20**:** 788. DOI 10.1186/s12864-019-6175-2

Charo-Karisa, H., Rezk, M.A., Bovenhuis, H. & Komen, H. (2005). Heritability of cold tolerance in Nile tilapia, *Oreochromis niloticus*, juveniles. *Aquaculture,* 249**:** 115-123. DOI https://doi.org/10.1016/j.aquaculture.2005.04.029

Choyam, S., Suresh, P., Pandey, R. & Kammara, R. (2019). Ocins database: a database of bug-busters from *Bifidobacterium, Lactobacillus*, and *Enterococcus*. *Access microbiol,* 1.

Christensen, G.J.M. & Brüggemann, H. (2014). Bacterial skin commensals and their role as host guardians. *Benef Microbes,* 5**:** 201-215.

Craft, K.M., Thomas, H.C. & Townsend, S.D. (2018). Interrogation of human milk oligosaccharide fucosylation patterns for antimicrobial and antibiofilm trends in group B *Streptococcus*. *ACS Infectious Diseases,* 4**:** 1755-1765. DOI 10.1021/acsinfecdis.8b00234

Crespi, V. & New, M. (2009). *Oreochromis niloticus*. *FAO cultured aquatic species fact sheets*.

Danley, P.D., Husemann, M., Ding, B., Dipietro, L.M., Beverly, E.J. & Peppe, D.J. (2012). The impact of the geologic history and paleoclimate on the diversification of East African Cichlids. *International Journal of Evolutionary Biology,* 2012**:** 574851. DOI 10.1155/2012/574851

Davis, E.M. (2016). Gene Sequence Analyses of the Healthy Oral Microbiome in Humans and Companion Animals:A Comparative Review. *Journal of Veterinary Dentistry,* 33**:** 97-107. DOI 10.1177/0898756416657239

De Bruijn, I., Liu, Y., Wiegertjes, G.F. & Raaijmakers, J.M. (2017). Exploring fish microbial communities to mitigate emerging diseases in aquaculture. *FEMS Microbiol Ecol,* 94. DOI 10.1093/femsec/fix161

De Verdal, H., Komen, H., Quillet, E., Chatain, B., Allal, F., Benzie, J.a.H. & Vandeputte, M. (2018). Improving feed efficiency in fish using selective breeding: a review. *Rev Aquac,* 10**:** 833-851. DOI https://doi.org/10.1111/raq.12202

Delicio, H.C., Barreto, R.E., Normandes, E.B., Luchiari, A.C. & Marcondes, A.L. (2006). A place preference test in the fish Nile tilapia. *J Exp Anim Sci,* 43**:** 141-148. DOI https://doi.org/10.1016/j.jeas.2006.01.001

Deng, Y., Kokou, F., Eding, E.H. & Verdegem, M.C.J. (2021). Impact of early-life rearing history on gut microbiome succession and performance of Nile tilapia. *Anim Microbiome,* 3**:** 81. DOI 10.1186/s42523-021-00145-w

Dhanasiri, A.K., Brunvold, L., Brinchmann, M.F., Korsnes, K., Bergh, Ø. & Kiron, V. (2011). Changes in the intestinal microbiota of wild Atlantic cod *Gadus morhua L.* upon captive rearing. *Microb Ecol,* 61**:** 20-30. DOI 10.1007/s00248-010-9673-y

Di Bella, J.M., Bao, Y., Gloor, G.B., Burton, J.P. & Reid, G. (2013). High throughput sequencing methods and analysis for microbiome research. *J Microbiol Methods,* 95**:** 401-14. DOI 10.1016/j.mimet.2013.08.011

Dubos, R., Schaedler, R.W., Costello, R. & Hoet, P. (1965). Indigenous, normal, and autochthonous flora of the gastrointestinal tract. *J Exp Med,* 122**:** 67-76.

Dvergedal, H., Ødegård, J., Øverland, M., Mydland, L.T. & Klemetsdal, G. (2019). Selection for feed efficiency in Atlantic salmon using individual indicator traits based on stable isotope profiling. *Genet Sel Evol,* 51**:** 13. DOI 10.1186/s12711-019-0455-9

Edgar, R.C. (2018). Accuracy of taxonomy prediction for 16S rRNA and fungal ITS sequences. *PeerJ,* 6**:** e4652. DOI 10.7717/peerj.4652

Egerton, S., Culloty, S., Whooley, J., Stanton, C. & Ross, R.P. (2018). The gut microbiota of marine fish. *Front Microbiol,* 9**:** 873.

Eknath, A., Dey, M., Rye, M., Gjerde, B., Abella, T., Sevilleja, R., Tayamen, M., Reyes, R. & Bentsen, H. (1998). Selective breeding of Nile tilapia for Asia. *6th World Congress on Genetics Applied to Livestock Production.* University of New England Armidale, Australia.

El-Gohary, F.A., Zahran, E., Abd El-Gawad, E.A., El-Gohary, A.H., M. Abdelhamid, F., El-Mleeh, A., Elmahallawy, E.K. & Elsayed, M.M. (2020). Investigation of the prevalence, virulence genes, and antibiogram of motile aeromonads isolated from Nile tilapia fish farms in Egypt and assessment of their water quality. *Animals,* 10**:** 1432.

El-Khaldi, A.T.F. (2010). Effect of different stress factors on some physiological parameters of Nile tilapia (*Oreochromis niloticus*). *Saudi J Biol Sci,* 17**:** 241-246. DOI https://doi.org/10.1016/j.sjbs.2010.04.009

Elsaied, H.E., Soliman, T., Abu-Taleb, H.T., Goto, H. & Jenke-Kodam, H. (2019). Phylogenetic characterization of eukaryotic and prokaryotic gut flora of Nile tilapia, *Oreochromis niloticus,* along niches of Lake Nasser, Egypt, based on rRNA gene high-throughput sequences. *Ecol Genet Genom,* 11**:** 100037. DOI https://doi.org/10.1016/j.egg.2019.100037

Eren, A.M., Morrison, H.G., Lescault, P.J., Reveillaud, J., Vineis, J.H. & Sogin, M.L. (2015). Minimum entropy decomposition: Unsupervised oligotyping for sensitive partitioning of high-throughput marker gene sequences. *ISME J,* 9**:** 968-979. DOI 10.1038/ismej.2014.195

Escherich, T. (1882). Die marantische Sinusthrombose bei Cholera infantum. Julius-Maximilians-Universität Würzburg.

Estellé, J. (2019). Benefits from the joint analysis of host genomes and metagenomes: Select the holobiont. *J Anim Breed Genet,* 136**:** 75-76. DOI https://doi.org/10.1111/jbg.12383

Estes, J.A., Terborgh, J., Brashares, J.S., Power, M.E., Berger, J., Bond, W.J., Carpenter, S.R., Essington, T.E., Holt, R.D., Jackson, J.B.C., Marquis, R.J., Oksanen, L., Oksanen, T., Paine, R.T., Pikitch, E.K., Ripple, W.J., Sandin, S.A., Scheffer, M., Schoener, T.W., Shurin, J.B., Sinclair, A.R.E., Soulé, M.E., Virtanen, R. & Wardle, D.A. (2011). Trophic downgrading of planet earth. *Science,* 333**:** 301-306. DOI 10.1126/science.1205106

Faith, J.J., Guruge, J.L., Charbonneau, M., Subramanian, S., Seedorf, H., Goodman, A.L., Clemente, J.C., Knight, R., Heath, A.C., Leibel, R.L., Rosenbaum, M. & Gordon, J.I. (2013). The long-term stability of the human gut microbiota. *Science,* 341**:** 1237439. DOI 10.1126/science.1237439

FAO (2020). Sustainability in action. *State of world fisheries and aquaculture Rome,* 200. DOI https://doi.org/10.4060/ca9229en

Ferretti, P., Pasolli, E., Tett, A., Asnicar, F., Gorfer, V., Fedi, S., Armanini, F., Truong, D.T., Manara, S. & Zolfo, M. (2018). Mother-to-infant microbial transmission from different body sites shapes the developing infant gut microbiome. *Cell Host & Microbe,* 24**:** 133-145. e5.

Fishbase (2015). Convention on the conservation of migratory species of wild animals (CMS). FishBase. https://www.fishbase.se/summary/oreochromis-niloticus.html#:~:text=Short%20description%20Morphology%20%7C%20Morphometrics,118638). Access date.04.03.2022

Frank, J.A., Arntzen, M.Ø., Sun, L., Hagen, L.H., Mchardy, A.C., Horn, S.J., Eijsink, V.G.H., Schnürer, A., Pope, P.B. & Summers, Z.M. (2016). Novel syntrophic populations dominate an ammonia-tolerant methanogenic microbiome. *mSystems,* 1**:** e00092-16. DOI 10.1128/mSystems.00092-16

Fry, J.P., Mailloux, N.A., Love, D.C., Milli, M.C. & Cao, L. (2018). Feed conversion efficiency in aquaculture: do we measure it correctly? *Environ Res Lett,* 13**:** 024017. DOI 10.1088/1748-9326/aaa273

Fujimura, K. & Okada, N. (2008). Shaping of the lower jaw bone during growth of Nile tilapia *Oreochromis niloticus* and a Lake Victoria Cichlid *Haplochromis chilotes*: A geometric morphometric approach. *Development, Growth & Differentiation,* 50**:** 653-663. DOI https://doi.org/10.1111/j.1440-169X.2008.01063.x

Fuks, G., Elgart, M., Amir, A., Zeisel, A., Turnbaugh, P.J., Soen, Y. & Shental, N. (2018). Combining 16S rRNA gene variable regions enables high-resolution microbial community profiling. *Microbiome,* 6**:** 17. DOI 10.1186/s40168-017-0396-x

Funkhouser, L.J. & Bordenstein, S.R. (2013). Mom knows best: the universality of maternal microbial transmission. *PLoS Biol,* 11**:** e1001631. DOI 10.1371/journal.pbio.1001631

Gao, B., Chi, L., Tu, P., Gao, N. & Lu, K. (2019). The carbamate aldicarb altered the gut microbiome, metabolome, and lipidome of C57BL/6J mice. *Chem Res Toxicol,* 32**:** 67-79. DOI 10.1021/acs.chemrestox.8b00179

Gatesoupe, F.-J., Huelvan, C., Le Bayon, N., Le Delliou, H., Madec, L., Mouchel, O., Quazuguel, P., Mazurais, D. & Zambonino-Infante, J.-L. (2016). The highly variable microbiota associated to intestinal mucosa

correlates with growth and hypoxia resistance of sea bass, *Dicentrarchus labrax*, submitted to different nutritional histories. *BMC Microbiol,* 16**:** 266. DOI 10.1186/s12866-016-0885-2

Gesheva, V. & Vasileva-Tonkova, E. (2012). Production of enzymes and antimicrobial compounds by halophilic Antarctic *Nocardioides sp.* grown on different carbon sources. *World Journal of Microbiology and Biotechnology,* 28**:** 2069-2076. DOI 10.1007/s11274-012-1009-2

Ghanbari, M., Kneifel, W. & Domig, K.J. (2015). A new view of the fish gut microbiome: Advances from next-generation sequencing. *Aquaculture,* 448**:** 464-475. DOI https://doi.org/10.1016/j.aquaculture.2015.06.033

Gilbert, J.A., Jansson, J.K. & Knight, R. (2014). The Earth Microbiome project: successes and aspirations. *BMC Biol,* 12**:** 69. DOI 10.1186/s12915-014-0069-1

Globefish (2020). Tilapia sector growth to resume after shaking off pandemic effects. Food and Agriculture Organization of the United Nations Access date.13/01/2022.2022

Golding, J., Gregory, S., Clark, R., Iles-Caven, Y., Ellis, G., Taylor, C.M. & Hibbeln, J. (2021). Maternal prenatal vitamin B12 intake is associated with speech development and mathematical abilities in childhood. *Nutr Res,* 86**:** 68-78. DOI https://doi.org/10.1016/j.nutres.2020.12.005

Gonçalves-De-Freitas, E., Bolognesi, M.C., Gauy, A.C.D.S., Brandão, M.L., Giaquinto, P.C. & Fernandes-Castilho, M. (2019). Social behavior and welfare in Nile tilapia. *Fishes,* 4**:** 23.

Gupta, S., Fernandes, J. & Kiron, V. (2019). Antibiotic-induced perturbations are manifested in the dominant intestinal bacterial phyla of Atlantic salmon. *Microorganisms,* 7**:** 233.

Guyon, R., Rakotomanga, M., Azzouzi, N., Coutanceau, J.P., Bonillo, C., D'cotta, H., Pepey, E., Soler, L., Rodier-Goud, M., D'hont, A., Conte, M.A., Van Bers, N.E.M., Penman, D.J., Hitte, C., Crooijmans, R.P.M.A., Kocher, T.D., Ozouf-Costaz, C., Baroiller, J.F. & Galibert, F. (2012). A high-resolution map of the Nile tilapia genome: a resource for studying cichlids and other percomorphs. *BMC Genomics,* 13**:** 222. DOI 10.1186/1471-2164-13-222

Hayes, W. & Sahu, S. (2020). The human microbiome: history and future: microbiome. *Journal of Pharmacy & Pharmaceutical Sciences,* 23**:** 406-411. DOI 10.18433/jpps31525

Henry, L.P., Bruijning, M., Forsberg, S.K.G. & Ayroles, J.F. (2021). The microbiome extends host evolutionary potential. *Nat Commun,* 12**:** 5141. DOI 10.1038/s41467-021-25315-x

Hon, T., Mars, K., Young, G., Tsai, Y.-C., Karalius, J.W., Landolin, J.M., Maurer, N., Kudrna, D., Hardigan, M.A., Steiner, C.C., Knapp, S.J., Ware, D., Shapiro, B., Peluso, P. & Rank, D.R. (2020). Highly accurate long-read HiFi sequencing data for five complex genomes. *Scientific Data,* 7**:** 399. DOI 10.1038/s41597-020-00743-4

Horsley, R.W. (1977). A review of the bacterial flora of teleosts and *elasmobranchs*, including methods for its analysis. *J Fish Biol,* 10**:** 529-553. DOI https://doi.org/10.1111/j.1095-8649.1977.tb04086.x

Huang, Q., Sham, R.C., Deng, Y., Mao, Y., Wang, C., Zhang, T. & Leung, K.M.Y. (2020). Diversity of gut microbiomes in marine fishes is shaped by host-related factors. *Mol Ecol,* 29**:** 5019-5034. DOI https://doi.org/10.1111/mec.15699

Huang, Y., Zheng, Q., Wang, Z., Tang, J., Lu, Y., Qin, Q., Cai, J. & Jian, J. (2021). Fish natural killer enhancing factor-A (NKEF-A) enhance cytotoxicity of nonspecific cytotoxic cells against bacterial infection. *Mol Immunol,* 133**:** 184-193. DOI https://doi.org/10.1016/j.molimm.2021.02.017

Iq, K.C. & Shu-Chien, A.C. (2011). Proteomics of buccal cavity mucus in female tilapia fish (*Oreochromis* spp.): A comparison between parental and non-parental fish. *PLoS One,* 6**:** e18555. DOI 10.1371/journal.pone.0018555

Isaiah, A., Hoffmann, A.R., Kelley, R., Mundell, P., Steiner, J.M. & Suchodolski, J.S. (2017). Characterization of the nasal and oral microbiota of detection dogs. *PLoS One,* 12**:** e0184899. DOI 10.1371/journal.pone.0184899

Janek, D., Zipperer, A., Kulik, A., Krismer, B. & Peschel, A. (2016). High frequency and diversity of antimicrobial activities produced by nasal *Staphylococcus* strains against bacterial competitors. *PLoS Path,* 12**:** e1005812. DOI 10.1371/journal.ppat.1005812

Johny, T.K., Puthusseri, R.M. & Bhat, S.G. (2021). A primer on metagenomics and next-generation sequencing in fish gut microbiome research. *Aquacult Res,* 52**:** 4574-4600. DOI https://doi.org/10.1111/are.15373

Joshi, R., Skaarud, A., Alvarez, A.T., Moen, T. & Ødegård, J. (2021a). Bayesian genomic models boost prediction accuracy for survival to *Streptococcus agalactiae* infection in Nile tilapia (*Oreochromus nilioticus*). *Genet Sel Evol,* 53**:** 37. DOI 10.1186/s12711-021-00629-y

Joshi, R., Skaaurd, A. & Tola Alvarez, A. (2021b). Experimental validation of genetic selection for resistance against *Streptococcus agalactiae* via different routes of infection in the commercial Nile tilapia breeding programme. *J Anim Breed Genet,* 138**:** 338-348. DOI https://doi.org/10.1111/jbg.12516

Junior, E.M.L., Bandeira, T.D.J.P.G., De Miranda, M.J.B., Ferreira, G.E., Parente, E.A., Piccolo, N.S. & De Moraes Flho, M.O. (2016). Characterization of the microbiota of the skin and oral cavity of *Oreochromis niloticus* Caracterização da microbiota da pele e cavidade oral de *Oreochromis niloticus*. *Journal of Health & Biological Sciences,* 4**:** 193-197.

Keller, I.S., Bayer, T., Salzburger, W. & Roth, O. (2018). Effects of parental care on resource allocation into immune defense and buccal microbiota in mouthbrooding cichlid fishes*. *Evolution,* 72**:** 1109-1123. DOI https://doi.org/10.1111/evo.13452

Kim, A., Kim, N., Roh, H.J., Chun, W.-K., Ho, D.T., Lee, Y. & Kim, D.-H. (2019). Administration of antibiotics can cause dysbiosis in fish gut. *Aquaculture,* 512**:** 734330. DOI https://doi.org/10.1016/j.aquaculture.2019.734330

Kim, D.H., Brunt, J. & Austin, B. (2007). Microbial diversity of intestinal contents and mucus in rainbow trout (*Oncorhynchus mykiss*). *J Appl Microbiol,* 102**:** 1654-64. DOI 10.1111/j.1365-2672.2006.03185.x

Kim, P.S., Shin, N.-R., Lee, J.-B., Kim, M.-S., Whon, T.W., Hyun, D.-W., Yun, J.-H., Jung, M.-J., Kim, J.Y. & Bae, J.-W. (2021). Host habitat is the major determinant of the gut microbiome of fish. *Microbiome,* 9**:** 166. DOI 10.1186/s40168-021-01113-x

Kokou, F., Sasson, G., Friedman, J., Eyal, S., Ovadia, O., Harpaz, S., Cnaani, A. & Mizrahi, I. (2019). Core gut microbial communities are maintained by beneficial interactions and strain variability in fish. *Nat Microbiol,* 4**:** 2456-2465. DOI 10.1038/s41564-019-0560-0

Kokou, F., Sasson, G., Nitzan, T., Doron-Faigenboim, A., Harpaz, S., Cnaani, A. & Mizrahi, I. (2018). Host genetic selection for cold tolerance shapes microbiome composition and modulates its response to temperature. *eLife,* 7**:** e36398.

Krajmalnik-Brown, R., Ilhan, Z.-E., Kang, D.-W. & Dibaise, J.K. (2012). Effects of gut microbes on nutrient absorption and energy regulation. *Nutr Clin Pract,* 27**:** 201-214. DOI https://doi.org/10.1177/0884533611436116

Kunath, B.J., Bremges, A., Weimann, A., Mchardy, A.C. & Pope, P.B. (2017). Metagenomics and CAZyme discovery. *Methods Mol Biol,* 1588**:** 255-277. DOI 10.1007/978-1-4939-6899-2_20

Kunath, B.J., Delogu, F., Naas, A.E., Arntzen, M.Ø., Eijsink, V.G.H., Henrissat, B., Hvidsten, T.R. & Pope, P.B. (2019). From proteins to polysaccharides: lifestyle and genetic evolution of *Coprothermobacter proteolyticus*. *ISME J,* 13**:** 603-617. DOI 10.1038/s41396-018-0290-y

Lacerda, S., Batlouni, S., Silva, S., Homem, C. & França, L. (2018). Germ cells transplantation in fish: the Nile-tilapia model. *Anim Prod,* 3**:** 146-159.

Lacerda, S.M.S.N., Batlouni, S.R., Costa, G.M.J., Segatelli, T.M., Quirino, B.R., Queiroz, B.M., Kalapothakis, E. & França, L.R. (2010). A new and fast technique to generate offspring after germ cells transplantation in adult fish: the Nile tilapia (*Oreochromis niloticus*) model. *PLoS One,* 5**:** e10740. DOI 10.1371/journal.pone.0010740

Langille, M.G.I., Zaneveld, J., Caporaso, J.G., Mcdonald, D., Knights, D., Reyes, J.A., Clemente, J.C., Burkepile, D.E., Vega Thurber, R.L., Knight, R., Beiko, R.G. & Huttenhower, C. (2013). Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nat Biotechnol,* 31**:** 814-821. DOI 10.1038/nbt.2676

Le, M.H. & Wang, D. (2020). Structure and membership of gut microbial communities in multiple fish cryptic species under potential migratory effects. *Sci Rep,* 10**:** 7547. DOI 10.1038/s41598-020-64570-8

Lederberg, J. & Mccray, A.T. (2001). Ome SweetOmics--A genealogical treasury of words. *The scientist,* 15**:** 8-8.

Lee, S., La, T.-M., Lee, H.-J., Choi, I.-S., Song, C.-S., Park, S.-Y., Lee, J.-B. & Lee, S.-W. (2019). Characterization of microbial communities in the chicken oviduct and the origin of chicken embryo gut microbiota. *Sci Rep,* 9**:** 6838. DOI 10.1038/s41598-019-43280-w

Leftwich, P.T., Edgington, M.P. & Chapman, T. (2020). Transmission efficiency drives host–microbe associations. *Proc R Soc B,* 287**:** 20200820.

Legrand, T.P., Wynne, J.W., Weyrich, L.S. & Oxley, A.P. (2020). A microbial sea of possibilities: current knowledge and prospects for an improved understanding of the fish microbiome. *Rev Aquac,* 12**:** 1101-1134.

Lev Fishelson, W.L.M., Arthur A. Myrberg, Jr. (1985). A unique symbiosis in the gut of tropical herbivorous surgeonfish (acanthuridae: teleostei) from the red sea. *Science,* 229**:** 49-51. DOI 10.1126/science.229.4708.49

Li, F., Li, C., Chen, Y., Liu, J., Zhang, C., Irving, B., Fitzsimmons, C., Plastow, G. & Guan, L.L. (2019). Host genetics influence the rumen microbiota and heritable rumen microbial features associate with feed efficiency in cattle. *Microbiome,* 7**:** 92. DOI 10.1186/s40168-019-0699-1

Li, H., Jiao, X., Sun, Y., Sun, S., Feng, Z., Zhou, W. & Zhu, H. (2016). The preparation and characterization of a novel sphingan WL from marine *Sphingomonas sp.* WG. *Sci Rep,* 6**:** 37899. DOI 10.1038/srep37899

Li, T., Long, M., Li, H., Gatesoupe, F.-J., Zhang, X., Zhang, Q., Feng, D. & Li, A. (2017). Multi-omics analysis reveals a correlation between the host phylogeny, gut microbiota and metabolite profiles in cyprinid fishes. *Front Microbiol,* 8. DOI 10.3389/fmicb.2017.00454

Li, W., Liu, J., Tan, H., Yang, C., Ren, L., Liu, Q., Wang, S., Hu, F., Xiao, J., Zhao, R., Tao, M., Zhang, C., Qin, Q. & Liu, S. (2018). Genetic effects on the gut microbiota assemblages of hybrid fish from parents with different feeding habits. *Front Microbiol,* 9. DOI 10.3389/fmicb.2018.02972

Li, X., Yan, Q., Xie, S., Hu, W., Yu, Y. & Hu, Z. (2013). Gut microbiota contributes to the growth of fast-growing transgenic common carp (*Cyprinus carpio L.*). *PLoS One,* 8**:** e64577. DOI 10.1371/journal.pone.0064577

Liao, J., Cao, X., Zhao, L., Wang, J., Gao, Z., Wang, M.C. & Huang, Y. (2016). The importance of neutral and niche processes for bacterial community assembly differs between habitat generalists and specialists. *FEMS Microbiol Ecol,* 92. DOI 10.1093/femsec/fiw174

Liston, J. (1957). The occurrence and distribution of bacterial types on flatfish. *J Gen Microbiol,* 16**:** 205-16. DOI 10.1099/00221287-16-1-205

Llewellyn, M.S., Boutin, S., Hoseinifar, S.H. & Derome, N. (2014). Teleost microbiomes: the state of the art in their characterization, manipulation and importance in aquaculture and fisheries. *Front Microbiol,* 5. DOI 10.3389/fmicb.2014.00207

Llewellyn, M.S., Leadbeater, S., Garcia, C., Sylvain, F.E., Custodio, M., Ang, K.P., Powell, F., Carvalho, G.R., Creer, S., Elliot, J. & Derome, N. (2017). Parasitism perturbs the mucosal microbiome of Atlantic salmon. *Sci Rep,* 7**:** 43465. DOI 10.1038/srep43465

Lokesh, J., Kiron, V., Sipkema, D., Fernandes, J.M.O. & Moum, T. (2019). Succession of embryonic and the intestinal bacterial communities of Atlantic salmon (*Salmo salar*) reveals stage-specific microbial signatures. *Microbiologyopen,* 8**:** e00672. DOI https://doi.org/10.1002/mbo3.672

Loman, N.J. & Pallen, M.J. (2015). Twenty years of bacterial genome sequencing. *Nat Rev Microbiol,* 13**:** 787-794. DOI 10.1038/nrmicro3565

Lu, Z. (2020). Microbiota research: from history to advances. *E3S Web of Conferences.* EDP Sciences.

Maja, M.M. & Ayano, S.F. (2021). The impact of population growth on natural resources and farmers' capacity to adapt to climate change in low-income countries. *Earth Syst Environ,* 5**:** 271-283. DOI 10.1007/s41748-021-00209-6

Malacrinò, A. (2018). Meta-omics tools in the world of insect-microorganism interactions. *Biology,* 7**:** 50.

Martinez, J.E., Kahana, D.D., Ghuman, S., Wilson, H.P., Wilson, J., Kim, S.C.J., Lagishetty, V., Jacobs, J.P., Sinha-Hikim, A.P. & Friedman, T.C. (2021). Unhealthy lifestyle and gut dysbiosis: A better understanding of

the effects of poor diet and nicotine on the intestinal microbiome. *Front Endocrinol,* 12. DOI 10.3389/fendo.2021.667066

Mashaii, N., Rajabipour, F., Mohammadi, M., Sarsangi, H., Bitaraf, A., Hossein-Zadeh, H. & Sharif-Rohani, M. (2016). Reproduction of Nile tilapia, *Oreochromis niloticus* in brackish water. *J Appl Aquacult,* 28**:** 1-8. DOI 10.1080/10454438.2015.1104943

Melo-Bolívar, J.F., Ruiz Pardo, R.Y., Hume, M.E., Nisbet, D.J., Rodríguez-Villamizar, F., Alzate, J.F., Junca, H. & Villamil Díaz, L.M. (2019). Establishment and characterization of a competitive exclusion bacterial culture derived from Nile tilapia (*Oreochromis niloticus*) gut microbiomes showing antibacterial activity against pathogenic *Streptococcus agalactiae*. *PLoS One,* 14**:** e0215375.

Méric, G., Hitchings, M.D., Pascoe, B. & Sheppard, S.K. (2016). From *Escherich* to the *Escherichia coli* genome. *The Lancet Infectious Diseases,* 16**:** 634-636.

Metchnikoff, I.I. (2004). *The prolongation of life: optimistic studies*: Springer Publishing Company.

Miao, W. & Wang, W. (2020). Trends of aquaculture production and trade: Carp, tilapia, and shrimp. *Asian Fish Sci,* 33**:** 1-10.

Mika, K., Okamoto, A.S., Shubin, N.H. & Mark Welch, D.B. (2021). Bacterial community dynamics during embryonic development of the little skate (*Leucoraja erinacea*). *Anim Microbiome,* 3**:** 72. DOI 10.1186/s42523-021-00136-x

Minty, M., Loubières, P., Canceill, T., Azalbert, V., Burcelin, R., Tercé, F. & Blasco-Baque, V. (2021). Gender-associated differences in oral microbiota and salivary biochemical parameters in response to feeding. *J Physiol Biochem,* 77**:** 155-166. DOI 10.1007/s13105-020-00757-x

Moses, M., Chauka, L.J., De Koning, D.J., Palaiokostas, C. & Mtolera, M.S.P. (2021). Growth performance of five different strains of Nile tilapia (*Oreochromis niloticus*) introduced to Tanzania reared in fresh and brackish waters. *Sci Rep,* 11**:** 11147. DOI 10.1038/s41598-021-90505-y

Mueller, N.T., Bakacs, E., Combellick, J., Grigoryan, Z. & Dominguez-Bello, M.G. (2015). The infant microbiome development: mom matters. *Trends Mol Med,* 21**:** 109-117.

Muhamad Rizal, N.S., Neoh, H.-M., Ramli, R., A/L K Periyasamy, P.R., Hanafiah, A., Abdul Samat, M.N., Tan, T.L., Wong, K.K., Nathan, S., Chieng, S., Saw, S.H. & Khor, B.Y. (2020). Advantages and limitations of 16S rRNA next-generation sequencing for pathogen identification in the diagnostic microbiology laboratory: perspectives from a middle-income country. *Diagnostics,* 10**:** 816.

Nayfach, S., Shi, Z.J., Seshadri, R., Pollard, K.S. & Kyrpides, N.C. (2019). New insights from uncultivated genomes of the global human gut microbiome. *Nature,* 568**:** 505-510. DOI 10.1038/s41586-019-1058-x

Negash, K.B., Curto, M., Domig, K.J., Abebe, G.B., Fanta, S.W., Waidbacher, H. & Meimberg, H. (2020). Metabarcoding analyses of gut microbiota of Nile tilapia (*Oreochromis niloticus*) from lake Awassa and lake Chamo, Ethiopia. *Microorganisms,* 8**:** 1040.

Nikouli, E., Meziti, A., Antonopoulou, E., Mente, E. & Kormas, K.A. (2018). Gut bacterial communities in geographically distant populations of farmed sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*). *Microorganisms,* 6**:** 92.

Nikouli, E., Meziti, A., Antonopoulou, E., Mente, E. & Kormas, K.A. (2019). Host-associated bacterial succession during the early embryonic stages and first feeding in farmed gilthead sea bream (*Sparus aurata*). *Genes,* 10**:** 483.

Nikouli, E., Meziti, A., Smeti, E., Antonopoulou, E., Mente, E. & Kormas, K.A. (2021). Gut microbiota of five sympatrically farmed marine fish species in the Aegean sea. *Microb Ecol,* 81**:** 460-470. DOI 10.1007/s00248-020-01580-z

Nitzan, T., Slosman, T., Gutkovich, D., Weller, J.I., Hulata, G., Zak, T., Benet, A. & Cnaani, A. (2016). Maternal effects in the inheritance of cold tolerance in blue tilapia (*Oreochromis aureus*). *Environ Biol Fishes,* 99**:** 975-981. DOI 10.1007/s10641-016-0539-0

Njiru, M., Ojuok, J.E., Okeyo-Owuor, J.B., Muchiri, M., Ntiba, M.J. & Cowx, I.G. (2006). Some biological aspects and life history strategies of Nile tilapia *Oreochromis niloticus* (L.) in Lake Victoria, Kenya. *Afr J Ecol,* 44**:** 30-37. DOI https://doi.org/10.1111/j.1365-2028.2006.00610.x

Nofima (2022). Unlocks genetic value of feed intake. Nofima. https://nofima.com/results/unlocks-genetic-value-of-feed-intake/. Access date.05.02.2022

Nygaard, A.B., Tunsjø, H.S., Meisal, R. & Charnock, C. (2020). A preliminary study on the potential of Nanopore MinION and Illumina MiSeq 16S rRNA gene sequencing to characterize building-dust microbiomes. *Sci Rep,* 10**:** 3209. DOI 10.1038/s41598-020-59771-0

Olafsen, J.A. (2001). Interactions between fish larvae and bacteria in marine aquaculture. *Aquaculture,* 200**:** 223-247. DOI https://doi.org/10.1016/S0044-8486(01)00702-5

Olsen, I. & Yamazaki, K. (2019). Can oral bacteria affect the microbiome of the gut? *J Oral Microbiol,* 11**:** 1586422. DOI 10.1080/20002297.2019.1586422

Omasaki, S., Charo-Karisa, H., Kahi, A. & Komen, H. (2016). Genotype by environment interaction for harvest weight, growth rate and shape between monosex and mixed sex Nile tilapia (*Oreochromis niloticus*). *Aquaculture,* 458**:** 75-81.

Owatari, M.S., Cardoso, L., Pereira, S.A., Pereira, U.D.P., Tachibana, L., Martins, M.L. & Mouriño, J.L.P. (2022). Laboratory-controlled challenges of streptococcosis in Nile tilapia using the oral route (infected-feed) for infection. *Fish Shellfish Immunol,* 120**:** 295-303. DOI https://doi.org/10.1016/j.fsi.2021.11.039

Parada, A.E., Needham, D.M. & Fuhrman, J.A. (2016). Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environ Microbiol,* 18**:** 1403-1414. DOI https://doi.org/10.1111/1462-2920.13023

Pękala, A., Paździor, E., Antychowicz, J., Bernad, A., Głowacka, H., Więcek, B. & Niemczuk, W. (2018). *Kocuria rhizophila* and *Micrococcus luteus* as emerging opportunist pathogens in brown trout (*Salmo trutta Linnaeus*, 1758) and rainbow trout (*Oncorhynchus mykiss Walbaum*, 1792). *Aquaculture,* 486**:** 285-289. DOI https://doi.org/10.1016/j.aquaculture.2017.12.028

Pike, L.J. & Forster, S.C. (2018). A new piece in the microbiome puzzle. *Nat Rev Microbiol,* 16**:** 186-186. DOI 10.1038/nrmicro.2018.24

Pope, P.B., Denman, S.E., Jones, M., Tringe, S.G., Barry, K., Malfatti, S.A., Mchardy, A.C., Cheng, J.-F., Hugenholtz, P., Mcsweeney, C.S. & Morrison, M. (2010). Adaptation to herbivory by the Tammar

wallaby includes bacterial and glycoside hydrolase profiles different from other herbivores. *Proc Natl Acad Sci U S A,* 107**:** 14793-14798. DOI 10.1073/pnas.1005297107

Popma, T. & Masser, M. (1999). Tilapia life history and biology. *Malawi Fisheries Repository*.

Puttaraksar, N. & Center, W. (2004). *GIFT technology manual: an aid to tilapia selective breeding*: WorldFish.

Rackaityte, E. & Lynch, S.V. (2020). The human microbiome in the 21st century. *Nat Commun,* 11**:** 5256. DOI 10.1038/s41467-020-18983-8

Radaic, A. & Kapila, Y.L. (2021). The oralome and its dysbiosis: New insights into oral microbiome-host interactions. *Comput Struct Biotechnol,* 19**:** 1335-1360. DOI https://doi.org/10.1016/j.csbj.2021.02.010

Rahman, M.L., Shahjahan, M. & Ahmed, N. (2021). Tilapia farming in Bangladesh: adaptation to climate change. *Sustainability,* 13**:** 7657.

Raju, S.C., Lagström, S., Ellonen, P., De Vos, W.M., Eriksson, J.G., Weiderpass, E. & Rounge, T.B. (2019). Gender-specific associations between saliva microbiota and body size. *Front Microbiol,* 10. DOI 10.3389/fmicb.2019.00767

Ramazzotti, M. & Bacci, G. (2018). Chapter 5 - 16S rRNA-based taxonomy profiling in the metagenomics era. IN Nagarajan, M. (Ed.) *Metagenomics.* Academic Press.

Relman, D.A. (2002). New technologies, human-microbe interactions, and the search for previously unrecognized pathogens. *J Infect Dis,* 186**:** S254-S258. DOI 10.1086/344935

Relman, D.A. & Falkow, S. (2001). The meaning and impact of the human genome sequence for microbiology. *Trends Microbiol,* 9**:** 206-8. DOI 10.1016/s0966-842x(01)02041-8

Rezk, M.A., Ponzoni, R.W., Khaw, H.L., Kamel, E., Dawood, T. & John, G. (2009). Selective breeding for increased body weight in a synthetic breed of Egyptian Nile tilapia, *Oreochromis niloticus*: Response to selection and genetic parameters. *Aquaculture,* 293**:** 187-194. DOI https://doi.org/10.1016/j.aquaculture.2009.03.019

Riiser, E.S., Haverkamp, T.H.A., Varadharajan, S., Borgan, Ø., Jakobsen, K.S., Jentoft, S., Star, B. & Elkins, C.A. (2020). Metagenomic shotgun analyses reveal complex patterns of intra- and interspecific variation in the intestinal microbiomes of codfishes. *Appl Environ Microbiol,* 86**:** e02788-19. DOI 10.1128/AEM.02788-19

Ringø, E. & Holzapfel, W. (2000). Identification and characterization of carnobacteria associated with the gills of Atlantic salmon (*Salmo salar L.*). *Syst Appl Microbiol,* 23**:** 523-7. DOI 10.1016/s0723-2020(00)80026-0

Ringø, E. & Strøm, E. (1994). Microflora of Arctic charr, *Salvelinus alpinus* (L.): gastrointestinal microflora of free-living fish and effect of diet and salinity on intestinal microflora. *Aquacult Res,* 25**:** 623-629. DOI https://doi.org/10.1111/j.1365-2109.1994.tb00726.x

Rosner, J.L. (2014). Ten times more microbial cells than body cells in humans. *Microbe Magazine,* 9**:** 47-47.

Rowe, M., Veerus, L., Trosvik, P., Buckling, A. & Pizzari, T. (2020). The reproductive microbiome: An emerging driver of sexual selection, sexual conflict, mating systems, and reproductive isolation. *Trends Ecol Evol,* 35**:** 220-234. DOI 10.1016/j.tree.2019.11.004

Ruthsatz, K., Lyra, M.L., Lambertini, C., Belasen, A.M., Jenkinson, T.S., Da Silva Leite, D., Becker, C.G., Haddad, C.F.B., James, T.Y., Zamudio, K.R., Toledo, L.F. & Vences, M. (2020). Skin microbiome correlates with bioclimate and *Batrachochytrium dendrobatidis* infection intensity in Brazil's Atlantic Forest treefrogs. *Sci Rep,* 10**:** 22311. DOI 10.1038/s41598-020-79130-3

Sabater, C., Fara, A., Palacios, J., Corzo, N., Requena, T., Montilla, A. & Zárate, G. (2019). Synthesis of prebiotic galactooligosaccharides from lactose and lactulose by dairy *propionibacteria*. *Food Microbiol,* 77**:** 93-105. DOI https://doi.org/10.1016/j.fm.2018.08.014

Sangwan, N., Xia, F. & Gilbert, J.A. (2016). Recovering complete and draft population genomes from metagenome datasets. *Microbiome,* 4**:** 8. DOI 10.1186/s40168-016-0154-5

Savage, D.C. (1977). Microbial ecology of the gastrointestinal tract. *Annu Rev Microbiol,* 31**:** 107-133. DOI 10.1146/annurev.mi.31.100177.000543

Schedel, F.D.B., Musilova, Z. & Schliewen, U.K. (2019). East African Cichlid lineages (Teleostei: Cichlidae) might be older than their ancient host lakes: new divergence estimates for the East African Cichlid radiation. *BMC Evol Biol,* 19**:** 94. DOI 10.1186/s12862-019-1417-0

Schloss, P.D. (2010). The effects of alignment quality, distance calculation method, sequence filtering, and region on the analysis of 16S rRNA gene-based studies. *PLoS Comput Biol,* 6**:** e1000844. DOI 10.1371/journal.pcbi.1000844

Schultzmarcolla, C., Sosaalvarado, C., Peterwilling, B. & Filippomiglior (2019). Early life microbial exposure shapes subsequent animal health. *Can J Anim Sci,* 99**:** 661-677. DOI 10.1139/cjas-2019-0029

Sender, R., Fuchs, S. & Milo, R. (2016). Revised estimates for the number of human and bacteria cells in the body. *PLoS Biol,* 14**:** e1002533. DOI 10.1371/journal.pbio.1002533

Shiffman, M.E., Soo, R.M., Dennis, P.G., Morrison, M., Tyson, G.W. & Hugenholtz, P. (2017). Gene and genome-centric analyses of koala and wombat fecal microbiomes point to metabolic specialization for *Eucalyptus* digestion. *PeerJ,* 5**:** e4075. DOI 10.7717/peerj.4075

Shu, M., Wang, Y., Yu, J., Kuo, S., Coda, A., Jiang, Y., Gallo, R.L. & Huang, C.-M. (2013). Fermentation of *Propionibacterium acnes*, a commensal bacterium in the human skin microbiome, as skin probiotics against methicillin-resistant *Staphylococcus aureus*. *PLoS One,* 8**:** e55380. DOI 10.1371/journal.pone.0055380

Sibinelli-Sousa, S., De Araújo-Silva, A.L., Hespanhol, J.T. & Bayer-Santos, E. (2022). Revisiting the steps of *Salmonella* gut infection with a focus on antagonistic interbacterial interactions. *The FEBS Journal*. DOI https://doi.org/10.1111/febs.16211

Silva, B.R.D.S., Derami, M.S., Paixão, D.A., Persinoti, G.F., Dias Da Silveira, W. & Maluta, R.P. (2020). Comparison between the intestinal microbiome of healthy fish and fish experimentally infected with *Streptococcus agalactiae*. *Aquacult Res*.

Simpson, C.A., Adler, C., Du Plessis, M.R., Landau, E.R., Dashper, S.G., Reynolds, E.C., Schwartz, O.S. & Simmons, J.G. (2020). Oral microbiome composition, but not diversity, is associated with adolescent anxiety and depression symptoms. *Physiol Behav,* 226**:** 113126. DOI 10.1016/j.physbeh.2020.113126

Smith, C.C.R., Snowberg, L.K., Gregory Caporaso, J., Knight, R. & Bolnick, D.I. (2015). Dietary input of microbes and host genetic variation shape among-population differences in stickleback gut microbiota. *ISME J,* 9**:** 2515-2526. DOI 10.1038/ismej.2015.64

Stephens, W.Z., Burns, A.R., Stagaman, K., Wong, S., Rawls, J.F., Guillemin, K. & Bohannan, B.J.M. (2016). The composition of the zebrafish intestinal microbial community varies across development. *ISME J,* 10**:** 644-654. DOI 10.1038/ismej.2015.140

Su, S.-C., Chang, L.-C., Huang, H.-D., Peng, C.-Y., Chuang, C.-Y., Chen, Y.-T., Lu, M.-Y., Chiu, Y.-W., Chen, P.-Y. & Yang, S.-F. (2020). Oral microbial dysbiosis and its performance in predicting oral cancer. *Carcinogenesis,* 42**:** 127-135. DOI 10.1093/carcin/bgaa062

Sun, S., Jones, R.B. & Fodor, A.A. (2020). Inference-based accuracy of metagenome prediction tools varies across sample types and functional categories. *Microbiome,* 8**:** 46. DOI 10.1186/s40168-020-00815-y

Sunagawa, S., Coelho, L.P., Chaffron, S., Kultima, J.R., Labadie, K., Salazar, G., Djahanschiri, B., Zeller, G., Mende, D.R., Alberti, A., Cornejo-Castillo, F.M., Costea, P.I., Cruaud, C., D'ovidio, F., Engelen, S., Ferrera, I., Gasol, J.M., Guidi, L., Hildebrand, F., Kokoszka, F., Lepoivre, C., Lima-Mendez, G., Poulain, J., Poulos, B.T., Royo-Llonch, M., Sarmento, H., Vieira-Silva, S., Dimier, C., Picheral, M., Searson, S., Kandels-Lewis, S., Bowler, C., Vargas, C.D., Gorsky, G., Grimsley, N., Hingamp, P., Iudicone, D., Jaillon, O., Not, F., Ogata, H., Pesant, S., Speich, S., Stemmann, L., Sullivan, M.B., Weissenbach, J., Wincker, P., Karsenti, E., Raes, J., Acinas, S.G., Bork, P., Boss, E., Bowler, C., Follows, M., Karp-Boss, L., Krzic, U., Reynaud, E.G., Sardet, C., Sieracki, M. & Velayoudon, D. (2015). Structure and function of the global ocean microbiome. *Science,* 348**:** 1261359. DOI 10.1126/science.1261359

Sylvain, F.-É. & Derome, N. (2017). Vertically and horizontally transmitted microbial symbionts shape the gut microbiota ontogenesis of a skin-mucus feeding discus fish progeny. *Scientific Reports,* 7**:** 1-14.

Tamaki, H., Wright, C.L., Li, X., Lin, Q., Hwang, C., Wang, S., Thimmapuram, J., Kamagata, Y. & Liu, W.-T. (2011). Analysis of 16S rRNA amplicon sequencing options on the Roche/454 next-generation titanium sequencing platform. *PLoS One,* 6**:** e25263. DOI 10.1371/journal.pone.0025263

Tan, H.Y., Chen, S.-W. & Hu, S.-Y. (2019a). Improvements in the growth performance, immunity, disease resistance, and gut microbiota by the probiotic *Rummeliibacillus stabekisii* in Nile tilapia (*Oreochromis niloticus*). *Fish Shellfish Immunol,* 92**:** 265-275. DOI https://doi.org/10.1016/j.fsi.2019.06.027

Tan, S.M., Yung, P.Y.M., Hutchinson, P.E., Xie, C., Teo, G.H., Ismail, M.H., Drautz-Moses, D.I., Little, P.F.R., Williams, R.B.H. & Cohen, Y. (2019b). Primer-free FISH probes from metagenomics/metatranscriptomics data permit the study of uncharacterised taxa in complex microbial communities. *NPJ Biofilms Microbiomes,* 5**:** 17. DOI 10.1038/s41522-019-0090-9

Tarnecki, A.M., Burgos, F.A., Ray, C.L. & Arias, C.R. (2017). Fish intestinal microbiome: diversity and symbiosis unravelled by metagenomics. *J Appl Microbiol,* 123**:** 2-17. DOI https://doi.org/10.1111/jam.13415

Tlaskalová-Hogenová, H., Štěpánková, R., Kozáková, H., Hudcovic, T., Vannucci, L., Tučková, L., Rossmann, P., Hrnčíř, T., Kverka, M., Zákostelská, Z., Klimešová, K., Přibylová, J., Bártová, J., Sanchez, D., Fundová, P., Borovská, D., Šrůtková, D., Zídek, Z., Schwarzer, M., Drastich, P. & Funda, D.P. (2011). The role of gut microbiota (commensal bacteria) and the mucosal barrier in the pathogenesis of inflammatory and autoimmune diseases and cancer: contribution of germ-free and gnotobiotic animal models of human diseases. *Cell Mol Immunol,* 8**:** 110-120. DOI 10.1038/cmi.2010.67

Trewavas, E. (1983). Tilapiine fishes of the genera *Sarotherodon*, *Oreochromis* and *Danakilia*. *British Museum of Natural History, London, UK*.

Trust, T.J. & Sparrow, R.A. (1974). The bacterial flora in the alimentary tract of freshwater *salmonid* fishes. *Can J Microbiol,* 20**:** 1219-28. DOI 10.1139/m74-188

Tsuchiya, C., Sakata, T. & Sugita, H. (2008). Novel ecological niche of *Cetobacterium somerae*, an anaerobic bacterium in the intestinal tracts of freshwater fish. *Lett Appl Microbiol,* 46**:** 43-48.

Turnbaugh, P.J., Ley, R.E., Hamady, M., Fraser-Liggett, C.M., Knight, R. & Gordon, J.I. (2007). The human microbiome project. *Nature,* 449**:** 804-810. DOI 10.1038/nature06244

Vilela, D.a.R., Silva, S.G.B., Peixoto, M.T.D., Godinho, H.P. & França, L.R. (2003). Spermatogenesis in teleost: insights from the Nile tilapia (*Oreochromis niloticus*) model. *Fish Physiol Biochem,* 28**:** 187-190. DOI 10.1023/B:FISH.0000030523.16010.62

Wang, A.R., Ran, C., Ringø, E. & Zhou, Z.G. (2018). Progress in fish gastrointestinal microbiota research. *Rev Aquac,* 10**:** 626-640. DOI https://doi.org/10.1111/raq.12191

Wang, Q., Fu, T., Li, X., Luo, Q., Huang, J., Sun, Y. & Wang, X. (2020). Cross-immunity in Nile tilapia vaccinated with *Streptococcus agalactiae* and *Streptococcus iniae* vaccines. *Fish Shellfish Immunol,* 97**:** 382-389. DOI https://doi.org/10.1016/j.fsi.2019.12.021

Washburne, A.D., Morton, J.T., Sanders, J., Mcdonald, D., Zhu, Q., Oliverio, A.M. & Knight, R. (2018). Methods for phylogenetic analysis of microbiome data. *Nat Microbiol,* 3**:** 652-661. DOI 10.1038/s41564-018-0156-0

Wei, S., Bahl, M.I., Baunwall, S.M.D., Hvas, C.L. & Licht, T.R. (2021). Determining gut microbial dysbiosis: a review of applied indexes for assessment of intestinal microbiota imbalances. *Appl Environ Microbiol,* 87**:** e00395-21.

Whipps J, Lewis K & R, C. (1988). Mycoparasitism and plant disease control. IN Burge M (Ed.) Manchester University Press: Fungi Biol Control Syst.

Wikoff, W.R., Anfora, A.T., Liu, J., Schultz, P.G., Lesley, S.A., Peters, E.C. & Siuzdak, G. (2009). Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proc Natl Acad Sci U S A,* 106**:** 3698-703. DOI 10.1073/pnas.0812874106

Wong, C.B., Odamaki, T. & Xiao, J.-Z. (2019). Beneficial effects of *Bifidobacterium longum subsp. longum BB536* on human health: Modulation of gut microbiome as the principal action. *J Funct Foods,* 54**:** 506-519. DOI https://doi.org/10.1016/j.jff.2019.02.002

Worldfish (2016). Genetically improved farmed tilapia (GIFT). WorldFish. https://www.worldfishcenter.org/pages/gift/. Access date.11/12/2021.2021

Wu, X., Gordon, O., Jiang, W., Antezana, B.S., Angulo-Zamudio, U.A., Rio, C.D., Moller, A., Brissac, T., Tierney, A.R.P., Warncke, K., Orihuela, C.J., Read, T.D., Vidal, J.E. & Federle, M.J. (2019). Interaction between *Streptococcus pneumoniae* and *Staphylococcus aureus* generates · OH radicals that rapidly kill *Staphylococcus aureus* strains. *J Bacteriol,* 201**:** e00474-19. DOI 10.1128/JB.00474-19

Wu, Z., Zhang, Q., Lin, Y., Hao, J., Wang, S., Zhang, J. & Li, A. (2021). Taxonomic and functional characteristics of the gill and gastrointestinal microbiota and its correlation with intestinal metabolites in new GIFT strain of farmed adult nile tilapia (*Oreochromis niloticus*). *Microorganisms,* 9**:** 617.

Wynne, J.W., Thakur, K.K., Slinger, J., Samsing, F., Milligan, B., Powell, J.F.F., Mckinnon, A., Nekouei, O., New, D., Richmond, Z., Gardner, I. & Siah, A. (2020). Microbiome profiling reveals a microbial dysbiosis during a natural outbreak of Tenacibaculosis (Yellow Mouth) in Atlantic Salmon. *Front Microbiol,* 11. DOI 10.3389/fmicb.2020.586387

Xia, Y., Lu, M., Chen, G., Cao, J., Gao, F., Wang, M., Liu, Z., Zhang, D., Zhu, H. & Yi, M. (2018). 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,* 76**:** 368-379. DOI https://doi.org/10.1016/j.fsi.2018.03.020

Xia, Y., Yu, E., Lu, M. & Xie, J. (2020). Effects of probiotic supplementation on gut microbiota as well as metabolite profiles within Nile tilapia, *Oreochromis niloticus*. *Aquaculture,* 527**:** 735428. DOI https://doi.org/10.1016/j.aquaculture.2020.735428

Xie, Q., Pan, M., Huang, R., Tian, X., Tao, X., Shah, N.P., Wei, H. & Wan, C. (2016). Short communication: modulation of the small intestinal microbial community composition over short-term or long-term administration with *Lactobacillus plantarum* ZDY2013. *J Dairy Sci,* 99**:** 6913-6921. DOI https://doi.org/10.3168/jds.2016-11141

Xu, D.-H., Shoemaker, C.A. & Klesius, P.H. (2007). Evaluation of the link between gyrodactylosis and streptococcosis of Nile tilapia, *Oreochromis niloticus* (L.). *J Fish Dis,* 30**:** 233-238. DOI https://doi.org/10.1111/j.1365-2761.2007.00806.x

Youssef, N., Sheik, C.S., Krumholz, L.R., Najar, F.Z., Roe, B.A. & Elshahed, M.S. (2009). Comparison of species richness estimates obtained using nearly complete fragments and simulated pyrosequencing-generated fragments in 16S rRNA gene-based environmental surveys. *Appl Environ Microbiol,* 75**:** 5227-5236. DOI 10.1128/AEM.00592-09

Zapién-Campos, R., Olmedo-Álvarez, G. & Santillán, M. (2015). Antagonistic interactions are sufficient to explain self-assemblage of bacterial communities in a homogeneous environment: a computational modeling approach. *Front Microbiol,* 6. DOI 10.3389/fmicb.2015.00489

Zhang, D., Ke, X., Liu, Z., Cao, J., Su, Y., Lu, M., Gao, F., Wang, M., Yi, M. & Qin, F. (2019). Capsular polysaccharide of *Streptococcus agalactiae* is an essential virulence factor for infection in Nile tilapia (*Oreochromis niloticus Linn.*). *J Fish Dis,* 42**:** 293-302. DOI https://doi.org/10.1111/jfd.12935

Zhang, Z. (2021). Research Advances on Tilapia Streptococcosis. *Pathogens,* 10**:** 558.

Zhu, L., Wu, Q., Dai, J., Zhang, S. & Wei, F. (2011). Evidence of cellulose metabolism by the giant panda gut microbiome. *Proc Natl Acad Sci U S A,* 108**:** 17714-17719. DOI 10.1073/pnas.1017956108

Zilber-Rosenberg, I. & Rosenberg, E. (2008). Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *FEMS Microbiol Rev,* 32**:** 723-735. DOI 10.1111/j.1574-6976.2008.00123.x

Paper I

frontiers in Microbiology

ORIGINAL RESEARCH
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Power Play of Commensal Bacteria in the Buccal Cavity of Female Nile Tilapia

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Fish are widely exposed to higher microbial loads compared to land and air animals. It is known that the microbiome plays an essential role in the health and development of the host. The oral microbiome is vital in females of different organisms, including the maternal mouthbrooding species such as Nile tilapia (*Oreochromis niloticus*). The present study reports for the first time the microbial composition in the buccal cavity of female and male Nile tilapia reared in a recirculating aquaculture system. Mucus samples were collected from the buccal cavity of 58 adult fish (∼1 kg), and 16S rRNA gene amplicon sequencing was used to profile the microbial communities in females and males. The analysis revealed that opportunistic pathogens such as *Streptococcus* sp. were less abundant in the female buccal cavity. The power play of certain bacteria such as *Acinetobacter*, Acidobacteria (GP4 and GP6), and Saccharibacteria that have known metabolic advantages was evident in females compared to males. Association networks inferred from relative abundances showed few microbe–microbe interactions of opportunistic pathogens in female fish. The findings of opportunistic bacteria and their interactions with other microbes will be valuable for improving Nile tilapia rearing practices. The presence of bacteria with specific functions in the buccal cavity of female fish points to their ability to create a protective microbial ecosystem for the offspring.

Keywords: Nile tilapia, buccal cavity, bacteria, 16S rRNA gene sequencing, commensal

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INTRODUCTION

The buccal cavity harbors a complex and diverse microbiota, and parallels can be drawn between human oral and gut bacterial communities (Maki et al., 2021). Although the oral microbiome composition is different between individuals and there exist differences between their micro-habitats, the principal function of the microbiome remains the same (Caselli et al., 2020). Microbial communities play a vital role in the physiological functions, immune system, and growth of the host.

The potential beneficial/commensal microbes of the oral cavity are essential for the wellbeing of the host. The composition of the oral microbiome in healthy individuals is generally stable. Imbalance in the microbial community is known as dysbiosis, and this condition can be associated with diseases (Zaura et al., 2009; Caselli et al., 2020; Su et al., 2020; Wynne et al., 2020). Although the composition of the oral microbiome changes with the host health status (as observed in the case of adolescence-related depression and anxiety), its diversity remains the same (Simpson et al., 2020). Pregnant women harbor more oral cavity microbes than non-pregnant women, and pathogenic

taxa proliferate during early periods of pregnancy (Fujiwara et al., 2017; Lin et al., 2018). Such periodontal pathogens can cause oral diseases, which in turn can complicate the pregnancy and lead to adverse outcomes (Farrell et al., 2006; Salih et al., 2020; Saadaoui et al., 2021). Moreover, it is now known that chewing of betel-areca preparations and the use of tobacco and alcohol can have cytogenetic effects, jeopardize oral health and shift the microbial population; these health-risk factors are linked to oral cancer (Wang et al., 2019; Su et al., 2020).

As mentioned above, the association of the oral microbiome and the health of humans is well studied compared to those in fish. Hence, more information about the symbionts of fishes is essential because dysbiosis may also occur in the fish mouth and may cause a natural outbreak of various diseases (Wynne et al., 2020). To our knowledge, only one study has reported the importance of microbiome balance in the buccal cavity of a fish; a dysbiosis event that occurred in Atlantic salmon (*Salmo salar*) was linked to yellow mouth disease (Wynne et al., 2020).

Nile tilapia (*Oreochromis niloticus*) is a preferred farmed species because it exhibits excellent growth and robustness under culture conditions. Wild Nile tilapia are sexually mature when they attain a total length of 20–30 cm (Gómez-Márquez et al., 2003; Shoko et al., 2015). However, under captivity, sexual maturity is reached at a relatively smaller size of 8–13 cm (Gómez-Márquez et al., 2003; Shoko et al., 2015). Nile tilapia is a mouthbrooder species, and the females protect the eggs by incubating them in their mouth until hatching (Konstantinidis et al., 2020). This form of parental care increases offspring survival and fitness; the epidermal mucus of female tilapia changes to ensure protection, development and capacity enhancement of the embryos/fry under different situations, for example, during transport to new locations/environments (Iq and Shu-Chien, 2011; Orlando et al., 2017). Buccal cavity mucus of female tilapia has an array of proteins, namely antioxidant enzymes such as peroxiredoxin and stress proteins like heat shock proteins that are upregulated during infection and parental care (Iq and Shu-Chien, 2011). A possibility of passive immune transfer from mother to offspring during mouthbrooding rather than via eggs has been reported, based on a higher survival rate against ectoparasites compared to those raised through artificial incubation (Subasinghe, 1993; Sin et al., 1994).

There is growing evidence that the microbiome can be horizontally or vertically transmitted from mother to infant (Ferretti et al., 2018) and from parent fish to progeny (Sylvain and Derome, 2017). Hence, we wanted to understand the differences in buccal cavity microbiome profiles in female and male Nile tilapia to understand if the mouthbrooders have specific microbes to protect their offspring.

MATERIALS AND METHODS

Ethics Statement

The study was conducted after obtaining the license from the Norwegian Animal Research Authority (FOTS ID 1042). The guidelines for research using experimental animals were strictly followed during Nile tilapia rearing, handling and tissue sampling.

Experimental Fish and Set Up

In the present experiment, we employed Nile tilapia that were the offspring of the fish obtained by hatching eggs from wild fish that were captured from the Nile River, Luxor, Egypt (location GPS: 25°39′56″ N, 32°37′07″ E). The stocking density was 27 fish/m^3, and the fish were reared in a freshwater recirculating system in a tilapia rearing facility at the Research Station of Nord University, Bodø, Norway. The rearing conditions were: dissolved oxygen – 8.33 mg/l, ammonia – 0.06 mg/l, nitrite – 0.03 mg/l, alkalinity – 53.92 mg/l as $CaCO_3$, water temperature – 29.3 ± 0.4°C, photoperiod – LD 13:11. The fish were fed commercial pellets (Skretting, Norway) during the rearing period (Podgorniak et al., 2019). We collected sexually mature males (n = 30) and females (n = 28) (both of average weight 1000 g, average total length 37.48 cm, 8 month-old) from the above mentioned stock by carefully distinguishing them based on the tapered shape or rounded shape below the anus. The sex of the fish was further confirmed by dissection and observation of the gonads.

Prior to sampling, fish were not fed for 48 h, and they were sacrificed by exposing them to an emulsion containing 12 mL of clove oil (Sigma-Aldrich, St. Louis, MO, United States), 96% ethanol (1:10 v/v) and 10 L of water (Konstantinidis et al., 2020). Mouth mucus samples from the buccal cavity were taken using swabs (Copan Italia, Brescia, Italy), which were transferred to cryotubes and immediately frozen in liquid nitrogen (Caselli et al., 2020; Wynne et al., 2020). The collected samples were stored at −80°C until further use.

Microbial DNA Extraction and Library Preparation

Each individual swab sample was transferred to a 5 ml tube containing 1.4 mm zirconium oxide beads (Cayman Chemical, Ann Arbor, MI, United States), and two ml of InhibitEX buffer (Qiagen, Hilden, Germany) were added into the tube. DNA was extracted using QIAamp DNA stool Mini Kit (Qiagen) according to the manufacturer's protocol. The extracted DNA was eluted in 75 μl ATE buffer. Then, the quality and quantity of the extracted DNA were checked with the NanoDrop spectrophotometer ND-8000 (Thermo Fisher Scientific Inc., Waltham, MA, United States).

Library preparation was performed under sterile conditions. The 16S rRNA gene library was constructed from the extracted DNA using the specific bacterial primers 341F (5′ CCTACGGGNGGCWGCAG 3′) and 805R (5′ GACTACNVGGGTWTCTAATCC 3′) (Klindworth et al., 2013) flanked by overhang Illumina adapters targeting the hypervariable V3-V4 region (∼ 460 bp). The primer concentration was 10 nM and 1 μl was used for the library preparation. PCR reactions were prepared (25 μl total volume) using (12.5 μl) AmpliTag gold Master Mix (Thermo Fisher Scientific Inc.) and 2.5 μl of DNA template (5 ng/μl). PCR conditions consisted of an initial denaturation step at 95°C for 10 min (1 cycle), 30 cycles at 95°C for 30 s, 57°C for 30 s, 72°C for 1 min, and a final extension step at 72°C for 7 min (1 cycle).

Agarose gel (1.5%, 4.5 g/300 ml) electrophoresis was employed to check the amplified products. The purified PCR products obtained using the CleanNGS system (CleanNA, Waddinxveen,

Netherlands) were subjected to a second PCR (8 cycles, 16S Metagenomic Sequencing Library Preparation, Illumina, San Diego, CA, United States). CleanNGS system (CleanNA) was used to purify the obtained amplicon libraries. The quality of the libraries was checked on a TapeStation 2200 platform (Agilent Technologies, Santa Clara, CA, United States). Thereafter, the libraries were quantified using the Quant-IT PicoGreen dsDNA assay kit (Thermo Fisher Scientific) on a Synergy 2 microplate reader (BioTek, Winooski, VT, United States). Next, the pooled libraries were quantified using the KAPA library quantification kit (Roche, Basel, Switzerland) on a real-time qPCR LightCycler 480 (Roche). They were then sequenced on an Illumina® MiSeq (PE300) platform (MiSeq Control Software 2.5.0.5 and Real-Time Analysis software 1.18.54.0).

Data Processing and Analyses

The generated paired-end reads were truncated at 270 bp using VSEARCH (Rognes et al., 2016), and then processed using MICCA pipeline (v1.7.2) (Albanese et al., 2015). Sequences with a minimum overlap length of 60 bp and a maximum mismatch of 20 bp were merged. Next, the forward and reverse primers were trimmed off the merged reads and reads that did not contain the primers were discarded. Thereafter, the sequences with an expected error rate (Edgar and Flyvbjerg, 2015) >0.75 were filtered out, and sequences shorter than 400 bp were discarded. The filtered reads were denoised using the "*de novo* unoise" method implemented in MICCA, which utilizes the UNOISE3 algorithm (Edgar, 2016). The denoising method, which is based on correcting sequencing errors and determining true biological sequences at single-nucleotide resolution, generates amplicon sequence variants (ASVs). The taxonomic assignment of the representative bacterial ASVs was performed using the RDP classifier (Lan et al., 2012). The sequences were aligned using the NAST (DeSantis et al., 2006) multiple sequence aligner, and a phylogenetic tree was prepared using the FastTree software available in the MICCA pipeline.

Statistical Analysis

The similarities/differences in α-diversity were checked by Wilcoxon rank-sum test. Bacterial β-diversity was determined using unweighted and weighted UniFrac distances (Lozupone and Knight, 2005). Differences between bacterial communities in male and female groups were visualized by Principal Coordinates Analysis (PCoA). After checking the dispersions within the data set of each group, statistically significant differences between the groups were assessed using Permutational Multivariate Analysis of Variance Using Distance Matrices (PERMANOVA) (Anderson, 2001) (with 9,999 permutations), implemented in adonis function of the vegan R-package (Oksanen et al., 2013). The DESeq2 (Love et al., 2014) package was employed to detect the differentially abundant ASVs in the non-rarefied data (McMurdie and Holmes, 2014). It is believed that rarefied data reduce statistical power and make it difficult to assess the differences in the actual composition (Weiss et al., 2017).

Microbial Network Analysis

Microbial communities are complex, and their function and structure are greatly influenced by microbe-host and microbe-microbe interactions. To investigate the latter connections, we

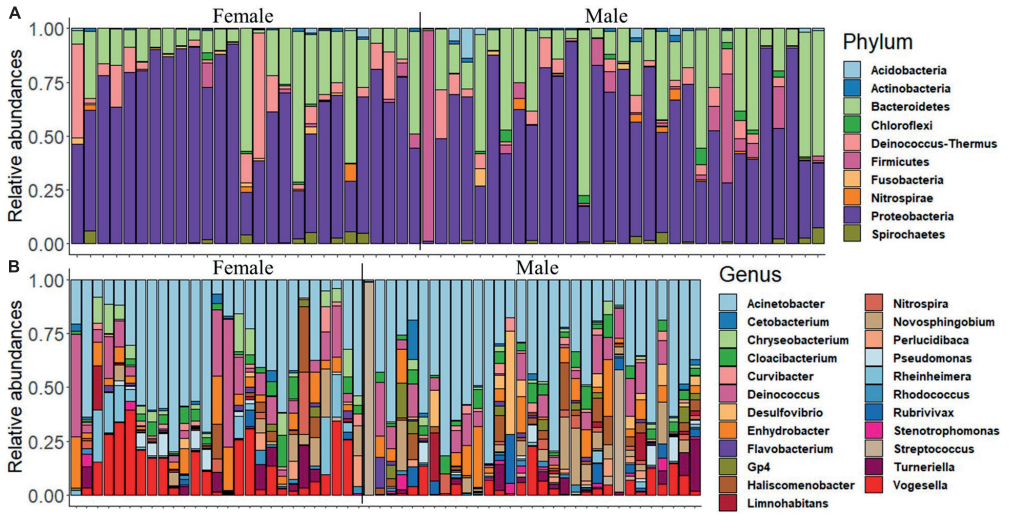

FIGURE 1 | The relative abundance of the microbial composition in the buccal cavity of female and male Nile tilapia. **(A)** Top 10 dominant phyla. **(B)** Top 23 dominant genera.

calculated pairwise relationships from the relative abundances of ASVs associated with the two types of samples (females and males). The networks were constructed at the phylum level using the SpiecEasi package, which considers an inverse covariance matrix and conditional independence (Kurtz et al., 2015). The differences in degrees and betweenness of nodes in the network of female and male fish were checked using the Wilcoxon rank-sum test.

RESULTS

Microbial Composition

To characterize the microbial composition in the buccal cavity of female and male Nile tilapia, the fish were reared in a common garden. The environmental conditions and diet that are known to affect the microbiota were kept constant throughout the experimental period. The amplicon sequencing of the 16S rRNA libraries generated 8706006 high-quality reads with an average of 150104 reads per sample. The reads were rarefied to 53575 reads per sample (without replacement), to take the read count variation in the different samples into account. A total of 1367 denoised ASVs were identified across all samples. Their taxonomic classification revealed the presence of bacteria belonging to 26 phyla and 272 genera.

First, we delineated the microbial composition in the mouth of female and male fish, and then we investigated the abundance of the dominant ASVs/taxa in females and males. The analysis revealed that Proteobacteria, Bacteroidetes, Firmicutes, Deinococcus–Thermus, Actinobacteria, and Acidobacteria were the most dominant phyla in both groups (**Figure 1A** and **Supplementary Figure 1A**). The dominance of Proteobacteria was also reflected in the microbial composition at the genus level, i.e., *Acinetobacter*, *Enhydrobacter*, *Novosphingobium*, *Pseudomonas*, *Haliscomenobacter*, *Rheinheimera,* and *Vogesella* (**Figure 1B**). However, the abundance of certain dominant genera such as *Acinetobacter* and *Enhydrobacter* was higher in females compared to males (**Supplementary Figure 1B**).

The proportions of the most dominant phyla and genera in both sexes are provided in **Table 1**. The proportions of Proteobacteria were 29.95 and 35.71% in females and males, respectively. The corresponding values of the phylum Bacteroidetes were 10.20 and 6.64%. Furthermore, *Acinetobacter* was the most abundant genus in the buccal cavity of female and male fish. The abundance of this genus in females was 28.07% compared to 26.19% males. The proportions (females vs. males) of the other genera belonging to the phylum Proteobacteria were: *Enhydrobacter* (5.01% vs. 2.87%), *Novosphingobium* (1.19% vs. 2.98%), *Pseudomonas* (1.37% vs. 3.15%), *Haliscomenobacter* (0.4% vs. 0.2%), *Rheinheimera* (0.99% vs. 4.83%), and *Vogesella* (4.57% vs. 8.80%).

Alpha diversity analysis of microbial communities in female and male buccal cavities was based on three ecological diversity measures, namely, the Chao1 estimator of the number of species, which is a measure of richness, the Shannon diversity, which measures the evenness of the microbial populations, and the Simpson diversity, which measures the dominant species (Marcon and Hérault, 2015; Hsieh et al., 2016). Wilcoxon rank-sum test did not detect any statistical differences in species richness ($P = 0.75$), microbial evenness ($P = 0.48$), and dominant species ($P = 0.55$) of the microbial communities in the two groups (**Figure 2**). Beta diversity analysis also did not reveal the differences between the microbial communities in the two groups, based on PCoA and PERMANOVA test using both weighted and unweighted UniFrac distances ($P > 0.05$) (**Figure 3**). In the case of unweighted UniFrac distance, we observed a statistical trend ($P = 0.08$) that could be indicating a difference between rare microbial communities in female and male buccal cavities.

Differential Abundance of Amplicon Sequence Variants Present in Female and Male Tilapia

The differences in the abundances of ASVs of the buccal cavity samples of female and male Nile tilapia were evaluated employing the Wald-test in DESeq2. The results revealed significant differences between the two groups. The abundance of many opportunistic pathogens such as *Streptococcus, Gemella, Veillonella*, *Kocuria,* and *SR1*, which belong to *Firmicutes*, *Actinobacteria,* and *SR1* was found to be significantly lower in the female buccal cavity compared to that in male tilapia; fold changes ranged between −10 and −25 (**Figure 4**). On the other hand, the abundance of *Acinetobacter* that belongs to the phylum Proteobacteria was five-fold higher in female tilapia. Furthermore, the abundance of *Nitrospira* was nine-fold higher in females (**Figure 4**). Acidobacteria Gp6 and Gp4 had significantly higher abundance (25-fold and five-fold, respectively) in females

TABLE 1 | The proportion (%) of different bacteria in the buccal cavity of female and male Nile tilapia.

Taxa	Female	Male
Phyla		
Proteobacteria	29.95	35.71
Bacteroidetes	10.20	6.64
Firmicutes	0.10	3.47
Deinococcus–Thermus	1.18	3.13
Nitrospirae	0.48	3.13
Actinobacteria	0.67	1.23
Acidobacteria	1.76	0.97
Fusobacteria	0.32	0.30
Genera		
Acinetobacter	28.07	26.19
Turneriella	1.18	0.54
Vogesella	4.57	8.80
Pseudomonas	1.37	3.15
Enhydrobacter	5.01	2.87
Rheinheimera	0.99	4.83
Novosphingobium	1.19	2.98
Streptococcus	0.01	3.02
Chryseobacterium	2.21	2.93

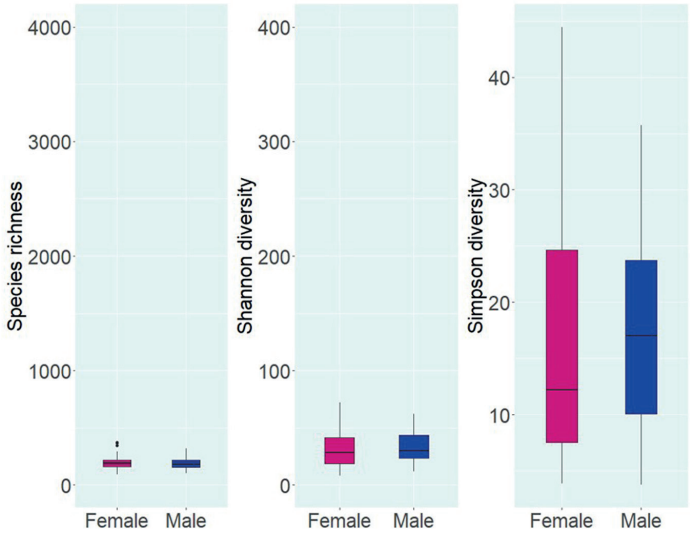

FIGURE 2 | Alpha diversity of the bacteria in the buccal cavity of female and male Nile tilapia. Species richness, Shannon diversity, and Simpson diversity of the groups are not significantly different. The boxplots show minimum, lower quartile, median, upper quartile, and maximum values.

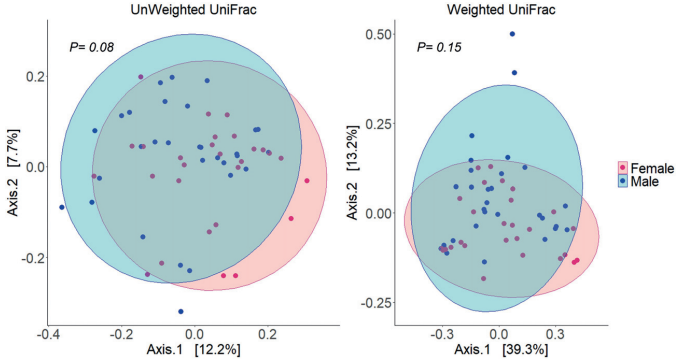

FIGURE 3 | Principal coordinates analyses (PCoA) using distance (Unweighted and weighted UniFrac) matrices of the bacteria in the buccal cavity of female and male Nile tilapia. The ellipses were generated assuming that the data are from a multivariate normal distribution.

compared to males. *Saccharibacteria* was also abundant (10-fold) in females compared to males.

Microbial Network

Microbial networks were generated using the information from the abundance of the 150 dominant ASVs in both females and males. The microbial connections between the nodes in the networks were different in females and males (**Figures 5A,C**). The ASVs in female fish had fewer connections compared to males. These results are evident in the degree histograms (**Figures 5B,D**). Wilcoxon rank-sum test revealed that the node degrees as well as betweenness in female and male fish were significantly different, with a P-value < 0.05. In a network, each node has a degree which refers to the number of connections it has to other nodes. On the other hand, betweenness reveals the ability of a node to act as a bridge along the shortest path. In the present microbial network analysis, the degree of distribution in female fish was lower compared to males (1.73 and 2.96). Similarly, the betweenness in female fish was lower than male fish (75.37 and 329.32). These results indicate that the microbial community in the buccal cavity of male fish has more inter-taxa associations/microbe-microbe interactions compared to that in

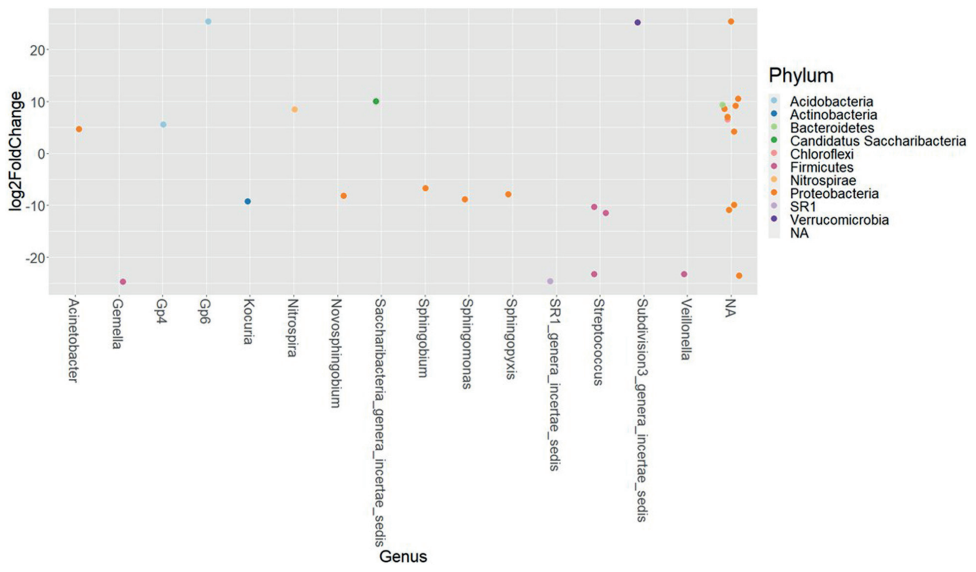

FIGURE 4 | The significantly abundant amplicon sequence variants in the buccal cavity of female Nile tilapia compared to males. The *X*-axis labels are genus-level annotations of the microbes identified in the buccal cavity.

female fish. In addition, *Staphylococcus* and *Streptococcus* had a higher degree and betweenness in the bacterial network of males compared to females.

DISCUSSION

To our knowledge, this is the first study that shows differences in the oral microbiome in female and male Nile tilapia that were reared in a recirculating aquaculture system. The early stages of embryonic development occur in the mouth of females, i.e., until they become hatchlings. Furthermore, fry seek shelter in the mouth of their mothers even after they start feeding on exogenous feeds (Popma and Masser, 1999; Coward and Bromage, 2000). This close association of fry and their maternal mouthbrooders indicate the importance of the oral microbiome in egg development. Moreover, the oral microbiome that is more exposed to the external environment has an indirect connection with the gut microbiome (Olsen and Yamazaki, 2019). Nevertheless, coaggregation of genetically distinct oral bacterial strains are strong, and a previous study has reported the weak interaction between oral and gut bacteria (Ledder et al., 2008). Although there is ample information about the gut microbiome of fishes, very little is known about their oral microbiome.

Bacterial Diversity in the Buccal Cavity of Female and Male Nile Tilapia

Alpha diversity analysis revealed low differences (not significant) in species richness (191 vs. 186) and evenness (28 vs. 29.7) between females and males, while Simpson diversity was 12.2 in females and 17 in males. The non-significant differences that we observed are similar to those described during a Tenacibaculosis outbreak in Atlantic salmon (Wynne et al., 2020). Nevertheless, the authors reported that dysbiosis in the oral microbiome of the fish was due to the dominance of *Tenacibaculum* spp. (Wynne et al., 2020). Furthermore, studies on the oral microbiome of healthy human subjects (Caselli et al., 2020), adolescents suffering from anxiety and depression (Simpson et al., 2020), and patients with esophageal carcinoma (Wang et al., 2019) reported non-significant statistical differences in the microbial composition (Wang et al., 2019; Caselli et al., 2020; Simpson et al., 2020). In the present study, the fishes used were apparently healthy, and our results showed that the microbial composition (based on the proportions) in females and males was different.

Microbial Composition in the Buccal Cavity of Female Nile Tilapia Tilts the Abundance of *Streptococcus*

The most dominant microbial phyla in the buccal cavity were Proteobacteria, Bacteroidetes, Firmicutes, Deinococcus–Thermus, Actinobacteria, and Acidobacteria. These phyla, except Deinococcus–Thermus, were also dominant in the gut and skin of Nile tilapia and Atlantic salmon (Kuebutornye et al., 2020; Sakyi et al., 2020). The presence of these dominant phyla is not affected by factors such as diet, salinity and rearing systems, but their abundances are affected by such environmental parameters (Giatsis et al., 2015; Souza et al., 2020; Yukgehnaish et al., 2020).

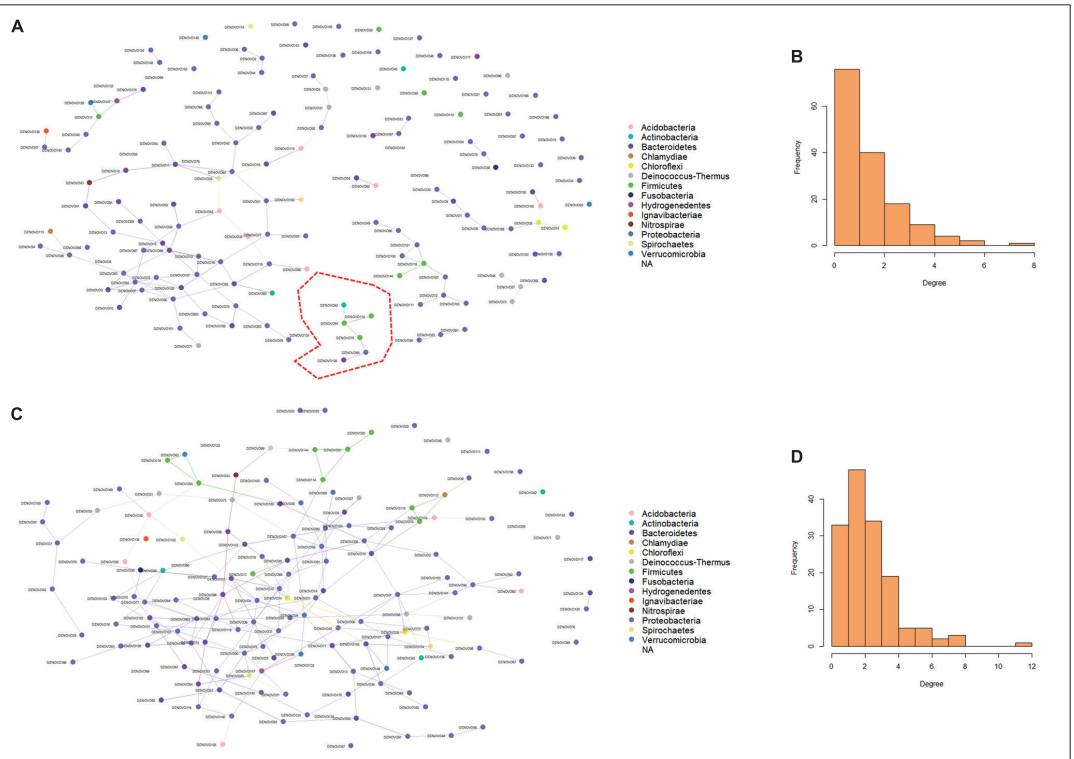

FIGURE 5 | Predicted association of bacteria in the buccal cavity of female and male Nile tilapia. Network of bacteria in the female **(A)** and male **(C)** buccal cavity was constructed using the SpiecEasi package. Each node represents a taxon (ASV) and connections between nodes are shown using edges. The degree distribution in the network of bacteria of the female **(B)** and male **(D)** buccal cavity indicates the high node degree in the males **(D)**. The cluster in red is detailed in **Figure 6**.

Proteobacteria, Bacteroidetes and Firmicutes are the most dominant phyla in the oral microbiome of dolphins and sea lions (Bik et al., 2016). Proteobacteria, Bacteroidetes, Firmicutes and Actinobacteria are also dominant in the buccal cavity of humans (Zaura et al., 2009; Almeida et al., 2020; Caselli et al., 2020). At the genus level, the most abundant bacteria in the human mouth is *Streptococcus*, and in children, there exists a significant negative correlation between the counts of *S. mutans* and secretory IgA (S-IgA), pH and flow rate of saliva (Sood et al., 2014). Furthermore, the abundance of many other opportunistic pathogens such as *Pseudomonas*, *Gemella*, and *Veillonella* was lower in female fish (1.37%) compared to male fish (3.15%). The proportion of bacteria belonging to the genus *Rheinheimera* was lower in female Nile tilapia. Diketopiperazines from *Rheinheimera japonica*, isolated from marine sediments, have been reported to exert antimicrobial activity against *Bacillus subtilis*, *Enterococcus faecium,* and *Staphylococcus aureus* (Kalinovskaya et al., 2017). Furthermore, the diketopiperazine factor in another marine bacterium, *Rheinheimera aquimaris* QSI02 is efficient in controlling quorum sensing systems of *Chromobacterium violaceum* and *Pseudomonas aeruginosa* (Sun et al., 2016). These previous reports indicate the ability of the opportunistic bacteria to suppress the growth of other bacteria and the activity of host defense molecules to regulate the abundance of opportunistic bacteria such as those belonging to *Streptococcus* sp.

Although the abundances of some of the microbes were higher in males compared to females, the analysis did not detect any statistical differences in beta diversity. This finding is also similar to that observed in the human oral microbiome studies (Almeida et al., 2020; Simpson et al., 2020). In our case, we found a statistical trend in the case of the unweighted UniFrac distance, which could be linked to the near absence of *Streptococcus* bacteria in female fish.

We found that many opportunistic pathogens had significantly lower abundance in the female fish, namely *Streptococcus* with about −20 fold-change. *Streptococcus* is abundant in the human buccal cavity, and many commensal bacteria belonging to this genus play a vital role in maintaining the microbiota balance and ensuring human oral cavity health (Zaura et al., 2009; Caselli et al., 2020). Members of this genus are reactive against S-IgA in saliva, and it is known that certain

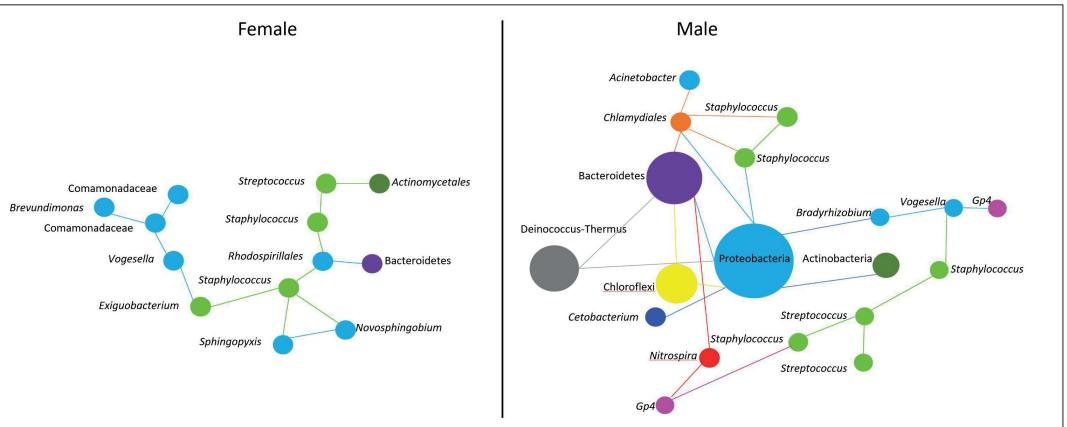

FIGURE 6 | Predicted association of *Streptococcus* and *Staphylococcus* in the buccal cavity of female and male Nile tilapia. In females, we find a single cluster (**Figure 5A**) of microbe-microbe interaction. While in males, microbe interactions are complex (**Figure 5C**). Bacteria belonging to a particular phylum are color coded. The size of the nodes are based on the abundance of the ASVs.

species of *Streptococcus* can cause diseases in the human oral cavity and infections in the respiratory tract (Kilian et al., 1996; Zaura et al., 2009). Streptococcal infection caused by the major bacterial pathogen *Streptococcus* sp. was reported in freshwater fish such as Nile tilapia and marine fish species (Xu et al., 2007; Jantrakajorn et al., 2014), and the disease has caused significant losses in tilapia farming (Xu et al., 2007). However, a study reported that the prevalence of *Streptococcus* sp. was relatively low in nursing Nile tilapia (Jantrakajorn et al., 2014). Interestingly, in the present study, the abundance of *Streptococcus* was much lower (0.01%) in females compared to males (3.02%) (**Supplementary Figure 2**). Similarly, in the oral cavity of pregnant women, the abundance of *Streptococcus* and *Veillonella* was lower compared to non-pregnant women (Lin et al., 2018). Therefore, we speculate that the lower abundance of *Streptococcus* in the buccal cavity of female tilapia could be due to the mouthbrooding nature of this species. Opportunistic pathogenic members of this genus might cause egg mortality. Moreover, Streptococcosis disease can affect any stage of Nile tilapia, and one of the clinical signs is hemorrhage at the base of the mouth (Jantrakajorn et al., 2014).

There were also differences in the abundance of other pathogenic bacteria such as *Gemella* and *Veillonella*. Bacteria belonging to these 3 genera (*Streptococcus*, *Gemella,* and *Veillonella*) form biofilms in the human oral cavity (Zaura et al., 2009; Caselli et al., 2020). Interestingly in the human oral microbiome, coaggregation occurs between genetically distinct bacteria (Kolenbrander, 1988), and in children, metabolic cooperation between *Veillonella* and *Streptococcus* species occurs at the early stage of biofilm formation (Mashima and Nakazawa, 2015; Mutha et al., 2019). Furthermore, *Veillonella* was associated with many human dental diseases such as chronic periodontitis (Mashima and Nakazawa, 2015), and the presence of *Veillonella* can reduce the biofilm formation capacity of certain *Streptococcus* sp. (Mashima and Nakazawa, 2014). *Gemella* and *Streptococcus* species were found in oral plaques of patients without periodontitis (Eberhard et al., 2017), and these microbes are part of the oral microbiota in humans (Welch et al., 2019). In the present study, we found that these microbes are members of the buccal cavity of both females and males, but their abundances were different. Furthermore, the abundance of species belonging to *Streptococcus*, *S. agalactiae* was higher in the intestine of Streptococcus-infected Nile tilapia compared to healthy fish (Silva et al., 2020). Streptococci can produce hydrogen peroxide (H_2O_2), and it is known that while certain oxidative stress-resistant bacteria such as *Rheinheimera* sp. can benefit from H_2O_2 treatment, others like Verrucomicrobia may find it difficult to survive (Piel et al., 2021). In our study, we found that when ASVs of *Streptococcus* had lower abundance in the buccal cavity of female fish, Verrucomicrobia thrived. Another bacterial genus that had higher abundance in female fish was *Acinetobacter*, which is a member of microbiota in healthy human gum area (Costalonga and Herzberg, 2014), and this bacteria can be exploited for beneficial applications because of their ability in biodegradation, to synthesize high molecular weight molecules, and to enhance growth (Adegoke et al., 2012). However, it should be noted that the benefits of *Acinetobacter* are not yet exploited in aquaculture, for example, their ability to produce lipase (Adegoke et al., 2012). A study that investigated the oral bacteria in Atlantic salmon reported that the abundance of *Acinetobacter* was higher in the oral microbiome of yellow mouth disease survivors (Wynne et al., 2020). Bacteria of the genus *Acinetobacter* need low pH and nitrogen (Baumann, 1968), and the higher abundance of *Nitrospira* in female tilapia indicates the presence of nitrogen sources in the mucus of the females. In addition, Acidobacteria that are considered *K*-strategists can thrive in low pH environments (Männistö et al., 2007), and it is presumed that along with

Nitrospira, Acidobacteria contribute to nitrification (Gülay et al., 2019). Hence, the hormonal changes that suppress appetite and reproductive functions during mouthbrooding (Das et al., 2019) could also create a conducive environment for bacteria that feeds on nitrogen. Yet another bacterial genus that had significantly higher abundance in the female Nile tilapia was Saccharibacteria. These bacteria are ultrasmall obligate parasites that lack the ability to synthesize their own amino acids and vitamins. It was reported that bacteria from this phylum parasitize other oral bacteria in humans (Bor et al., 2019; McLean et al., 2020). Furthermore, Saccharibacteria is reported to be a parasite of Actinobacteria, and this association causes slow growth of its host (Bor et al., 2020). Our results showed that the abundance of Saccharibacteria was high in the female buccal cavity, while the abundance of the *Kocuria* which belongs to the phylum Actinobacteria was lower. This could be due to the parasitic activity of Saccharibacteria. *Kocuria* is an opportunistic pathogen that was reported to be the agent of rainbow trout fry syndrome in salmonids (Pękala et al., 2018). Interestingly, *Sphingomonas*, *Sphingobium*, *Novosphingobium*, and *Sphingopyxis* belonging to Sphingomonadaceae that are hydrocarbon degraders (Kertesz and Kawasaki, 2010) had lower abundance, and Saccharibacteria that are organic carbon sinks in hydrocarbon-fueled environments (Figueroa-Gonzalez et al., 2020) and starch degraders (Baker, 2021) had higher abundance in the buccal cavity of female Nile tilapia.

We found that the bacteria in the female buccal cavity with few potential opportunistic pathogens probably create an environment that could likely aid the host in fighting invasive pathogens such as *Streptococcus*; for example, by reducing the biofilm-forming and H_2O_2-producing ability of *Streptococcus*, maintaining a balance between the growth of organic and inorganic compound degraders and lipase producers. This could be a strategy adopted by the parent fish to create a stable egg incubation environment, which eventually would have an effect on the climax community of the juveniles (Krishnan et al., 2017). This climax community may have microorganisms that depend on each other via established cross-feeding strategies or other communication tactics to maintain stability over time (Díaz and Kolenbrander, 2009). However, further investigation is needed to support this hypothesis.

Microbial Networks in the Buccal Cavity of Female Nile Tilapia Disfavor the Abundance of *Streptococcus*

Network analysis has been extensively used by biologists and computer scientists to explore interactions between entities by analyzing nodes and their connections through edges. This approach offers insight into the structure of complex inter-taxa association. In the present study, microbial network analysis was used to identify the inter-taxa association of the communities of the buccal cavities of female and male tilapia. The strong microbe-microbe interaction in male fish and the presence of more opportunists indicate the importance of cautious monitoring for the early detection of disease outbreaks in male tilapia rearing systems. It should be noted that the abundance of opportunistic pathogens is considerably higher in males, and the network analysis also indicated better microbe–microbe interactions. It was reported that opportunistic pathogens are part of the oral microbiome and their low abundance is not usually related to any disease. Nevertheless, the overgrowth of these pathogens might result in dysbiosis, which increases the risk of diseases (Radaic and Kapila, 2021).

As in any other environment, the oral cavity favors microbe-microbe interactions. Early colonizers are biofilm producers and feed on oral glycoproteins and salivary mucins (Radaic and Kapila, 2021). Around 80% of these microbes in the oral cavity of humans are represented by *Streptococcus* species (Velsko et al., 2019; Radaic and Kapila, 2021). There is growing evidence that biofilm-producing bacteria can interact physically and metabolically to form the initial biofilm community (Lamont et al., 2018). A study in lumpfish reported that a high abundance of *Tenacibaculum* on eggs can be an indication of egg mortality (Roalkvam et al., 2019). Hence, the presence of opportunistic bacteria could affect the quality of eggs and eventually the progeny. As stated earlier, *Streptococcus* spp. can produce hydrogen peroxide (H_2O_2), which is sufficient to kill many oral microbes, including *Staphylococcus* spp. (Jakubovics et al., 2008; Janek et al., 2016). However, it has been reported that the majority of *Staphylococcus* spp. in humans are commensal bacteria and can produce antimicrobial compounds known as bacteriocins with widely diverse activity spectra (Janek et al., 2016). *Staphylococcus*-derived bacteriocins can inhibit the action of H_2O_2 from *Streptococcus* spp., thereby limiting the growth of the latter in the human nasal cavity (Janek et al., 2016). The network analysis in the current study showed that *Streptococcus* spp. have a limited microbe-microbe interaction in the female buccal cavity. In the female fish-associated network, there were two *Streptococcus* spp. One (DENOVO91) that interacted with other microbes and another (DENOVO20) that did not. The interaction of *Streptococcus* (DENOVO91) in females was found in a separate cluster away from the main network (**Figure 6**), and in the subset we observed two *Staphylococcus* bacteria interacting with *Streptococcus*. The presence of many *Staphylococcus* ASVs compared to *Streptococcus* may indicate competition between these microbes. In contrast, in males, *Streptococcus* interacted with *Staphylococcus* and many other bacteria in the main network (**Figure 6**). Thus, we found that microbe-microbe interactions were less and the abundance of opportunistic bacteria was lower in the female buccal cavity. This could be due to the mouthbrooding nature of the fish to keep a suitable growth and incubation environment for the eggs.

CONCLUSION

We successfully profiled the microbial communities in the buccal cavity of female and male Nile tilapia. Our results suggest that opportunistic pathogens such as *Streptococcus* are much less abundant in the female buccal cavity compared to male fish. In addition, the abundance of certain bacteria that have metabolic advantages over others was higher in female Nile tilapia. This is the first report that highlights the importance of the presence of

presumed beneficial community in the oral microbiome of female Nile tilapia that are mouthbrooders.

DATA AVAILABILITY STATEMENT

The data used in this study is available at Sequence Read Archive (SRA) with accession no. PRJNA763184.

ETHICS STATEMENT

The animal study was reviewed and approved by the Norwegian Animal Research Authority (FOTS ID 1042).

AUTHOR CONTRIBUTIONS

VK, JF, and YA designed the study. YA carried out the sampling, lab work, and prepared the library, analyzed the data, and wrote the manuscript. CD analyzed the data. ES performed sequencing and data generation. DA involved in initial data analyses. YA, JF, CD, and VK interpreted the data. VK, JF, and CD reviewed and edited the manuscript. All authors approved the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2021.773351/full#supplementary-material

REFERENCES

Adegoke, A. A., Mvuyo, T., and Okoh, A. I. (2012). Ubiquitous *Acinetobacter* species as beneficial commensals but gradually being emboldened with antibiotic resistance genes. *J. Basic Microbiol.* 52, 620–627. doi: 10.1002/jobm.201100323

Albanese, D., Fontana, P., De Filippo, C., Cavalieri, D., and Donati, C. (2015). MICCA: a complete and accurate software for taxonomic profiling of metagenomic data. *Sci. Rep.* 5:9743. doi: 10.1038/srep09743

Almeida, V. D. S. M., Azevedo, J., Leal, H. F., Queiroz, A. T. L., daSilvaFilho, H. P., and Reis, J. N. (2020). Bacterial diversity and prevalence of antibiotic resistance genes in the oral microbiome. *PLoS One* 15:e0239664. doi: 10.1371/journal.pone.0239664

Anderson, M. J. (2001). A new method for non−parametric multivariate analysis of variance. *Austral. Ecol.* 26, 32–46. doi: 10.1111/j.1442-9993.2001.01070.pp.x

Baker, J. L. (2021). Complete genomes of clade G6 *Saccharibacteria* suggest a divergent ecological niche and lifestyle. *mSphere* 6, e0053021–e0053021. doi: 10.1128/mSphere.00530-21

Baumann, P. (1968). Isolation of *Acinetobacter* from soil and water. *J. Bacteriol.* 96, 39–42. doi: 10.1128/jb.96.1.39-42.1968

Bik, E. M., Costello, E. K., Switzer, A. D., Callahan, B. J., Holmes, S. P., Wells, R. S., et al. (2016). Marine mammals harbor unique microbiotas shaped by and yet distinct from the sea. *Nat. Commun.* 7:10516. doi: 10.1038/ncomms10516

Bor, B., Bedree, J. K., Shi, W., McLean, J. S., and He, X. (2019). *Saccharibacteria* (TM7) in the Human oral microbiome. *J. Dent. Res.* 98, 500–509. doi: 10.1177/0022034519831671

Bor, B., Collins, A. J., Murugkar, P. P., Balasubramanian, S., To, T. T., Hendrickson, E. L., et al. (2020). Insights obtained by culturing *Saccharibacteria* with their bacterial hosts. *J. Dent. Res.* 99, 685–694. doi: 10.1177/0022034520905792

Caselli, E., Fabbri, C., D'Accolti, M., Soffritti, I., Bassi, C., Mazzacane, S., et al. (2020). Defining the oral microbiome by whole-genome sequencing and resistome analysis: the complexity of the healthy picture. *BMC Microbiol.* 20:1801. doi: 10.1186/s12866-020-01801-y

Costalonga, M., and Herzberg, M. C. (2014). The oral microbiome and the immunobiology of periodontal disease and caries. *Immunol. Lett.* 162, 22–38. doi: 10.1016/j.imlet.2014.08.017

Coward, K., and Bromage, N. R. (2000). Reproductive physiology of female tilapia broodstock. *Rev. Fish Biol. Fish* 10, 1–25. doi: 10.1023/A:1008942318272

Das, K., Ogawa, S., Kitahashi, T., and Parhar, I. S. (2019). Expression of neuropeptide Y and gonadotropin-releasing hormone gene types in the brain of female Nile tilapia (*Oreochromis niloticus*) during mouthbrooding and food restriction. *Peptides* 112, 67–77. doi: 10.1016/j.peptides.2018.10.009

DeSantis, T., Hugenholtz, P., Keller, K., Brodie, E., Larsen, N., Piceno, Y., et al. (2006). NAST: a multiple sequence alignment server for comparative analysis of 16S rRNA genes. *Nucleic Acids Res.* 34, W394–W399. doi: 10.1093/nar/gkl244

Díaz, P. I., and Kolenbrander, P. E. (2009). Subgingival biofilm communities in health and disease. *Revista Clínica de Periodoncia Implantología y Rehabilitación Oral* 2, 187–192. doi: 10.1016/S0718-5391(09)70033-3

Eberhard, J., Stumpp, N., Winkel, A., Schrimpf, C., Bisdas, T., Orzak, P., et al. (2017). *Streptococcus mitis* and *Gemella haemolysans* were simultaneously found in atherosclerotic and oral plaques of elderly without periodontitis—a pilot study. *Clin. Oral Invest.* 21, 447–452. doi: 10.1007/s00784-016-1811-6

Edgar, R. C. (2016). UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. *bioRxiv* 2016:081257. doi: 10.1101/081257

Edgar, R. C., and Flyvbjerg, H. (2015). Error filtering, pair assembly and error correction for next-generation sequencing reads. *Bioinformatics* 31, 3476–3482. doi: 10.1093/bioinformatics/btv401

Farrell, S., Ide, M., and Wilson, R. F. (2006). The relationship between maternal periodontitis, adverse pregnancy outcome and miscarriage in never smokers. *J. Clin. Periodontol.* 33, 115–120. doi: 10.1111/j.1600-051X.2005.00884.x

Ferretti, P., Pasolli, E., Tett, A., Asnicar, F., Gorfer, V., Fedi, S., et al. (2018). Mother-to-infant microbial transmission from different body sites shapes the

developing infant gut microbiome. *Cell Host Microbe.* 24, 133–145. doi: 10.1016/j.chom.2018.06.005

Figueroa-Gonzalez, P. A., Bornemann, T. L. V., Adam, P. S., Plewka, J., Revesz, F., von Hagen, C. A., et al. (2020). Saccharibacteria as organic carbon sinks in hydrocarbon-fueled communities. *Front. Microbiol.* 11:587782. doi: 10.3389/fmicb.2020.587782

Fujiwara, N., Tsuruda, K., Iwamoto, Y., Kato, F., Odaki, T., Yamane, N., et al. (2017). Significant increase of oral bacteria in the early pregnancy period in Japanese women. *J. Investig. Clin. Dent.* 8:e12189. doi: 10.1111/jicd.12189

Giatsis, C., Sipkema, D., Smidt, H., Heilig, H., Benvenuti, G., Verreth, J., et al. (2015). The impact of rearing environment on the development of gut microbiota in tilapia larvae. *Sci. Rep.* 5:18206. doi: 10.1038/srep18206

Gómez-Márquez, J. L., Peña-Mendoza, B., Salgado-Ugarte, I. H., and Guzmán-Arroyo, M. (2003). Reproductive aspects of *Oreochromis niloticus* (Perciformes: Cichlidae) at coatetelco lake, morelos, mexico. *Rev. Biol. Trop.* 51, 221–228.

Gülay, A., Fowler, S. J., Tatari, K., Thamdrup, B., Albrechtsen, H.-J., Al-Soud, W. A., et al. (2019). DNA- and RNA-SIP reveal *Nitrospira* spp. as key drivers of nitrification in groundwater-fed biofilters. *mBio* 10, e1819–e1870. doi: 10.1128/mBio.01870-19

Hsieh, T., Ma, K., and Chao, A. (2016). iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers). *Methods Ecol. Evol.* 7, 1451–1456. doi: 10.1111/2041-210X.12613

Iq, K. C., and Shu-Chien, A. C. (2011). Proteomics of buccal cavity mucus in female tilapia fish (*Oreochromis* spp.): a comparison between parental and non-parental fish. *PLoS One* 6:e18555. doi: 10.1371/journal.pone.0018555

Jakubovics, N. S., Gill, S. R., Vickerman, M. M., and Kolenbrander, P. E. (2008). Role of hydrogen peroxide in competition and cooperation between *Streptococcus gordonii* and *Actinomyces naeslundii*. *FEMS Microbiol. Ecol.* 66, 637–644. doi: 10.1111/j.1574-6941.2008.00585.x

Janek, D., Zipperer, A., Kulik, A., Krismer, B., and Peschel, A. (2016). High frequency and diversity of antimicrobial activities produced by nasal *Staphylococcus* strains against bacterial competitors. *PLoS Path.* 12:e1005812. doi: 10.1371/journal.ppat.1005812

Jantrakajorn, S., Maisak, H., and Wongtavatchai, J. (2014). Comprehensive investigation of Streptococcosis outbreaks in cultured Nile tilapia. *Oreochromis niloticus*, and red tilapia, *Oreochromis* sp., of Thailand. *J. World Aquacult. Soc.* 45, 392–402. doi: 10.1111/jwas.12131

Kalinovskaya, N. I., Romanenko, L. A., and Kalinovsky, A. I. (2017). Antibacterial low-molecular-weight compounds produced by the marine bacterium *Rheinheimera japonica* KMM 9513T. *Antonie Van Leeuwenhoek* 110, 719–726. doi: 10.1007/s10482-017-0839-1

Kertesz, M. A., and Kawasaki, A. (2010). "Hydrocarbon-degrading *Sphingomonads*: *Sphingomonas*, *Sphingobium*, *Novosphingobium*, and *Sphingopyxis*," in *Handbook of Hydrocarbon and Lipid Microbiology*, ed. K. N. Timmis (Berlin: Springer).

Kilian, M., Reinholdt, J., Lomholt, H., Poulsen, K., and Frandsen, E. V. G. (1996). Biological significance of IgA1 proteases in bacterial colonization and pathogenesis: critical evaluation of experimental evidence. *APMIS* 104, 321–338. doi: 10.1111/j.1699-0463.1996.tb00724.x

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., et al. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res.* 41:808. doi: 10.1093/nar/gks808

Kolenbrander, P. E. (1988). Intergeneric coaggregation among human oral bacteria and ecology of dental plaque. *Annu. Rev. Microbiol.* 42, 627–656. doi: 10.1146/annurev.mi.42.100188.003211

Konstantinidis, I., Sætrom, P., Mjelle, R., Nedoluzhko, A. V., Robledo, D., and Fernandes, J. M. (2020). Major gene expression changes and epigenetic remodelling in Nile tilapia muscle after just one generation of domestication. *Epigenetics* 2020, 1–16. doi: 10.1080/15592294.2020.1748914

Krishnan, K., Chen, T., and Paster, B. J. (2017). A practical guide to the oral microbiome and its relation to health and disease. *Oral Dis.* 23, 276–286. doi: 10.1111/odi.12509

Kuebutornye, F. K. A., Wang, Z., Lu, Y., Abarike, E. D., Sakyi, M. E., Li, Y., et al. (2020). Effects of three host-associated *Bacillus* species on mucosal immunity and gut health of Nile tilapia, *Oreochromis niloticus* and its resistance against *Aeromonas hydrophila* infection. *Fish Shellfish Immunol.* 97, 83–95. doi: 10.1016/j.fsi.2019.12.046

Kurtz, Z. D., Müller, C. L., Miraldi, E. R., Littman, D. R., Blaser, M. J., and Bonneau, R. A. (2015). Sparse and compositionally robust inference of microbial ecological networks. *PLoS Comp. Biol.* 11:e1004226. doi: 10.1371/journal.pcbi.1004226

Lamont, R. J., Koo, H., and Hajishengallis, G. (2018). The oral microbiota: dynamic communities and host interactions. *Nat. Rev. Microbiol.* 16, 745–759. doi: 10.1038/s41579-018-0089-x

Lan, Y., Wang, Q., Cole, J. R., and Rosen, G. L. (2012). Using the RDP classifier to predict taxonomic novelty and reduce the search space for finding novel organisms. *PLoS One* 7:e32491. doi: 10.1371/journal.pone.0032491

Ledder, R. G., Timperley, A. S., Friswell, M. K., Macfarlane, S., and McBain, A. J. (2008). Coaggregation between and among human intestinal and oral bacteria. *FEMS Microbiol. Ecol.* 66, 630–636. doi: 10.1111/j.1574-6941.2008.00525.x

Lin, W., Jiang, W., Hu, X., Gao, L., Ai, D., Pan, H., et al. (2018). Ecological shifts of supragingival microbiota in association with pregnancy. *Front. Cell. Infect. Microbiol.* 8:24. doi: 10.3389/fcimb.2018.00024

Love, M. I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 15:550. doi: 10.1186/s13059-014-0550-8

Lozupone, C., and Knight, R. (2005). UniFrac: a new phylogenetic method for comparing microbial communities. *Appl. Environ. Microbiol.* 71, 8228–8235. doi: 10.1128/AEM.71.12.8228-8235.2005

Maki, K. A., Kazmi, N., Barb, J. J., and Ames, N. (2021). The oral and gut bacterial microbiomes: similarities, differences, and connections. *Biol. Res. Nurs.* 23, 7–20. doi: 10.1177/1099800420941606

Männistö, M. K., Tiirola, M., and Häggblom, M. M. (2007). Bacterial communities in Arctic fjelds of Finnish Lapland are stable but highly pH-dependent. *FEMS Microbiol. Ecol.* 59, 452–465. doi: 10.1111/j.1574-6941.2006.00232.x

Marcon, E., and Hérault, B. (2015). Entropart: An R package to measure and partition diversity. *J. Stat. Softw.* 67, 1–26. doi: 10.18637/jss.v067.i08

Mashima, I., and Nakazawa, F. (2014). The influence of oral *Veillonella* species on biofilms formed by *Streptococcus* species. *Anaerobe* 28, 54–61. doi: 10.1016/j.anaerobe.2014.05.003

Mashima, I., and Nakazawa, F. (2015). The interaction between *Streptococcus* spp. and *Veillonella tobetsuensis* in the early stages of oral biofilm formation. *J. Bacteriol.* 197, 2104–2111. doi: 10.1128/JB.02512-14

McLean, J. S., Bor, B., Kerns, K. A., Liu, Q., To, T. T., Solden, L., et al. (2020). Acquisition and adaptation of ultra-small parasitic reduced genome bacteria to mammalian hosts. *Cell Reports* 32:107939. doi: 10.1016/j.celrep.2020.107939

McMurdie, P. J., and Holmes, S. (2014). Waste not, want not: why rarefying microbiome data is inadmissible. *PLoS Comp. Biol.* 10:3531. doi: 10.1371/journal.pcbi.1003531

Mutha, N. V. R., Mohammed, W. K., Krasnogor, N., Tan, G. Y. A., Wee, W. Y., Li, Y., et al. (2019). Transcriptional profiling of coaggregation interactions between *Streptococcus gordonii* and *Veillonella parvula* by Dual RNA-Seq. *Sci. Rep.* 9:7664. doi: 10.1038/s41598-019-43979-w

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P., O'hara, R., et al. (2013). *Community ecology package. R package version*, 2.0-2. https://cran.r-project.org/web/packages/vegan/index.html.

Olsen, I., and Yamazaki, K. (2019). Can oral bacteria affect the microbiome of the gut? *J. Oral Microbiol.* 11, 1586422–1586422. doi: 10.1080/20002297.2019.1586422

Orlando, T. M., Moraes de Oliveira, M., Paulino, R. R., Costa, A. C., Allaman, I. B., and Rosa, P. V. (2017). Reproductive performance of female Nile tilapia (*Oreochromis niloticus*) fed diets with different digestible energy levels. *Revista Brasileira de Zootecnia* 46, 1–7. doi: 10.1590/S1806-92902017000100001

Pękala, A., Paździor, E., Antychowicz, J., Bernad, A., Głowacka, H., Wiêcek, B., et al. (2018). *Kocuria rhizophila* and *Micrococcus luteus* as emerging opportunist pathogens in brown trout (*Salmo trutta* Linnaeus, 1758) and rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792). *Aquaculture* 486, 285–289. doi: 10.1016/j.aquaculture.2017.12.028

Piel, T., Sandrini, G., Muyzer, G., Brussaard, C. P. D., Slot, P. C., van Herk, M. J., et al. (2021). Resilience of microbial communities after hydrogen peroxide treatment of a eutrophic lake to suppress harmful cyanobacterial blooms. *Microorganisms* 9:1495.

Podgorniak, T., Brockmann, S., Konstantinidis, I., and Fernandes, J. M. (2019). Differences in the fast muscle methylome provide insight into sex-specific epigenetic regulation of growth in Nile tilapia during early stages of domestication. *Epigenetics* 14, 818–836. doi: 10.1080/15592294.2019.1618164

Popma, T., and Masser, M. (1999). *Tilapia life history and biology*. Lexington: University of Kentucky.

Radaic, A., and Kapila, Y. L. (2021). The oralome and its dysbiosis: New insights into oral microbiome-host interactions. *Comput. Struct. Biotechnol* 19, 1335–1360. doi: 10.1016/j.csbj.2021.02.010

Roalkvam, I., Drønen, K., Dahle, H., and Wergeland, H. I. (2019). Microbial communities in a flow-through fish farm for lumpfish (*Cyclopterus lumpus* L.) during healthy rearing conditions. *Front. Microbiol.* 10:1594. doi: 10.3389/fmicb.2019.01594

Rognes, T., Flouri, T., Nichols, B., Quince, C., and Mahé, F. (2016). VSEARCH: a versatile open source tool for metagenomics. *PeerJ* 4:e2584. doi: 10.7717/peerj.2584

Saadaoui, M., Singh, P., and Al Khodor, S. (2021). Oral microbiome and pregnancy: A bidirectional relationship. *J. Reprod. Immunol.* 145:103293. doi: 10.1016/j.jri.2021.103293

Sakyi, M. E., Cai, J., Tang, J., Abarike, E. D., Xia, L., Li, P., et al. (2020). Effects of starvation and subsequent re-feeding on intestinal microbiota, and metabolic responses in Nile tilapia. *Oreochromis niloticus Aquac. Rep*. 17:100370. doi: 10.1016/j.aqrep.2020.100370

Salih, Y., Nasr, A. M., Ahmed, A. B. A., Sharif, M. E., and Adam, I. (2020). Prevalence of and risk factors for periodontal disease among pregnant women in an antenatal care clinic in Khartoum. *Sudan. BMC Res.* 13:147. doi: 10.1186/s13104-020-04998-3

Shoko, A. P., Limbu, S. M., Mrosso, H. D. J., and Mgaya, Y. D. (2015). Reproductive biology of female Nile tilapia *Oreochromis niloticus* (Linnaeus) reared in monoculture and polyculture with African sharptooth catfish *Clarias gariepinus* (Burchell). *SpringerPlus* 4:275. doi: 10.1186/s40064-015-1027-2

Silva, B. R. D. S., Derami, M. S., Paixao, D. A., Persinoti, G. F., DiasdaSilveira, W., and Maluta, R. P. (2020). Comparison between the intestinal microbiome of healthy fish and fish experimentally infected with *Streptococcus agalactiae*. *Aquacult. Res.* 51, 3412–3420. doi: 10.1111/are.14676

Simpson, C. A., Adler, C., du Plessis, M. R., Landau, E. R., Dashper, S. G., Reynolds, E. C., et al. (2020). Oral microbiome composition, but not diversity, is associated with adolescent anxiety and depression symptoms. *Physiol. Behav.* 226:113126. doi: 10.1016/j.physbeh.2020.113126

Sin, Y. M., Ling, K. H., and Lam, T. J. (1994). Passive transfer of protective immunity against ichthyophthiriasis from vaccinated mother to fry in tilapias, *Oreochromis aureus*. *Aquaculture* 120, 229–237. doi: 10.1016/0044-8486(94)90081-7

Sood, L. I., Al-Ezzy, M. Y., and Diajil, A. R. (2014). Correlation between *Streptococci mutans* and salivary IgA in relation to some oral parameters in saliva of children. *J. Baghdad Coll. Dent.* 26, 71–79. doi: 10.12816/0015168

Souza, F. P. D., Lima, E. C. S., Urrea-Rojas, A. M., Suphoronski, S. A., Facimoto, C. T., Bezerra Junior, J. D. S., et al. (2020). Effects of dietary supplementation with a microalga (*Schizochytrium* sp.) on the hemato-immunological, and intestinal histological parameters and gut microbiota of Nile tilapia in net cages. *PLoS One* 15:e0226977. doi: 10.1371/journal.pone.0226977

Su, S.-C., Chang, L.-C., Huang, H.-D., Peng, C.-Y., Chuang, C.-Y., Chen, Y.-T., et al. (2020). Oral microbial dysbiosis and its performance in predicting oral cancer. *Carcinogenesis* 42, 127–135. doi: 10.1093/carcin/bgaa062

Subasinghe, R. P. (1993). Effects of immunosuppression on protection of fry from *Ichthyophthirius multifiliis* Fouquet 1876 during mouthbrooding of *Oreochromis mossambicus* (Peters). *Fish Shellfish Immunol.* 3, 97–105. doi: 10.1006/fsim.1993.1011

Sun, S., Dai, X., Sun, J., Bu, X., Weng, C., Li, H., et al. (2016). A diketopiperazine factor from *Rheinheimera aquimaris* QSI02 exhibits anti-quorum sensing activity. *Sci. Rep.* 6:39637. doi: 10.1038/srep39637

Sylvain, F. E., and Derome, N. (2017). Vertically and horizontally transmitted microbial symbionts shape the gut microbiota ontogenesis of a skin-mucus feeding discus fish progeny. *Sci. Rep.* 7:5263. doi: 10.1038/s41598-017-05662-w

Velsko, I. M., Fellows Yates, J. A., Aron, F., Hagan, R. W., Frantz, L. A. F., Loe, L., et al. (2019). Microbial differences between dental plaque and historic dental calculus are related to oral biofilm maturation stage. *Microbiome* 7:102. doi: 10.1186/s40168-019-0717-3

Wang, Q., Rao, Y., Guo, X., Liu, N., Liu, S., Wen, P., et al. (2019). Oral microbiome in patients with oesophageal squamous cell carcinoma. *Sci. Rep.* 9:19055. doi: 10.1038/s41598-019-55667-w

Weiss, S., Xu, Z. Z., Peddada, S., Amir, A., Bittinger, K., Gonzalez, A., et al. (2017). Normalization and microbial differential abundance strategies depend upon data characteristics. *Microbiome* 5:27. doi: 10.1186/s40168-017-0237-y

Welch, J. L. M., Dewhirst, F. E., and Borisy, G. G. (2019). Biogeography of the oral microbiome: the site-specialist hypothesis. *Annu. Rev. Microbiol.* 73, 335–358. doi: 10.1146/annurev-micro-090817-062503

Wynne, J. W., Thakur, K. K., Slinger, J., Samsing, F., Milligan, B., Powell, J. F. F., et al. (2020). Microbiome profiling reveals a microbial dysbiosis during a natural outbreak of Tenacibaculosis (Yellow Mouth) in Atlantic salmon. *Front. Microbiol.* 11:586387. doi: 10.3389/fmicb.2020.586387

Xu, D.-H., Shoemaker, C. A., and Klesius, P. H. (2007). Evaluation of the link between gyrodactylosis and streptococcosis of Nile tilapia, *Oreochromis niloticus* (L.). *J. Fish Dis.* 30, 233–238. doi: 10.1111/j.1365-2761.2007.00806.x

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

Zaura, E., Keijser, B. J. F., Huse, S. M., and Crielaard, W. (2009). Defining the healthy "core microbiome" of oral microbial communities. *BMC Microbiol.* 9:259. doi: 10.1186/1471-2180-9-259

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Paper II

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Breeding Strategy Shapes the Composition of Bacterial Communities in Female Nile Tilapia Reared in a Recirculating Aquaculture System

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In industrial animal production, breeding strategies are essential to produce offspring of better quality and vitality. It is also known that host microbiome has a bearing on its health. Here, we report for the first time the influence of crossbreeding strategy, inbreeding or outbreeding, on the buccal and intestinal bacterial communities in female Nile tilapia (*Oreochromis niloticus*). Crossbreeding was performed within a family and between different fish families to obtain the inbred and outbred study groups, respectively. The genetic relationship and structure analysis revealed significant genetic differentiation between the inbred and outbred groups. We also employed a 16S rRNA gene sequencing technique to understand the significant differences between the diversities of the bacterial communities of the inbred and outbred groups. The core microbiota composition in the mouth and the intestine was not affected by the crossbreeding strategy but their abundance varied between the two groups. Furthermore, opportunistic bacteria were abundant in the buccal cavity and intestine of the outbred group, whereas beneficial bacteria were abundant in the intestine of the inbred group. The present study indicates that crossbreeding can influence the abundance of beneficial bacteria, core microbiome and the inter-individual variation in the microbiome.

Keywords: breeding, Nile tilapia, microbiome, 16S amplicon, whole-genome sequencing, core microbiome

INTRODUCTION

Animals are bred for food, fibers, transport, protection, company as well as for other purposes such as scientific research (Flint and Woolliams, 2008). Domestication of different animals, mainly livestock species started several years ago and presently crossbreeding programs are essential tools to improve the productivity, efficiency, and sustainability of domesticated animals (Hill, 2014, 2016). Initially, livestock were selected based on desired phenotypic traits. Over the past 50 years, there has been a remarkable increase

in livestock production due to the improvement in breeding practices and better understanding of genetics. Genetics plays an important role in modern breeding programs, which combine basic breeding concepts and emerging technologies (Schultz et al., 2020).

Crossbreeding of farmed animals and agricultural plants is well-established compared to those of farmed aquatic animals (D'ambrosio et al., 2019; Gratacap et al., 2019). However, the production of fish based on crossbreeding programs is expected to increase as the farming of fish such as Nile tilapia (*Oreochromis niloticus*) and Atlantic salmon (*Salmo salar*) is expanding rapidly (Gjedrem et al., 2012; Gjedrem and Rye, 2018; Mehar et al., 2019). Several strategies such as selective breeding have been implemented to increase the production of fast-growing fish species and their disease resistance (Lind et al., 2012; Ina-Salwany et al., 2019). Nevertheless, outbreak of many diseases such as Tenacibaculosis (yellow mouth), Streptococcosis and Vibriosis has led to high mortality in fish farms and the industry has suffered huge economic losses (Jantrakajorn et al., 2014; Ina-Salwany et al., 2019; Wynne et al., 2020). The industry has hardly taken steps to selectively breed fishes in order to shape the microbiota as an indicator of health. It has been reported that selective breeding can produce fishes with microbiota that can be manipulated to improve disease resistance (Piazzon et al., 2020).

Currently, there are many genetically improved tilapia and GIFT (Genetically Improved Farmed Tilapia) is the most known breed. Although many studies have employed genetically improved tilapia (Bolivar and Newkirk, 2002; Romana-Eguia et al., 2005; Santos et al., 2013; Mehar et al., 2019), to our knowledge there are only a couple of reports about the microbiome composition in selectively bred fish (Kokou et al., 2018; Brown et al., 2019). In mouse, selective breeding is known to increase the inter-individual gut microbiota similarity (Pang et al., 2012); variation is less in the case of inbred animals compared to their outbred counterparts (Hufeldt et al., 2010). Researchers have also succeeded in producing outbred mice with stable gut microbiota (Hart et al., 2018). Furthermore, the association between the gut microbiome and breeding was studied in mouse models by analysing the effect of the gut microbiome on different breeds (Pang et al., 2012; Kreisinger et al., 2014; Ericsson et al., 2015; Oriá et al., 2018). This link was also explored in plants by examining the impact of the microorganisms on host phenotype (Wagner et al., 2020). Moreover, the microbial taxa that is widespread among the host population is vertically transmitted, and host factors provide them with the optimum ecosystem for colonization (Risely, 2020).

Selective breeding affects host genetic selection, which in turn shapes the gut microbiome (Kokou et al., 2018) that has an important role in, among others, maintaining the host health. The paucity of information regarding the mating strategy-caused changes in fish microbiome that can signal disease propensity led us to examine the differences in the bacteria associated with inbred and outbred Nile tilapia using next-generation sequencing technology.

MATERIALS AND METHODS

Fish Husbandry and Sample Collection

Fertilized eggs ($n = 180$) of Nile tilapia, were obtained from wild fish captured from the Nile river, Luxor, Egypt (location GPS: 25°39'56" N, 32°37'07" E). These eggs were disinfected with hydrogen peroxide for 10 min and placed in egg rockers (Cobalt Aquatics, Rock Hill, South Carolina, United States) installed in a 60 L tank with UV treated water, containing 5% NaCl. Around 85% of the eggs were hatched at 28°C within 4 days. The hatched larvae were placed in fish transport bags filled with UV treated and 100% oxygen saturated water. These larvae were shipped, within approximately 18 h, to the Research Station of Nord University, Bodø, Norway *via* air and their survival rate exceeded 95%. The transported larvae were reared at a maximum density of 27 fish/m^3 for 5 months in a freshwater recirculating system. The rearing conditions were: dissolved oxygen – 100%, water temperature –28°C, photoperiod – LD 13:11. The fish were fed Amber Neptun pellets (0.15–0.8 mm, Skretting, Stavanger, Norway) during the rearing period. These fish were designated as the F0 generation and were used for the breeding study.

We randomly chose males and females and produced the inbred and outbred groups. When the fish reached 3,570 degree·days, we anesthetized and PIT-tagged them for tracing the individual families.

Prior to sampling, fish were not fed for 48 h. They were sacrificed by immersion in an emulsion containing 12 ml of clove oil (Sigma Aldrich, St. Louis, Missouri, United States), 96% ethanol (1:10 v/v) and 10 L of water (Simões et al., 2011; Konstantinidis et al., 2020). Female fish were used for the study as they are maternal mouthbrooders. Twenty fish each from the inbred and outbred groups were used in this study, and three body sites (mouth, anterior and posterior intestine) of female Nile tilapia were targeted for examining the bacterial communities. Mucus samples from the buccal cavity were taken using swabs (Copan Italia, Brescia, Italy), which were transferred to cryotubes and immediately frozen in liquid nitrogen. Then, the same fish were aseptically dissected to collect the anterior and posterior intestine. The intestine samples were also transferred to cryotubes and snap-frozen in liquid nitrogen. The collected samples were stored at −80°C until further use.

DNA Extraction for Whole-Genome Sequencing

DNA was extracted from fast muscle using DNeasy Blood and Tissue Kit based on the guidelines provided by the manufacturer (Qiagen, Hilden, Germany). The Invitrogen Qubit 3.0 fluorometer (ThermoFisher Scientific, Waltham, Massachusetts, United States) was used to quantify the concentration of DNA in the samples. Quality (based on 260/280 and 260/230 absorbance ratios) and integrity (based on DIN values) of the extracted DNA samples were checked using Nanodrop 1000 Spectrophotometer (ThermoFisher Scientific) and TapeStation 2200 DNA screen (Agilent Technologies, Santa Clara, California, United States), respectively.

DNA Extraction for 16S Amplicon Analysis

All the procedures mentioned here were performed under sterile conditions. Before extracting the DNA, intestine samples were transferred to a sterile Petri dish and placed on a cool-pack on dry ice. The intestine was opened and transferred to a 5 ml tube containing 1.4 mm Zirconium oxide beads (Cayman Chemical, Ann Arbor, Michigan, United States) and 2 ml of InhibitEX buffer (Qiagen). Thereafter, DNA was extracted immediately using QIAamp DNA stool Mini Kit (Qiagen) according to the manufacturer's protocol. The final elution volume was 75 µl (ATE buffer). The same extraction method was employed for the mouth samples. The quality and quantity of the extracted DNA were checked with NanoDrop spectrophotometer ND-8000 (ThermoFisher Scientific).

Libraries Preparation and Sequencing

Whole-Genome Sequences

The Nextera DNA Flex library preparation kit with dual indices was used to prepare whole genome libraries based on the manufacturer's protocol (Illumina, San Diego, California, United States). After tagmentation of the extracted gDNA samples using Bead-linked transposomes at 55°C for 15 min, the sheared and tagmented gDNA was washed at 30°C for 15 min. Amplification of the tagmented gDNA was performed using a 5-cycle PCR programme wherein the index 1 (i7) and index 2 (i5) adapters were added for sequencing cluster formation. The PCR program was started with an incubation at 68°C for 3 min and a subsequent pre-denaturation at 98°C for 3 min. In the following step, 5 cycles of denaturation at 98°C for 45 s, annealing at 62°C for 30 s and extension at 68°C for 2 min were first performed, followed by a final extension at 68°C for 1 min. In the final step of the library preparation, the amplified libraries were purified through a double-sided bead (Bead-linked transposome; Illumina) purification procedure. The quality and normality of the libraries were assessed with the Agilent Tapestation instrument using High Sensitivity D1000 screen tape. After normalization based on the minimum observed molarity, the barcoded samples were pooled before the sequencing run. The 75 bp paired-end sequencing was done on a NextSeq 500 sequencer (Illumina) at the sequencing platform of Nord University.

Bacterial 16S Sequences

Under sterile conditions, 16S rRNA gene libraries were constructed from DNA extracts using the specific bacterial primers 341F (5'CCTACGGGNGGCWGCAG 3') and 805R (5'GACTACNVGGGTWTCTAATCC 3'; Klindworth et al., 2013) flanked by overhang Illumina adapters targeting the hypervariable V3–V4 region (~460 bp). PCR reactions were performed for each sample in 25 µl, using Q5® High-Fidelity 2X Master Mix (New England Biolabs, Ipswich, Massachusetts, United States) and 2.5 µl of DNA template (5 ng/µl). PCR conditions consisted of an initial denaturation step at 95°C for 10 min (1 cycle), 30 cycles at 95°C for 30 s, 57°C for 30 s, 72°C for 1 min, and a final extension step at 72°C for 7 min (1 cycle).

An agarose gel (1.5%) was employed to check the amplified products. The PCR products were purified using the CleanNGS system (CleanNA, Waddinxveen, Netherlands) following the manufacturer's instructions. The purified product was subjected to a second PCR (8 cycles, 16S Metagenomic Sequencing Library Preparation, Illumina); this step was done to add dual indices and Illumina sequencing adapters Nextera XT Index Primer (Illumina). CleanNGS (CleanNA) was used to purify the obtained amplicon libraries. The quality of the libraries was checked on a Tapestation 2200 platform (Agilent Technologies). Thereafter, the libraries were quantified using the Quant-IT PicoGreen dsDNA assay kit (ThermoFisher Scientific) by the Synergy2 microplate reader (Biotek, Winooski, Vermont, United States). Next, the pooled libraries were quantified using the KAPA Library quantification kit (Roche, Basel, Switzerland). The libraries were checked by realtime qPCR LightCycler 480 (Roche) and then sequenced on an Illumina® MiSeq (PE300) platform (MiSeq Control Software 2.5.0.5 and Real-Time Analysis software 1.18.54.0).

Sequence Analysis

Whole-Genome Sequences

In order to perform demultiplexing and obtain the fastq files, the Illumina Experiment Manager v1.18.1 along with bcl2fastq v2.20.0.422 was used. Thereafter, dual adapter indexes and Ns from the 3' end of the raw reads were trimmed and the quality of the cleaned fastq files was assessed employing Trime_galore v0.4.4 (Babraham Bioinformatics; http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/). The clean reads were then aligned to the reference genome O_niloticus_UMD_NMBU, GCA_001858045.3 (Conte et al., 2017) using Bowtie2 v0.12.8 with the --very-sensitive option (Langmead and Salzberg, 2012). The bcftools pipeline was applied for variant calling (Li, 2011), and the generated SAM files were converted to the binary format and sorted based on coordinates using samtools v1.9. Also, the samtools markedup command was used to mark duplicate reads. Then variants were called using bcftools mpileup command (bcftools 1.9) with the minimum base and mapping quality of 20 (−q 20 −Q 20). Using bcftools filter command accompanied by the options --SnpGap 5 -i 'MQ > 20 and QUAL>20 and DP > 100 and DP < 450 and TYPE = "snp," only SNP variants were kept in the Variant Call Format (VCF). The missing genotypes were imputed using imp-states = 1,600 option in Beagle v5.0 (Browning and Browning, 2016). Thereafter, using vcftools, the non-biallelic SNP variants were omitted so that the generated VCF file had only the biallelic SNPs (Danecek et al., 2011). This VCF file was read by vcfR package (Knaus and Grünwald, 2017).

Bacterial 16S Amplicon Sequences

The generated reads were truncated at 270 bp using VSEARCH (Rognes et al., 2016), and then processed using MICCA pipeline (v1.7.2; Albanese et al., 2015). Sequences with a minimum overlap length of 60 bp and a maximum mismatch of 20 bp were merged. Next, the forward and reverse primers were trimmed off the merged reads and reads which did not contain

the primers were discarded. Thereafter, the sequences with an expected error rate (Edgar and Flyvbjerg, 2015) >0.75 were filtered out and shorter than 400 bp sequences were discarded. Filtered reads were denoised using the "*de novo* unoise" method implemented in MICCA, which utilise UNOISE3 algorithm (Edgar, 2016). The denoising method generates amplicon sequence variants (ASVs) which is based on correcting sequencing errors and determining true biological sequences at single-nucleotide resolution. The taxonomic assignment of the representative bacterial ASVs was performed using RDP classifier. The sequences were aligned using the NAST (Desantis et al., 2006) multiple sequence aligner, and a phylogenetic tree was prepared using the FastTree software available in the MICCA pipeline.

Statistical Analysis of Host Genetic Data

To quantify the genetic diversity of the inbred and outbred groups, we first determined the genetic diversity within members of the crossbred groups, and then the between groups genetic diversity. For this, we quantified the level of heterozygosity, using the population package of the Stacks 2.3b. Next, to assess the level of genetic differentiation based on allele frequencies between different groups, the F_{st} index was calculated using the StAMPP package (Pembleton et al., 2013). In order to quantify the genetic relationship between the inbred and outbred groups, Nei-based genetic distance between individuals was estimated using poppr (Kamvar et al., 2015) and adegenet (Jombart, 2008) packages and visualized using pheatmap package (Kolde and Kolde, 2015). Then the genetic relationship between the crossbred groups was assessed by PCoA (employing the abovementioned Nei-based genetic distance), also using the ape package (Paradis and Schliep, 2019). PERMANOVA (Permutational Multivariate Analysis of Variance) was performed to decipher the significance of genetic differences between the inbred and outbred groups. To further analyze the population structure of the inbred and outbred groups, admixture analysis was performed in adegenet for values of ancestries (K) from 1 to 10 with 10 repeats for each value of K, decided based on Bayesian Information Criteria. Four samples were removed due to the low quality of sequences.

Statistical Analysis of 16S Amplicon Data

Statistical analysis was conducted using R (version 3.6.3) software. The packages phyloseq (McMurdie and Holmes, 2013) and vegan (Oksanen et al., 2013) were employed to analyse the data. All plots were made using ggplot2 package (Wickham, 2011).

To understand the differences between the proportions of different bacteria in the inbred and outbred groups, we performed chi-square test and the associated *post hoc* analyses. A subset of the most dominant phyla was employed for this analysis. The similarities/differences in α-diversity were checked by Wilcoxon rank-sum test. Bacterial β-diversity was determined using unweighted and weighted UniFrac distances (Lozupone and Knight, 2005). Differences between the bacterial communities of the two groups were visualized by PCoA. After checking the dispersions within the data set of each group, statistically significant differences between the groups were assessed using PERMANOVA (Anderson, 2001; with 9,999 permutations), implemented in adonis function of the vegan R-package (Oksanen et al., 2013). DESeq2 (Love et al., 2014) package was employed to detect the differentially abundant ASVs in the non-rarefied data (McMurdie and Holmes, 2014). The core microbiota was analysed using the packages microbiome and microbiome utilities; at a detection level of 0.2% and prevalence level of 90%. The differences in the core bacterial community in the two crossbred groups were analysed by performing PERMANOVA on weighted and unweighted UniFrac distances. The abundances in the different ASVs which made up the core microbiome were analyzed using Spearman test (Zar, 2014) and correlation plot package (Wei and Simko, 2017).

RESULTS

Genetic Background-Associated Changes in the Microbiome of Nile Tilapia

A total of 11,578,530 SNPs were obtained after the initial SNP calling. Bcftools was employed to first calculate genotype likelihoods for each position and then filter out every position with actual sequence variant. Thus, 4,693,720 SNPs were filtered out and finally after biallelic filtration, 6,825,083 SNP variants with an average coverage of 1.74 per sample were used in the final VCF file.

The genetic diversity analysis based on nucleotide sequences revealed that the observed heterozygosity (Ho) values were slightly higher compared to the expected heterozygosity values (He; **Table 1**).

The fixation index (F_{st}) value within groups was 0.04 for both Inbred-S1 vs. Inbred-C6 and Outbred-S3 vs. Outbred-C9 comparisons. On the other hand, the F_{st} values between crossbred groups were in the range 0.06–0.08 (**Table 2**).

The Nei-based genetic distances between the inbred and outbred groups were employed to generate a heatmap to understand their genetic relationships; differences between the

TABLE 1 | Observed (Ho) and expected (He) heterozygosity of the crossbred female Nile tilapia.

	Ho	*He*
Outbred-S3	0.171	0.157
Inbred-S1	0.164	0.153
Inbred-C6	0.167	0.155
Outbred-C9	0.166	0.149

TABLE 2 | Genetic differentiation, based on *Fst* index, of the crossbred female Nile tilapia.

	Outbred-S3	Inbred-S1	Inbred-C6	Outbred-C9
Outbred-S3	-			
Inbred-S1	0.061	-		
Inbred-C6	0.077	0.041	-	
Outbred-C9	0.044	0.058	0.074	-

groups are seen in **Supplementary Figure 1**. Principal Coordinates Analysis (PCoA) based on the Nei-based genetic distance indicated that the first two components captured 17.7 and 7.8% of the variation in the data set (**Figure 1A**). Furthermore, a PERMANOVA test based on the same genetic distance showed that the inbred and outbred groups were significantly different ($p = 0.001$). The genetic sub-population clustering based on admixture analysis revealed that K = 2 was the optimal number to explain the genetic structure of the inbred and outbred groups (**Supplementary Figure 2**). The results also indicated that 4 inbred individuals are genetically similar to the outbred population.

To delineate the effect of genetic selection on gut microbiota composition, the inbred and outbred Nile tilapia were reared in a common garden and the environmental and nutritional factors that affect the microbiota were kept constant throughout the experimental period. The amplicon library of 16S rRNA gene, generated 12,034,190 high-quality reads with an average coverage of 54,016 reads per sample. Due to the variation in sample size, the reads were rarefied to 18,000 reads per sample (without replacement). Out of the 120 samples, six libraries with a number of reads below the cut off were discarded. After normalization we obtained 14,228 ASVs, distributed among 30 phyla and 695 genera.

First, we investigated the dominant communities in the two groups. In their order of dominance, the most dominant bacterial phyla in both the inbred and outbred fish groups were *Proteobacteria*, *Fusobacteria*, *Firmicutes*, *Bacteroidetes*, and *Actinobacteria* (**Figure 1B**). This order of dominance was reflected in the microbial composition at the genus level also. Most of the dominant genera in the two crossbred groups belonged to the phylum *Proteobacteria* (*Acinetobacter*,

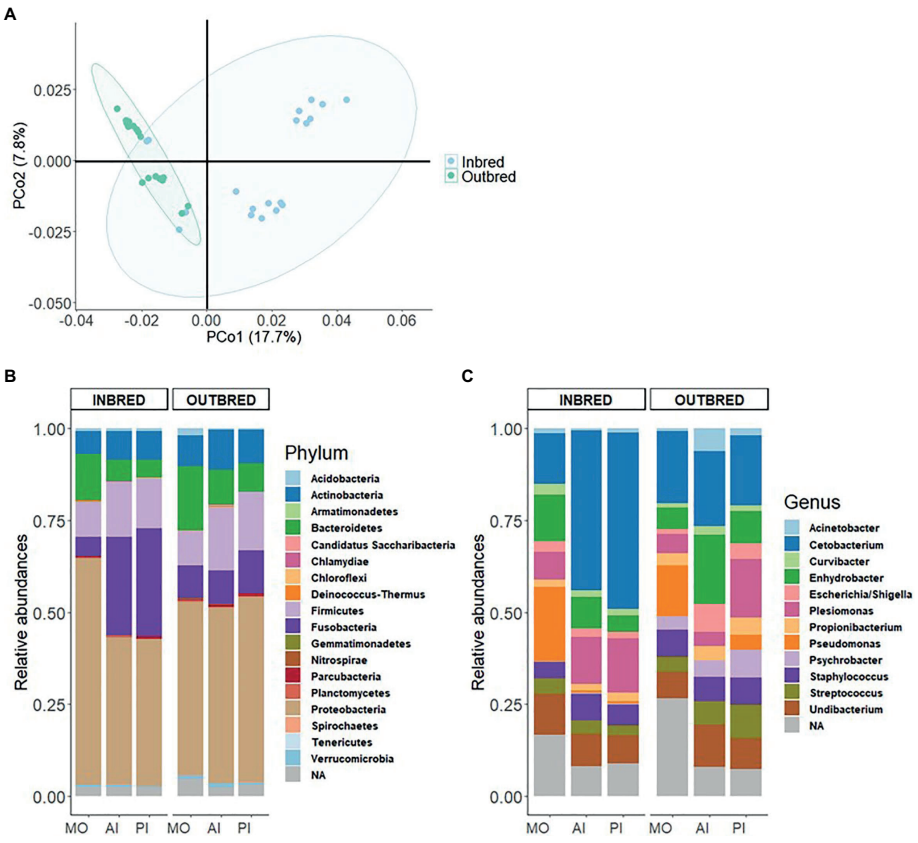

FIGURE 1 | Genetic differentiation and microbiome in the inbred and outbred groups of Nile tilapia. **(A)** Principal coordinates analysis (PCoA) plot based on 6,825,083 SNPs of the inbred and outbred groups. The ellipses were generated assuming that the data are from a multivariate normal distribution. **(B)** Phylum-level relative abundance of the microbial composition in the inbred and outbred groups. **(C)** Relative abundance of top 12 genera in the inbred and outbred groups.

Curvibacter, *Enhydrobacter*, *Escherichia/Shigella*, *Plesiomonas*, *Pseudomonas*, *Psychrobacter*, and *Undibacterium*). The most abundant genus was *Cetobacterium* which belongs to the phylum *Fusobacteria* (**Figure 1C**).

To understand the differences in proportions of the dominant communities in each study group, we performed chi-square test. The analyses revealed that the abundances of the most dominant phyla in both the inbred and outbred groups were significantly different (**Supplementary Table 1**).

To characterize the microbial diversity within the samples, we calculated three ecological indexes, namely the Chao1 estimator of the number of species, which is a measure of richness, the Shannon diversity which measures the evenness of the microbial populations and the Simpson diversity, which measures the importance of dominant species (Marcon and Hérault, 2015; Hsieh et al., 2016). Shannon diversity analysis showed that the microbial diversity in the mouth of the inbred group was lower compared to the outbred group (**Figure 2**, $p = 0.01$). The Simpson diversity analysis indicated that there were fewer dominant ASVs in the posterior intestine of the inbred group (**Figure 3**, $p = 0.04$). Although there were no significant differences in species richness of the communities associated with the two groups, in each body site (**Supplementary Table 2**), there was an increasing trend ($p = 0.08$; inbred higher richness) in the case of the anterior intestine (**Figure 4**). Furthermore, the diversity analysis of dominant bacteria (Simpson diversity) in the mouth and anterior intestine revealed a trend in differences ($p = 0.08$ and 0.06, respectively; **Figures 2, 4**).

Beta diversity analysis was performed to evaluate the overall dissimilarity between the two crossbred groups (**Figure 5**). The results of PERMANOVA on the unweighted UniFrac distances showed a significant difference between the bacterial composition in the posterior intestine of the inbred and outbred groups ($p = 0.003$). There was no significant difference between the communities in the mouth or the anterior intestine of the two groups ($p = 0.082$ and 0.311, respectively). In the mouth, there may exist a difference in composition between the two groups, based on the observed trend (**Table 3**).

Considering the weighted UniFrac distance, there was a significant difference in the community composition of the anterior intestine ($p = 0.001$). In addition, there was a significant difference in the community of posterior intestine ($p = 0.003$), but not in the case of mouth ($p = 0.37$; **Table 3**).

Differential Abundance of ASVs: Outbred Group vs. Inbred Group

The package DESeq2 was used to identify the ASVs with a significantly different abundance in the outbred group compared to the inbred group. In the mouth, the bacteria belonging to *Actinobacteria*, *Armatimonadetes*, *Firmicutes*, and *Proteobacteria* were differentially abundant. There were six genera that belonged to the phylum *Proteobacteria*. Bacteria belonging to two genera (*Psychrobacter* and *Polaromonas*) were 5-fold higher in the outbred group, while those of *Pseudomonas* and *Acinetobacter* were 20-fold higher in the same group. Furthermore, an ASV of the genus *Limnohabitans* was about 9-fold lower and *Comamonas* was 20-fold lower in the outbred group. *Lachnospiracea_incertae_sedis* were about 5-fold higher in the outbred group, whereas the genus *Bacillus* was 20-fold lower. These two genera belong to the phylum *Firmicutes*. Also, *Armatimonadetes_gp5* was 20-fold lower in the outbred group (**Supplementary Figure 3**). In the anterior intestine, the majority of the ASVs that were differentially abundant in the outbred group had fold changes between −5 and −15

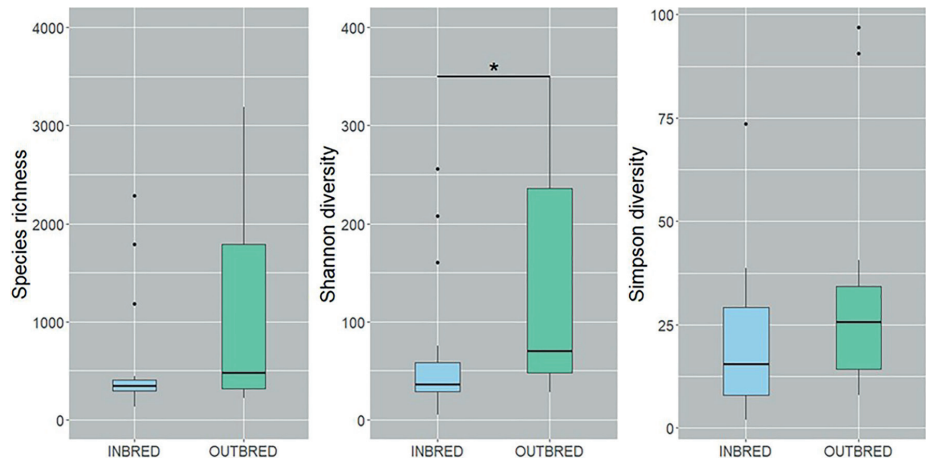

FIGURE 2 | Alpha diversity of the bacteria in the mouth of the inbred and outbred groups of Nile tilapia. Species richness of the groups is not significantly different. Shannon diversity is higher in the outbred group ($p = 0.007$, indicated with an asterisk). Simpson diversity indicated an increasing trend in the dominant ASVs of the outbred group ($p = 0.08$). The boxplots show minimum, lower quartile, median, upper quartile and maximum values.

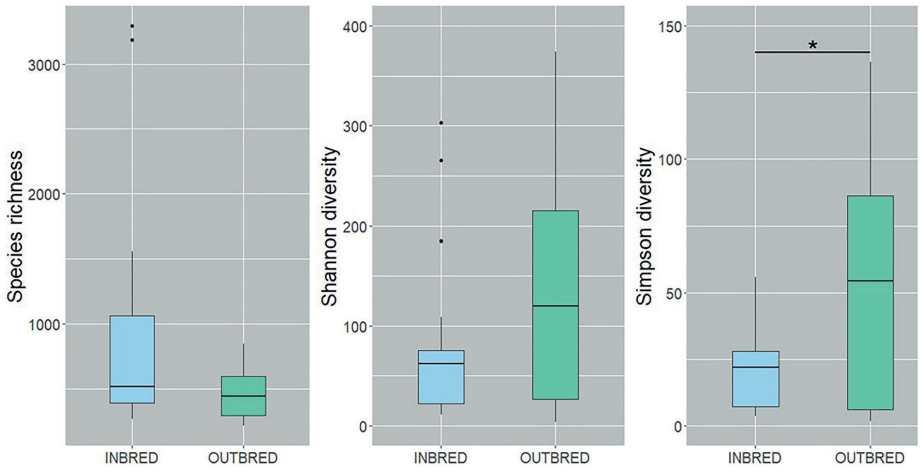

FIGURE 3 | Alpha diversity of the bacteria in the posterior intestine of the inbred and outbred groups of Nile tilapia. Simpson diversity analysis showed that the dominant ASVs are higher in the outbred groups ($p = 0.04$, indicated with an asterisk). The boxplots show minimum, lower quartile, median, upper quartile and maximum values.

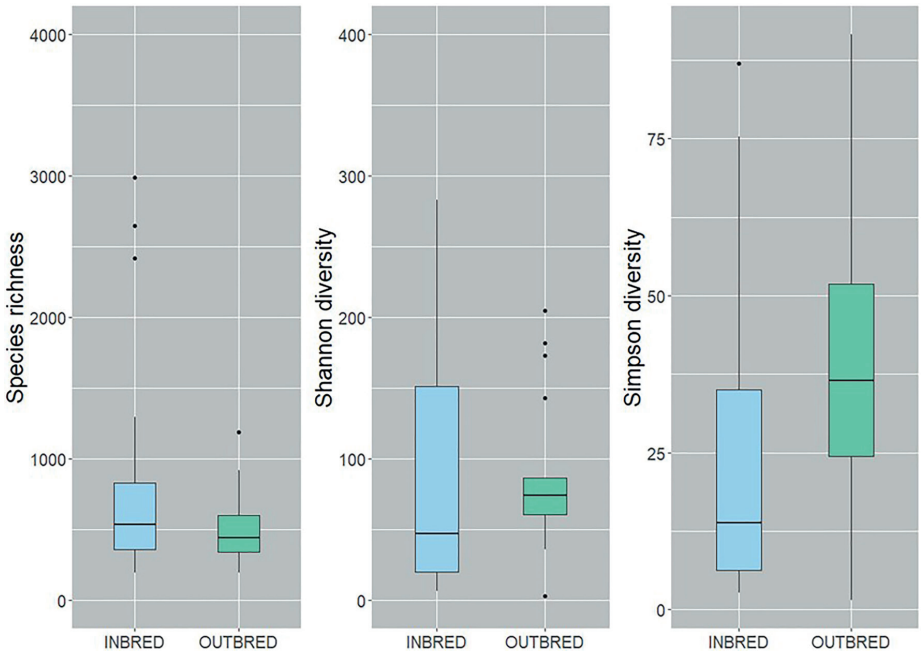

FIGURE 4 | Alpha diversity of the bacteria in the anterior intestine of the inbred and outbred groups of Nile tilapia. There is an increasing trend in the species richness of the inbred group ($p = 0.07$). Simpson diversity shows an increasing trend in the dominant ASVs of the outbred group ($p = 0.06$). The boxplots show minimum, lower quartile, median, upper quartile and maximum values.

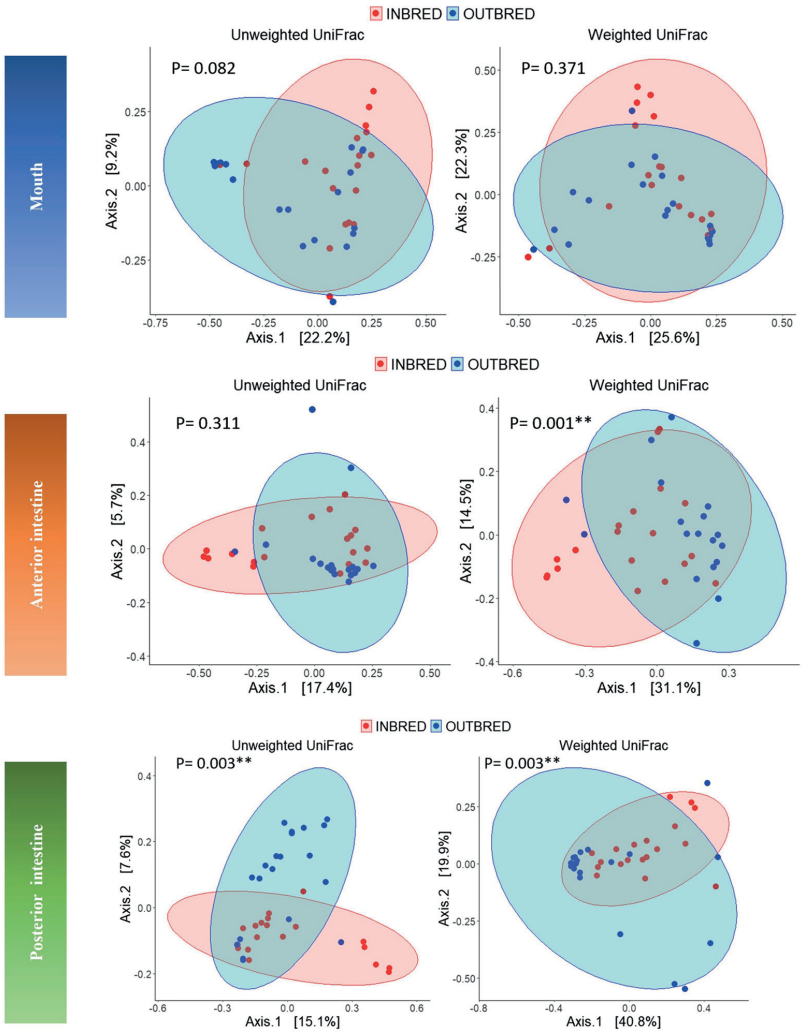

FIGURE 5 | Principal coordinates analyses (PCoA) using unweighted and weighted UniFrac distance matrices of the bacteria in the different body sites of the inbred and outbred groups of Nile tilapia. The ellipses were generated assuming that the data are from a multivariate normal distribution.

(**Supplementary Figures 4**, **5**) compared to the inbred group. However, the fold changes of the differentially abundant ASVs of *Bacteroidetes*, *Fusobacteria*, and *Proteobacteria* in the anterior intestine were between −5 and −10 (**Supplementary Figure 4** shows selected differentially abundant ASVs; **Supplementary Figure 5** shows all the differentially abundant ASVs). Similarly, in the posterior intestine, out of 31 ASVs that were differentially abundant, 30 ASVs had fold changes between −5 and −28 in the outbred group, while only one ASV that belongs to *Acinetobacter* was 20-fold higher in the outbred group (**Supplementary Figure 6**). Moreover, ASVs of *Pediococcus* and *Bifidobacterium* which belong to *Firmicutes* and *Actinobacteria*, respectively, were lower (log fold change; −5 and −8, respectively) in the posterior intestine of the outbred group (**Supplementary Figure 6**).

Core Microbiome and Variability in Taxa

In the mouth, 9 ASVs of the core microbiota belonged to the genera *Staphylococcus*, *Curvibacter*, *Undibacterium*, *Escherichia/Shigella*, *Enhydrobacter*, *Propionibacterium*, and

TABLE 3 | Results of the analysis of homogeneity of group dispersions and PERMANOVA using distance (unweighted and weighted UniFrac) matrices.

Comparison	Variable	Unweighted UniFrac distance			Weighted UniFrac distance		
		p-value dispersions	R^2	*p*-value adonis	*p*-value dispersions	R^2	*p*-value adonis
Outbred vs. Inbred	Mouth	0.86	0.08	0.08	0.62	0.029	0.37
	Anterior intestine	0.20	0.03	0.31	0.27	0.14	0.001**
	Posterior intestine	0.60	0.05	0.003**	0.05	0.10	0.003**

**Indicates $p < 0.05$.

Cetobacterium. However, two bacteria were classified only up to the order level – *Actinomycetales, Sphingobacteriales* (**Figure 6**). Taking all the 9 ASVs together, we observed a significant difference in the core microbiome in the inbred and outbred groups; only for unweighted UniFrac distance ($R^2 = 0.073$, $p = 0.043$; weighted UniFrac distance showed no significant difference; $R^2 = 0.024$, $p = 0.445$; **Table 4**). In the anterior and posterior intestine, the core ASVs were *Staphylococcus*, *Plesiomonas*, *Undibacterium*, *Enhydrobacter*, *Propionibacterium*, and *Cetobacterium* (**Figures 7A,B**). One extra genus was a member of the core microbiota in the anterior intestine (*Escherichia*/*Shigella*). One ASV in the anterior and posterior intestine was not classified up to the genus level, but was annotated as *Actinomycetales* (**Figures 7A,B**). The core microbiota in the anterior intestine of the inbred group was different from that of the outbred group; the weighted UniFrac distances-based assessment indicated the significant difference (PERMANOVA test; $R^2 = 0.155$, $p = 0.001$) between the two crossbred groups. As for the posterior intestine, we cannot specify that there is a significant difference between the crossbred groups (**Table 4**). The inter-individual variation in the abundance of the core microbiota in the intestine samples of the inbred group was less pronounced compared to the outbred groups (**Supplementary Figure 7**). On the other hand, the inter-individual variation in the abundances was more pronounced in the mouth of the inbred compared to the outbred group (**Supplementary Figure 8**).

DISCUSSION

The genetic structure of wild/domestic/experimental animals can be altered through breeding to retain desired phenotypic and genotypic traits across generations. It is known that selective breeding can preserve desired traits, which can affect the bacterial profile that is highly correlated to host health.

Gut microbiota in fish has been studied extensively in recent years considering mainly its importance in host health. In the present study, we used genetically distinct (based on SNP analysis) inbred and outbred Nile tilapia to investigate the impact of crossbreeding on the composition of the mouth and intestine bacteria.

Mouth and Intestine Bacterial Community Composition and Diversity in the Inbred and Outbred Groups

Although male Nile tilapia are widely farmed because of their higher growth rate, in the present study, we analyzed the microbial community in females, which are mouthbrooders. Hence, we believe that studying the microbial communities in its mouth will yield interesting results. In humans, microbiota is transferred from different body sites of mothers to infants (Ferretti et al., 2018). Moreover, microbial symbionts from discus (*Symphysodon aequifasciata*, another fish of the family Cichlidae) parents are vertically transferred to fry through feeding of a cutaneous mucus secretion (Sylvain and Derome, 2017). The most dominant phyla found in our samples were *Proteobacteria*, *Fusobacteria*, *Firmicutes*, *Bacteroidetes*, and *Actinobacteria* (**Supplementary Figure 9**). These are known to be the most represented phyla in model fishes such as zebrafish and threespine stickleback (Legrand et al., 2020). They are also dominant in farmed fishes like Nile tilapia even though many factors including diet (Ray et al., 2017; Souza et al., 2020), rearing systems (Giatsis et al., 2015; Yukgehnaish et al., 2020), and salinity (Zhang et al., 2016; Yukgehnaish et al., 2020) affect the abundance of these phyla in the gut. However, the role of crossbreeding in shaping microbial communities has not yet been reported in fish although it is studied in mice (Pang et al., 2012; Kreisinger et al., 2014), mammals (Alessandri et al., 2019), and plants (Wagner et al., 2020).

The dominant phyla were the same in both the inbred and outbred groups of Nile tilapia. *Proteobacteria* are facultative anaerobes, and they are the most abundant bacterial phylum in fish gut (Egerton et al., 2018). Furthermore, bacteria such as *Escherichia* and *Enhydrobacter* belonging to this phylum have the ability to make the gut environment conducive to strict anaerobes which colonize healthy gut (Shin et al., 2015). Although the aforementioned genera were present in the mouth and intestine of both the outbred and inbred fish, their abundances in the two groups were different. In addition, the genus *Curvibacter* which was present in both groups is known to have a critical role in colonization in freshwater invertebrates (Wein et al., 2018).

Alpha diversity analysis revealed that our crossbreeding strategy increased the microbial evenness in the mouth of the outbred group, in which we observed apparently higher species richness. The increasing trend in the dominant bacteria in the mouth and the anterior intestine of the outbred group along with the significant increase in the posterior intestine suggests that the dominant bacteria in the outbred groups are more diverse compared to the inbred group. On the other hand, the increasing trend in the species richness in the anterior intestine of the inbred group suggests that the bacterial

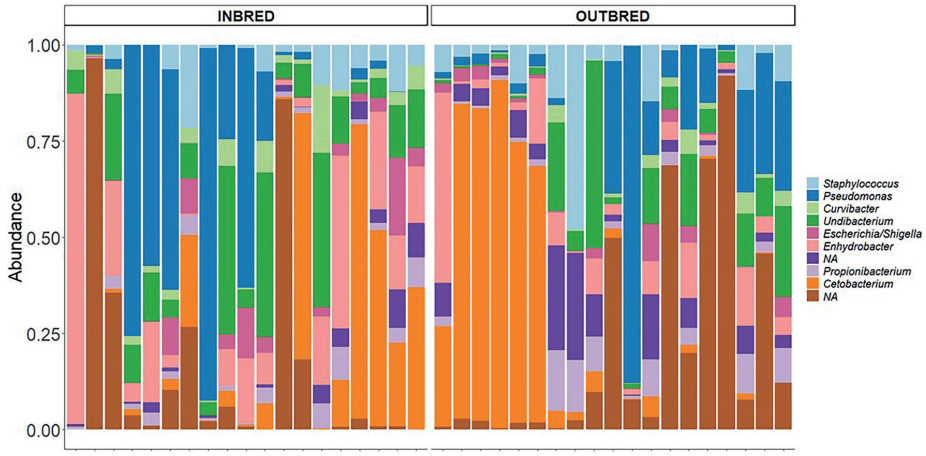

FIGURE 6 | Core microbiota in the mouth of the inbred and outbred groups of Nile tilapia. NAs: Not classified at the genus level, but at the order level, they are classified as *Actinomycetales*, *Sphingobacteriales*, in both groups.

TABLE 4 | Results of the analysis of homogeneity of group dispersions and PERMANOVA using distance (unweighted and weighted UniFrac) matrices of the core microbiota.

		Unweighted UniFrac distance			Weighted UniFrac distance		
Comparision	**Variable**	***p*-value dispersions**	**R^2**	***p*-value adonis**	***p*-value dispersions**	**R^2**	***p*-value adonis**
Outbred vs. Inbred	Mouth	0.834	0.0734	0.043*	0.742	0.0241	0.445
	Anterior intestine	0.08	0.0355	0.352	0.323	0.1553	0.0011**
	Posterior intestine	0.208	0.0181	0.541	0.003**	0.1238	0.0025**

*Indicates $p < 0.05$ and **indicates $p < 0.01$.

community is more diverse in this intestinal segment of the inbred group compared to the outbred group. The abovementioned findings are similar to the results of the PCoA analysis that used UniFrac distances. Microbial diversity is believed to have a positive correlation with host health (Deng et al., 2019). However, Reese and Dunn (2018) have stated that "understanding diversity in host-associated microbial communities will not be as simple as 'more diversity is better'." Hence, it is not ideal to correlate host health with the diversity in the outbred group. Studies in Nile tilapia have not reported a significant difference in the diversity of gut microbiota as a pathogenic effect (Suphoronski et al., 2019; Silva et al., 2020). On the other hand, while diet was shown to increase the species richness of bacteria in the gut, another environmental factor, salinity, was found to decrease the richness of bacteria in Nile tilapia (Zhang et al., 2016). The implication of the increasing trend in diversity in the anterior intestine of the inbred group should be clarified by conducting studies on the bacteria in this segment and their effect on nutritional physiology (Hallali et al., 2018). Thus, in addition to the aforementioned factors, we suggest that crossbreeding is a determinant of both the mouth and intestine bacterial diversity in female Nile tilapia.

Significant Differences Between the ASV Abundance of the Inbred and Outbred Groups

Fish gut harbors complex and diverse microbial communities, and the site is a reservoir of many opportunistic pathogens belonging to the genera *Acinetobacter*, *Aeromonas*, *Psychrobacter*, *Flavobacterium*, *Pseudomonas*, and *Pleisomonas*. Many commensal bacteria including *Cetobacterium*, *Methylobacterium*, *Sphingomonas*, and *Propionibacterium* (Suphoronski et al., 2019; Legrand et al., 2020; Silva et al., 2020) that colonise the fish gut are essential for the production of vitamin B12 and antimicrobial metabolites (Suphoronski et al., 2019; Legrand et al., 2020), protection against pathogens such as *Flavobacterium* (Boutin et al., 2014), and improving host health (Boutin et al., 2013). The differential ASV analysis revealed that the abundances of some of these opportunistic pathogens (*Psychrobacter*, *Pseudomonas*, and *Acinetobacter*) were more than 5-fold in the mouth of the outbred group compared to the inbred group. In the anterior and posterior intestine of the outbred group, although the opportunistic pathogens belonging to the genera *Acinetobacter*, *Aeromonas*, *Pleisomonas*, *Psychrobacter*, *Pseudomonas*, and *Flavobacterium* were differentially abundant,

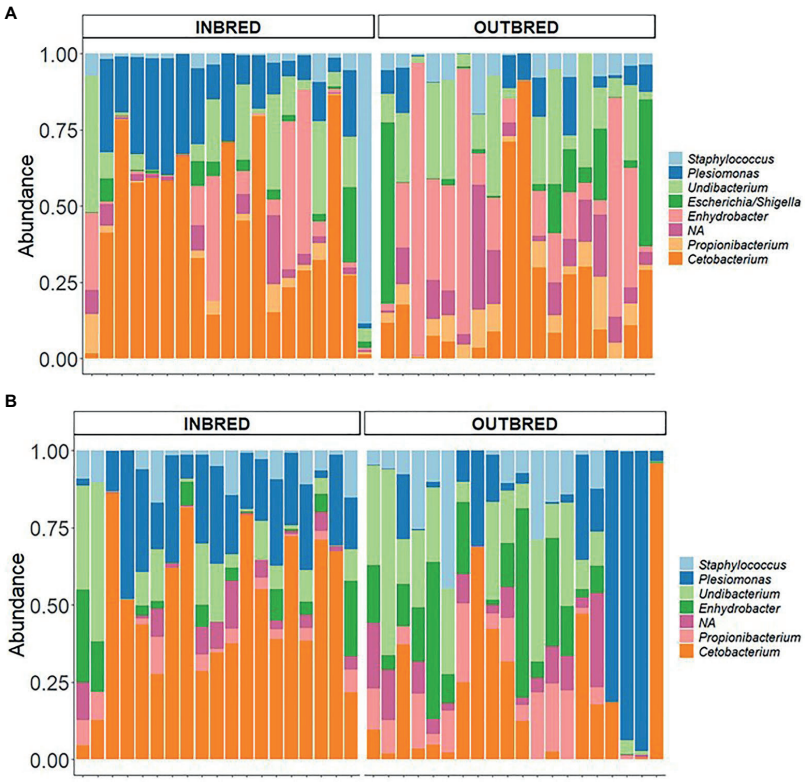

FIGURE 7 | Core microbiota in the anterior and posterior intestine of the inbred and outbred groups of Nile tilapia. **(A)** anterior intestine and **(B)** posterior intestine. NA: at the order level is classified as *Actinomycetales*.

their fold changes were less than 5-fold. The bacterial community in the mouth is extensively exposed to the external environment, and we found that the opportunistic pathogens in the mouth are more abundant in the outbred group. On the other hand, the abundance of potential pathogens was lower in the intestine of the inbred group. *Pseudomonas* sp. are opportunistic pathogens and they cause high mortality in farmed fishes (Oh et al., 2019). Moreover, bacteria belonging to *Flavobacterium* were reported to cause acute bacteremia primarily in small fishes or more chronic disease in larger fishes (Semple et al., 2020). Although the outbred fish had a more diverse microbiome, they appear to harbor potential opportunistic bacteria also.

Interestingly, the abundance of potential beneficial bacteria (*Cetobacterium*, *Methylobacterium*, *Sphingomonas*, and *Propionibacterium*; Boutin et al., 2013, 2014; Suphoronski et al., 2019; Legrand et al., 2020) was higher in the inbred group. Many studies report that commensal microbiota in the gut plays an important role in regulating the growth of other microbes by competing for space and nutrition. The mouth of the inbred fish had higher abundance of *Aeromonas* sp. which was found to compete for nutrients and play a negative role during infection (Wiles et al., 2016; Legrand et al., 2020). On the other hand, the bacteria that had higher abundance in the posterior intestine of the inbred tilapia, namely *Enhydrobacter* sp., is a commensal microbe in rainbow trout (*Oncorhynchus mykiss*), which is known to produce entericidin, and this antitoxin peptide inhibits the growth of certain pathogens such as those belonging to *Flavobacterium* (Legrand et al., 2020). Furthermore, *Pediococcus* and *Bifidobacterium* which were found to be more abundant in the anterior and posterior intestine of the inbred groups compared to the outbred group are known to outcompete some invasive pathogens, associated with tilapia intestinal mucosa (Ferguson et al., 2010; Standen et al., 2013) and promote fish growth (Ayyat et al., 2014). Thus, the inbred group had a higher abundance of potential beneficial commensal bacteria.

Changes in Core Microbiome

The transient allochthonous microbiome of fish is associated with digesta and is usually expelled after some period as they are predominantly influenced by diet. On the other hand, the

resident microbes that belong to the autochthonous microbiome colonise the mucus surface in the gut and make up the core microbiome (Egerton et al., 2018). These microbial communities, which are known to be vertically transmitted (Risely, 2020), associate with the host's cells (Egerton et al., 2018; Legrand et al., 2020). In the present study, the core microbiome in each body site was determined based on the ASVs present in all samples in each group. However, the inter-individual variation in abundance that we observed is similar to the learning from studies on zebrafish (Burns et al., 2016) and mice (Pang et al., 2012). In mice, inbreeding was found to reduce the inter-individual variation (Pang et al., 2012). The inter-individual variation in the core microbiome in the intestine of the inbred group is much lesser compared to the outbred group. In contrast, such similarity was not observed in the mouth of the inbred fish; this was attributed to the effect of external environment in other studies (Lokesh and Kiron, 2016; Krotman et al., 2020). However, in the present study, environmental factors were kept constant throughout the study period. In humans, the initial oral colonizers from the vagina and mother's milk and mouth can be perturbed by environmental factors (Kilian, 2018).

The most dominant bacterial phylum in the two study groups was *Proteobacteria*. Nevertheless, *Cetobacterium* (phylum *Fusobacteria)* was found to be dominant in the anterior and posterior intestine of the inbred group, while its proportion was reduced in the outbred group. Previous studies conducted on Nile tilapia showed that the composition of *Cetobacterium* spp., the most prevalent genera in tilapia gut, was not affected by diets (Ray et al., 2017) or presence of pathogens (Suphoronski et al., 2019; Silva et al., 2020). Other reports that studied the influence of factors including rearing environment (Giatsis et al., 2015), and salinity (Zhang et al., 2016) on the gut microbial composition substantiates our finding that *Cetobacterium* is a core member of the bacterial community. Based on the present study, it appears that the crossbreeding strategy does not impact the presence of this core member in the mouth and intestine of Nile tilapia.

Some of the commonly reported bacteria in the intestine of Nile tilapia (*Staphylococcus*, *Cetobacterium*, *Plesiomonas*, *Enhydrobacter*, *Undibacterium*, and *Propionibacterium*) were present in both groups. However, some core microbiome members such as *Pseudomonas* and *Curvibacter* were present only in the mouth of both groups. A study employing turbot (*Scophthalmus maximus*) showed that a similar microbiome community was present in the intestine of different breeds fed with different diets and reared in different water environments. In addition, it was reported that core microbiome could colonize fish gut for a long term and it could have a vital physiological significance to the host (Zhang et al., 2020). This suggests that fishes preserve their core microbiome community despite differences in environmental factors.

Host Genetics and Intestine Microbiome

Growing evidence shows that host genetics plays a key role in shaping the gut microbiome of mammals (Hufeldt et al., 2010; Miller et al., 2018; Alessandri et al., 2019), but not to the same degree as that of environmental factors (Davenport, 2016). While there are many reports on diet-based microbiota differences in fish, evidences of fish genetics-associated microbiota are sparse (Li et al., 2014; Kokou et al., 2018).

Our genetic diversity analysis indicated a small but significant difference between the inbred and outbred fish. Unexpectedly, the observed heterozygosity was slightly higher than the expected heterozygosity, probably arising from the low genetic diversity values in both the inbred and outbred groups. The Ho, He, and F_{st} results that we obtained are likely due to small number of founders with a similar genetic background since the F0 generation of the fish were caught from the same area. The F0 itself may have lost considerable genetic diversity, as noted for birds; a small number of founders in a population increased the probability of inbreeding and associated gene diversity loss (Jamieson, 2011).

Wild Nile tilapia populations in West Africa are reported to have low diversity, especially, the species within a particular region; for example in Gambia River and the far western region of the Niger River (Lind et al., 2019). Nile tilapia is seen as a range-limited species in these areas, and founder effect was reported to be the reason for their genetic diversity reduction (Lind et al., 2019). In addition, F_{st} results also indicated the low genetic differentiation within the inbred groups as well as the outbred groups.

Anthropogenic needs not only alter species behavior, feeding habits, rearing environment, and traits within the host genotype but also reshape the gut microbiota of domesticated/captivated animals (Li et al., 2014; Alessandri et al., 2019). A study on blue tilapia, which was selectively bred to retain a host genotype, has reported that gut microbiome was linked to host genotype as well as specific bacteria such as *Cetobacterium somerae* (Kokou et al., 2018). This bacterium is a cobalamin producer (Tsuchiya et al., 2008; Degnan et al., 2014) and fishes with high abundance of *C. somerae* do not require dietary vitamin B12 (Sugita et al., 1991; Tsuchiya et al., 2008).

In order to analyse the genetic effect (by controlling the mating strategy) on the mouth and gut microbiota, the fish were kept in the same environmental conditions and fed the same diet, since both these factors are determinants of host microbial communities. Thus, crossing strategy influenced the microbial alpha diversity and composition in Nile tilapia. A similar effect on the midgut microbiota composition was observed in selectively bred trout (Brown et al., 2019). In addition, a study conducted on mice suggested that the alpha diversity of the gastrointestinal tract microbiota is slightly decreased in the inbred individuals (Kreisinger et al., 2014). Thus, the differences in the diversities of the microbial communities of the two groups could be attributed to crossbreeding strategy.

The differences in abundance of the microbial composition of the core microbiome in the individual samples from the mouth of the inbred fish were more pronounced compared to the outbred groups. In the mouth, influence of an external environmental factor (water) appears to surpass that of the host genetics. On the other hand, there was more similarity in the abundance of the bacterial communities in the individual intestine samples of the inbred group compared to the outbred group of Nile tilapia.

Host genetics is known to have a long-lasting effect on the gut microbial communities and this is due to maternal transfer during early development (Kreisinger et al., 2014). A core microbiota is heritable in several species (Hauffe and Barelli, 2019), including cichlids (Baldo et al., 2017). The similarities in the abundances of the taxa in the inbred group of Nile tilapia, which is also a cichlid fish, suggest that the microbial composition in the gut is more established without being affected by the external environment. A study conducted in mice showed that the inter-individual variation in the gut microbiome of the inbred group is lower compared to the outbred animals (Hufeldt et al., 2010). Furthermore, in humans the similarity of the gut microbiome is higher among closer relatives in families (Zoetendal et al., 2001). Therefore, this finding suggests that the genetic factor is more prominent in the intestine of the inbred groups and the effect is likely the inheritance of the microbial profile to the offspring of the fish, especially the core microbiome.

We report for the first time the effect of inbreeding and outbreeding on the mouth and intestine microbiome in Nile tilapia. The genetic relationship and structure analysis indicated the genetic differentiation between the inbred and outbred groups. Differential ASV analysis revealed the abundance of the potential opportunistic pathogens such as *Flavobacterium* in the outbred group and beneficial bacteria like *Bifidobacterium* and *Pediococcus* in the inbred group. We also found that *Cetobacterium* is the core member in both groups, but its abundance was higher in the intestine of the inbred group. The inbred fish which has less inter-individual microbiome variability, could be a better choice for controlled studies that examine the maternal transfer of intestine microbiome to offspring. We highlight that crossbreeding can influence Nile tilapia bacterial communities.

DATA AVAILABILITY STATEMENT

The data used in this study is available at European Nucleotide Archive (ENA) with accession no PRJEB40093. Whole-genome data of the host are available at Sequence Read Archive (SRA) with accession no PRJNA719847.

ETHICS STATEMENT

The animal study was reviewed and approved by Norwegian Animal Research Authority, FOTS ID 1042.

AUTHOR CONTRIBUTIONS

VK, JF, and YA designed the study. YA carried out the sampling and lab work, analysed the data, and wrote the manuscript. CD also analysed the data. SL helped in sequencing and data generation. DA was involved in initial data analyses. OJ and AN prepared the whole genome shotgun sequencing libraries. OJ performed the SNPs analysis of the host. YA, JF, CD, and VK interpreted the data. VK, JF, and CD reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2021.709611/full#supplementary-material

REFERENCES

Albanese, D., Fontana, P., De Filippo, C., Cavalieri, D., and Donati, C. (2015). MICCA: a complete and accurate software for taxonomic profiling of metagenomic data. *Sci. Rep.* 5:9743. doi: 10.1038/srep09743

Alessandri, G., Milani, C., Mancabelli, L., Mangifesta, M., Lugli, G. A., Viappiani, A., et al. (2019). The impact of human-facilitated selection on the gut microbiota of domesticated mammals. *FEMS Microbiol. Ecol.* 95:fiz121. doi: 10.1093/femsec/fiz121

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral. Ecol.* 26, 32–46. doi: 10.1111/j.1442-9993.2001.01070.pp.x

Ayyat, M., Labib, H. M., and Mahmoud, H. K. (2014). A probiotic cocktail as a growth promoter in Nile tilapia (*Oreochromis niloticus*). *J. Appl. Aquac.* 26, 208–215. doi: 10.1080/10454438.2014.934164

Baldo, L., Pretus, J. L., Riera, J. L., Musilova, Z., Nyom, A. R. B., and Salzburger, W. (2017). Convergence of gut microbiotas in the adaptive radiations of African cichlid fishes. *ISME J.* 11, 1975–1987. doi: 10.1038/ismej.2017.62

Bolivar, R. B., and Newkirk, G. F. (2002). Response to within family selection for body weight in Nile tilapia (*Oreochromis niloticus*) using a single-trait animal model. *Aquaculture* 204, 371–381. doi: 10.1016/S0044-8486(01)00824-9

Boutin, S., Bernatchez, L., Audet, C., and Derôme, N. (2013). Network analysis highlights complex interactions between pathogen, host and commensal microbiota. *PLoS One* 8:e84772. doi: 10.1371/journal.pone.0084772

Boutin, S., Sauvage, C., Bernatchez, L., Audet, C., and Derome, N. (2014). Inter individual variations of the fish skin microbiota: host genetics basis of mutualism? *PLoS One* 9:e102649. doi: 10.1371/journal.pone.0102649

Brown, R. M., Wiens, G. D., and Salinas, I. (2019). Analysis of the gut and gill microbiome of resistant and susceptible lines of rainbow trout (*Oncorhynchus mykiss*). *Fish Shellfish Immunol.* 86, 497–506. doi: 10.1016/j.fsi.2018.11.079

Browning, B. L., and Browning, S. R. (2016). Genotype imputation with millions of reference samples. *Am. J. Hum. Genet.* 98, 116–126. doi: 10.1016/j.ajhg.2015.11.020

Burns, A. R., Stephens, W. Z., Stagaman, K., Wong, S., Rawls, J. F., Guillemin, K., et al. (2016). Contribution of neutral processes to the assembly of gut microbial communities in the zebrafish over host development. *ISME J.* 10, 655–664. doi: 10.1038/ismej.2015.142

Conte, M. A., Gammerdinger, W. J., Bartie, K. L., Penman, D. J., and Kocher, T. D. (2017). A high quality assembly of the Nile tilapia (*Oreochromis niloticus*) genome reveals the structure of two sex determination regions. *BMC Genomics* 18:341. doi: 10.1186/s12864-017-3723-5

D'ambrosio, J., Phocas, F., Haffray, P., Bestin, A., Brard-Fudulea, S., Poncet, C., et al. (2019). Genome-wide estimates of genetic diversity, inbreeding and effective size of experimental and commercial rainbow trout lines undergoing selective breeding. *Genet. Sel. Evol.* 51:26. doi: 10.1186/s12711-019-0468-4

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., Depristo, M. A., et al. (2011). The variant call format and VCFtools. *Bioinformatics* 27, 2156–2158. doi: 10.1093/bioinformatics/btr330

Davenport, E. R. (2016). Elucidating the role of the host genome in shaping microbiome composition. *Gut Microbes* 7, 178–184. doi: 10.1080/19490976.2016.1155022

Degnan, P. H., Taga, M. E., and Goodman, A. L. (2014). Vitamin B12 as a modulator of gut microbial ecology. *Cell Metab.* 20, 769–778. doi: 10.1016/j.cmet.2014.10.002

Deng, F., Li, Y., and Zhao, J. (2019). The gut microbiome of healthy long-living people. *Aging (Albany NY)* 11, 289–290. doi: 10.18632/aging.101771

Desantis, T., Hugenholtz, P., Keller, K., Brodie, E., Larsen, N., Piceno, Y., et al. (2006). NAST: a multiple sequence alignment server for comparative analysis of 16S rRNA genes. *Nucleic Acids Res.* 34, W394–W399. doi: 10.1093/nar/gkl244

Edgar, R. C. (2016). UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. bioRxiv [Preprint]. doi: 10.1101/081257

Edgar, R. C., and Flyvbjerg, H. (2015). Error filtering, pair assembly and error correction for next-generation sequencing reads. *Bioinformatics* 31, 3476–3482. doi: 10.1093/bioinformatics/btv401

Egerton, S., Culloty, S., Whooley, J., Stanton, C., and Ross, R. P. (2018). The gut microbiota of marine fish. *Front. Microbiol.* 9:873. doi: 10.3389/fmicb.2018.00873

Ericsson, A. C., Davis, J. W., Spollen, W., Bivens, N., Givan, S., Hagan, C. E., et al. (2015). Effects of vendor and genetic background on the composition of the fecal microbiota of inbred mice. *PLoS One* 10:e0116704. doi: 10.1371/journal.pone.0116704

Ferguson, R., Merrifield, D. L., Harper, G. M., Rawling, M. D., Mustafa, S., Picchietti, S., et al. (2010). The effect of *Pediococcus acidilactici* on the gut microbiota and immune status of on-growing red tilapia (*Oreochromis niloticus*). *J. Appl. Microbiol.* 109, 851–862. doi: 10.1111/j.1365-2672.2010.04713.x

Ferretti, P., Pasolli, E., Tett, A., Asnicar, F., Gorfer, V., Fedi, S., et al. (2018). Mother-to-infant microbial transmission from different body sites shapes the developing infant gut microbiome. *Cell Host Microbe* 24, 133.e135–145.e135. doi: 10.1016/j.chom.2018.06.005

Flint, A., and Woolliams, J. (2008). Precision animal breeding. *Philos. Trans. R. Soc. B Biol. Sci.* 363, 573–590. doi: 10.1098/rstb.2007.2171

Giatsis, C., Sipkema, D., Smidt, H., Heilig, H., Benvenuti, G., Verreth, J., et al. (2015). The impact of rearing environment on the development of gut microbiota in tilapia larvae. *Sci. Rep.* 5:18206. doi: 10.1038/srep18206

Gjedrem, T., Robinson, N., and Rye, M. (2012). The importance of selective breeding in aquaculture to meet future demands for animal protein: a review. *Aquaculture* 350, 117–129. doi: 10.1016/j.aquaculture.2012.04.008

Gjedrem, T., and Rye, M. (2018). Selection response in fish and shellfish: a review. *Rev. Aquac.* 10, 168–179. doi: 10.1111/raq.12154

Gratacap, R. L., Wargelius, A., Edvardsen, R. B., and Houston, R. D. (2019). Potential of genome editing to improve aquaculture breeding and production. *Trends Genet.* 35, 672–684. doi: 10.1016/j.tig.2019.06.006

Hallali, E., Kokou, F., Chourasia, T. K., Nitzan, T., Con, P., Harpaz, S., et al. (2018). Dietary salt levels affect digestibility, intestinal gene expression, and the microbiome, in Nile tilapia (*Oreochromis niloticus*). *PLoS One* 13:e0202351. doi: 10.1371/journal.pone.0202351

Hart, M. L., Ericsson, A. C., Lloyd, K. K., Grimsrud, K. N., Rogala, A. R., Godfrey, V. L., et al. (2018). Development of outbred CD1 mouse colonies with distinct standardized gut microbiota profiles for use in complex microbiota targeted studies. *Sci. Rep.* 8:10107. doi: 10.1038/s41598-018-28448-0

Hauffe, H. C., and Barelli, C. (2019). Conserve the germs: the gut microbiota and adaptive potential. *Conserv. Genet.* 20, 19–27. doi: 10.1007/s10592-019-01150-y

Hill, W. G. (2014). Applications of population genetics to animal breeding, from wright, fisher and lush to genomic prediction. *Genetics* 196, 1–16. doi: 10.1534/genetics.112.147850

Hill, W. G. (2016). Is continued genetic improvement of livestock sustainable? *Genetics* 202, 877–881. doi: 10.1534/genetics.115.186650

Hsieh, T., Ma, K., and Chao, A. (2016). iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers). *Methods Ecol. Evol.* 7, 1451–1456. doi: 10.1111/2041-210X.12613

Hufeldt, M. R., Nielsen, D. S., Vogensen, F. K., Midtvedt, T., and Hansen, A. K. (2010). Variation in the gut microbiota of laboratory mice is related to both genetic and environmental factors. *Comp. Med.* 60, 336–347.

Ina-Salwany, M. Y., Al-Saari, N., Mohamad, A., Mursidi, F.-A., Mohd-Aris, A., Amal, M. N. A., et al. (2019). Vibriosis in fish: a review on disease development and prevention. *J. Aquat. Anim. Health* 31, 3–22. doi: 10.1002/aah.10045

Jamieson, I. G. (2011). Founder effects, inbreeding, and loss of genetic diversity in four avian reintroduction programs. *Conserv. Biol.* 25, 115–123. doi: 10.1111/j.1523-1739.2010.01574.x

Jantrakajorn, S., Maisak, H., and Wongtavatchai, J. (2014). Comprehensive investigation of Streptococcosis outbreaks in cultured Nile tilapia, *Oreochromis niloticus*, and red tilapia, *Oreochromis* sp., of Thailand. *J. World Aquacult. Soc.* 45, 392–402. doi: 10.1111/jwas.12131

Jombart, T. (2008). Adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* 24, 1403–1405. doi: 10.1093/bioinformatics/btn129

Kamvar, Z. N., Brooks, J. C., and Grünwald, N. J. (2015). Novel R tools for analysis of genome-wide population genetic data with emphasis on clonality. *Front. Gent.* 6:208. doi: 10.3389/fgene.2015.00208

Kilian, M. (2018). The oral microbiome–friend or foe? *Eur. J. Oral Sci.* 126, 5–12. doi: 10.1111/eos.12527

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., et al. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res.* 41:e1. doi: 10.1093/nar/gks808

Knaus, B. J., and Grünwald, N. J. (2017). vcfr: a package to manipulate and visualize variant call format data in R. *Mol. Ecol.* 17, 44–53. doi: 10.1111/1755-0998.12549

Kokou, F., Sasson, G., Nitzan, T., Doron-Faigenboim, A., Harpaz, S., Cnaani, A., et al. (2018). Host genetic selection for cold tolerance shapes microbiome composition and modulates its response to temperature. *eLife* 7:e36398. doi: 10.7554/eLife.36398

Kolde, R., and Kolde, M. R. (2015). Package 'pheatmap'. R package 1, 790.

Konstantinidis, I., Sætrom, P., Mjelle, R., Nedoluzhko, A. V., Robledo, D., and Fernandes, J. M. (2020). Major gene expression changes and epigenetic remodelling in Nile tilapia muscle after just one generation of domestication. *Epigenetics* 15, 1052–1067. doi: 10.1080/15592294.2020.1748914

Kreisinger, J., Čížková, D., Vohánka, J., and Piálek, J. (2014). Gastrointestinal microbiota of wild and inbred individuals of two house mouse subspecies assessed using high-throughput parallel pyrosequencing. *Mol. Ecol.* 23, 5048–5060. doi: 10.1111/mec.12909

Krotman, Y., Yergaliyev, T. M., Shani, R. A., Avrahami, Y., and Szitenberg, A. (2020). Dissecting the factors shaping fish skin microbiomes in a heterogeneous inland water system. *Microbiome* 8:9. doi: 10.1186/s40168-020-0784-5

Langmead, B., and Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nat. Methods* 9:357. doi: 10.1038/nmeth.1923

Legrand, T. P., Wynne, J. W., Weyrich, L. S., and Oxley, A. P. (2020). A microbial sea of possibilities: current knowledge and prospects for an improved understanding of the fish microbiome. *Rev. Aquac.* 12, 1101–1134. doi: 10.1111/raq.12375

Li, H. (2011). A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. *Bioinformatics* 27, 2987–2993. doi: 10.1093/bioinformatics/btr509

Li, J., Ni, J., Li, J., Wang, C., Li, X., Wu, S., et al. (2014). Comparative study on gastrointestinal microbiota of eight fish species with different feeding habits. *J. Appl. Microbiol.* 117, 1750–1760. doi: 10.1111/jam.12663

Lind, C. E., Agyakwah, S. K., Attipoe, F. Y., Nugent, C., Crooijmans, R. P., and Toguyeni, A. (2019). Genetic diversity of Nile tilapia (*Oreochromis niloticus*) throughout West Africa. *Sci. Rep.* 9:16767. doi: 10.1038/s41598-019-53295-y

Lind, C., Ponzoni, R., Nguyen, N., and Khaw, H. (2012). Selective breeding in fish and conservation of genetic resources for aquaculture. *Reprod. Domest. Anim.* 47, 255–263. doi: 10.1111/j.1439-0531.2012.02084.x

Lokesh, J., and Kiron, V. (2016). Transition from freshwater to seawater reshapes the skin-associated microbiota of Atlantic salmon. *Sci. Rep.* 6:19707. doi: 10.1038/srep19707

Love, M. I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 15:550. doi: 10.1186/s13059-014-0550-8

Lozupone, C., and Knight, R. (2005). UniFrac: a new phylogenetic method for comparing microbial communities. *Appl. Environ. Microbiol.* 71, 8228–8235. doi: 10.1128/AEM.71.12.8228-8235.2005

Marcon, E., and Hérault, B. (2015). entropart: an R package to measure and partition diversity. *J. Stat. Softw.* 67, 1–26. doi: 10.18637/jss.v067.i08

McMurdie, P. J., and Holmes, S. (2013). phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* 8:e61217. doi: 10.1371/journal.pone.0061217

McMurdie, P. J., and Holmes, S. (2014). Waste not, want not: why rarefying microbiome data is inadmissible. *PLoS Comput. Biol.* 10:e1003531. doi: 10.1371/journal.pcbi.1003531

Mehar, M., Mekkawy, W., Mcdougall, C., and Benzie, J. A. (2019). Fish trait preferences: a review of existing knowledge and implications for breeding programmes. *Rev. Aquac.* 12, 1273–1296. doi: 10.1111/raq.12382

Miller, E. T., Svanbäck, R., and Bohannan, B. J. (2018). Microbiomes as metacommunities: understanding host-associated microbes through metacommunity ecology. *Trends Ecol. Evol.* 33, 926–935. doi: 10.1016/j.tree.2018.09.002

Oh, W. T., Kim, J. H., Jun, J. W., Giri, S. S., Yun, S., Kim, H. J., et al. (2019). Genetic characterization and pathological analysis of a novel bacterial pathogen, *Pseudomonas tructae*, in rainbow trout (*Oncorhynchus mykiss*). *Microorganisms* 7:432. doi: 10.3390/microorganisms7100432

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P., O'hara, R., et al. (2013). Community ecology package. R package version, 2.0–2.

Oriá, R. B., Malva, J. O., Foley, P. L., Freitas, R. S., Bolick, D. T., and Guerrant, R. L. (2018). Revisiting inbred mouse models to study the developing brain: the potential role of intestinal microbiota. *Front. Hum. Neurosci.* 12:358. doi: 10.3389/fnhum.2018.00358

Pang, W., Stradiotto, D., Krych, L., Karlskov-Mortensen, P., Vogensen, F. K., Nielsen, D. S., et al. (2012). Selective inbreeding does not increase gut microbiota similarity in BALB/c mice. *Lab. Anim.* 46, 335–337. doi: 10.1258/la.2012.012040

Paradis, E., and Schliep, K. (2019). ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* 35, 526–528. doi: 10.1093/bioinformatics/bty633

Pembleton, L. W., Cogan, N. O., and Forster, J. W. (2013). St AMPP: an R package for calculation of genetic differentiation and structure of mixed-ploidy level populations. *Mol. Ecol.* 13, 946–952. doi: 10.1111/1755-0998.12129

Piazzon, M. C., Naya-Català, F., Perera, E., Palenzuela, O., Sitjà-Bobadilla, A., and Pérez-Sánchez, J. (2020). Genetic selection for growth drives differences in intestinal microbiota composition and parasite disease resistance in gilthead sea bream. *Microbiome* 8:168. doi: 10.1186/s40168-020-00922-w

Ray, C., Bujan, N., Tarnecki, A., Davis, A. D., Browdy, C., and Arias, C. (2017). Analysis of the gut microbiome of Nile tilapia *Oreochromis niloticus* L. fed diets supplemented with Previda® and saponin. *J. Fishscicom* 11:36. doi: 10.21767/1307-234X.1000116

Reese, A. T., and Dunn, R. R. (2018). Drivers of microbiome biodiversity: a review of general rules, feces, and ignorance. *MBio* 9:e01294–18. doi: 10.1128/mBio.01294-18

Risely, A. (2020). Applying the core microbiome to understand host–microbe systems. *J. Anim. Ecol.* 89, 1549–1558. doi: 10.1111/1365-2656.13229

Rognes, T., Flouri, T., Nichols, B., Quince, C., and Mahé, F. (2016). VSEARCH: a versatile open source tool for metagenomics. *PeerJ* 4:e2584. doi: 10.7717/peerj.2584

Romana-Eguia, M. R. R., Ikeda, M., Basiao, Z. U., and Taniguchi, N. (2005). Genetic changes during mass selection for growth in Nile tilapia, *Oreochromis niloticus* (L.), assessed by microsatellites. *Aquac. Res.* 36, 69–78. doi: 10.1111/j.1365-2109.2004.01185.x

Santos, V. B. D., Mareco, E. A., and Dal Pai Silva, M. (2013). Growth curves of Nile tilapia (*Oreochromis niloticus*) strains cultivated at different temperatures. *Acta Sci. Anim. Sci.* 35, 235–242. doi: 10.4025/actascianimsci.v35i3.19443

Schultz, B., Serão, N., and Ross, J. W. (2020). "Genetic improvement of livestock, from conventional breeding to biotechnological approaches," in *Animal Agriculture*. eds. F. W. Bazer, G. C. Lamb and G. Wu (London, UK: Elsevier), 393–405.

Semple, S. L., Bols, N. C., Lumsden, J. S., and Dixon, B. (2020). Understanding the pathogenesis of *Flavobacterium psychrophilum* using the rainbow trout monocyte/macrophage-like cell line, RTS11, as an infection model. *Microb. Pathog.* 139:103910. doi: 10.1016/j.micpath.2019.103910

Shin, N.-R., Whon, T. W., and Bae, J.-W. (2015). *Proteobacteria*: microbial signature of dysbiosis in gut microbiota. *Trends Biotechnol.* 33, 496–503. doi: 10.1016/j.tibtech.2015.06.011

Silva, B. R. D. S., Derami, M. S., Paixão, D. A., Persinoti, G. F., Dias Da Silveira, W., and Maluta, R. P. (2020). Comparison between the intestinal microbiome of healthy fish and fish experimentally infected with *Streptococcus agalactiae*. *Aquac. Res.* 51, 3412–3420. doi: 10.1111/are.14676

Simões, L. N., Lombardi, D. C., Gomide, A. T. M., and Gomes, L. C. (2011). Efficacy of clove oil as anesthetic in handling and transportation of Nile tilapia, *Oreochromis niloticus* (Actinopterygii: Cichlidae) juveniles. *Zoologia* 28, 285–290. doi: 10.1590/S1984-46702011000300001

Souza, F. P. D., Lima, E. C. S. D., Urrea-Rojas, A. M., Suphoronski, S. A., Facimoto, C. T., Bezerra Júnior, J. D. S., et al. (2020). Effects of dietary supplementation with a microalga (*Schizochytrium* sp.) on the hemato-immunological, and intestinal histological parameters and gut microbiota of Nile tilapia in net cages. *PLoS One* 15:e0226977. doi: 10.1371/journal.pone.0226977

Standen, B., Rawling, M., Davies, S., Castex, M., Foey, A., Gioacchini, G., et al. (2013). Probiotic *Pediococcus acidilactici* modulates both localised intestinal-and peripheral-immunity in tilapia (*Oreochromis niloticus*). *Fish Shellfish Immunol.* 35, 1097–1104. doi: 10.1016/j.fsi.2013.07.018

Sugita, H., Miyajima, C., and Deguchi, Y. (1991). The vitamin B12-producing ability of the intestinal microflora of freshwater fish. *Aquaculture* 92, 267–276. doi: 10.1016/0044-8486(91)90028-6

Suphoronski, S., Chideroli, R., Facimoto, C., Mainardi, R., Souza, F., Lopera-Barrero, N., et al. (2019). Effects of a phytogenic, alone and associated with potassium diformate, on tilapia growth, immunity, gut microbiome and resistance against Francisellosis. *Sci. Rep.* 9:6045. doi: 10.1038/s41598-019-42480-8

Sylvain, F.-É., and Derome, N. (2017). Vertically and horizontally transmitted microbial symbionts shape the gut microbiota ontogenesis of a skin-mucus feeding discus fish progeny. *Sci. Rep.* 7:5263. doi: 10.1038/s41598-017-05662-w

Tsuchiya, C., Sakata, T., and Sugita, H. (2008). Novel ecological niche of *Cetobacterium somerae*, an anaerobic bacterium in the intestinal tracts of freshwater fish. *Lett. Appl. Microbiol.* 46, 43–48. doi: 10.1111/j.1472-765X.2007.02258.x

Wagner, M. R., Roberts, J. H., Balint-Kurti, P. J., and Holland, J. B. (2020). Microbiome composition differs in hybrid and inbred maize. bioRxiv [Preprint]. doi: 10.1101/2020.01.13.904979

Wei, T., and Simko, V. (2017). R package "corrplot": Visualization of a correlation matrix (Version 0.84). Available at: https://github.com/taiyun/corrplot (Accessed April 28, 2021).

Wein, T., Dagan, T., Fraune, S., Bosch, T. C., Reusch, T. B., and Hülter, N. F. (2018). Carrying capacity and colonization dynamics of *Curvibacter* in the hydra host habitat. *Front. Microbiol.* 9:443. doi: 10.3389/fmicb.2018.00443

Wickham, H. (2011). ggplot2. *Wiley Interdiscip. Rev. Comput. Stat.* 3, 180–185. doi: 10.1002/wics.147

Wiles, T. J., Jemielita, M., Baker, R. P., Schlomann, B. H., Logan, S. L., Ganz, J., et al. (2016). Host gut motility promotes competitive exclusion within a model intestinal microbiota. *PLoS Biol.* 14:e1002517. doi: 10.1371/journal.pbio.1002517

Wynne, J. W., Thakur, K. K., Slinger, J., Samsing, F., Milligan, B., Powell, J. F. F., et al. (2020). Microbiome profiling reveals a microbial dysbiosis during a natural outbreak of Tenacibaculosis (yellow mouth) in Atlantic Salmon. *Front. Microbiol.* 11:586387. doi: 10.3389/fmicb.2020.586387

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

Zar, J. H. (2014). Spearman rank correlation: overview. Wiley StatsRef: Statistics Reference Online.

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

Zhang, Z., Yu, Y., Jiang, Y., Wang, Y., Liao, M., Rong, X., et al. (2020). The intestine of artificially bred larval turbot (*Scophthalmus maximus*) contains a stable core group of microbiota. *Arch. Microbiol.* 202, 2619–2628. doi: 10.1007/s00203-020-01984-y

Zoetendal, E. G., Akkermans, A. D., Akkermans-Van Vliet, W. M., De Visser, J. A. G., and De Vos, W. M. (2001). The host genotype affects the bacterial community in the human gastronintestinal tract. *Microb. Ecol. Health Dis.* 13, 129–134. doi: 10.3402/mehd.v13i3.8013

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Paper III

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Intergenerational Transfer of Persistent Bacterial Communities in Female Nile Tilapia

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Resident microbial communities that can support various host functions play a key role in their development and health. In fishes, microbial symbionts are vertically transferred from the parents to their progeny. Such transfer of microbes in mouthbrooder fish species has not been reported yet. Here, we employed Nile tilapia (*Oreochromis niloticus*) to investigate the vertical transmission of microbes across generations using a 16S rRNA amplicon sequencing approach, based on the presence of bacteria in different generations. Our analysis revealed that the core microbiome in the buccal cavity and posterior intestine of parents shapes the gut microbiome of the progeny across generations. We speculate that the route of this transmission is *via* the buccal cavity. The identified core microbiome bacteria, namely *Nocardioides, Propionibacterium*, and *Sphingomonas* have been reported to play an essential role in the health and development of offspring. These core microbiome members could have specific functions in fish, similar to mammals.

Keywords: microbiome, buccal cavity, intestine, Nile tilapia, vertical microbe transfer, *Nocardioides*, *Propionibacterium*, *Sphingomonas*

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INTRODUCTION

Microbial colonization and assemblage on various niches of hosts is a complex process, which is dependent on genetic as well as environmental background. Many studies have reported the significant role of these microbial communities in humans (Rackaityte and Lynch, 2020), fish (Legrand et al., 2020), and livestock (Cholewińska et al., 2021); they are composed of bacteria, fungi, and archaea (Berg et al., 2020). Different aspects of microbiota have been intensively studied to report valuable information about their influence on human health (Rackaityte and Lynch, 2020). Microbiota supports many functions to satisfy the nutritional needs of the host, mainly due to the ability of the microorganisms to produce vitamins (Nagy-Szakal et al., 2012) and valuable metabolites such as short chain fatty acids (García-Mantrana et al., 2019; Silva et al., 2020). In addition, host-associated microbes train and modulate the immune system to establish tolerance to commensal bacteria (Chai et al., 2014) as well as ward off invasive pathogens (Sylvain and Derome, 2017; Ferretti et al., 2018; Yukgehnaish et al., 2020; Cholewińska et al., 2021). It is noteworthy that early life food components can promote the colonization of specific microbes and their syntrophy with beneficial microbes (Kostopoulos et al., 2020). In fact, microbiome development during the early life of hosts helps in the intrinsic training of the immune functions and shaping of microbiome

composition (Sylvain and Derome, 2017; Ferretti et al., 2018). Therefore, in the past years, scientists have been studying the vertical transfer of microbes from mother to infant (Ferretti et al., 2018; Rackaityte and Lynch, 2020) and from parents to progeny in animals (Sylvain and Derome, 2017; McGrath-Blaser et al., 2021; Mika et al., 2021). Microbe transfer in most organisms occurs in three ways: (i) vertical transfer of essential maternal microbes that aid host development at the early stage of life; (ii) horizontal transfer *via* ingestion of microbes from diet or surrounding environment to which host is exposed to; (iii) environmental transfer between conspecific organisms during social or sexual interaction (Leftwich et al., 2020). Nevertheless, the transfer routes vary across species. For example, in chicken, successive transfer of resident microbes takes place from the oviduct and cloaca to eggshell, egg white, and then the embryo (Lee et al., 2019). In livestock, microbes present in the birth canal colonize newborns (Estellé, 2019). In humans, microbes from the skin and vagina of mothers colonize different body sites of infants, and this type of vertical transmission continues through direct contact (Ferretti et al., 2018).

The mechanism of microbial transmission in aquatic animals differs from those of mammals. In most fish species, the early stage microbiome is shaped by the environment (Llewellyn et al., 2014). As the fish grows, the environmental influence will be overshadowed by other factors (Llewellyn et al., 2014). Atlantic salmon (*Salmo salar*) embryos were reported to have lower diversity compared to hatchlings, probably indicating the impact of factors other than the original determinant (Lokesh et al., 2019). In zebrafish (*Danio rerio*) larvae, horizontal transmission of microbial symbionts occurs from the surrounding environment (Stephens et al., 2016). In another fish model, discus (*Symphysodon aequifasciata*), also the larvae obtain their microbial symbionts *via* horizontal transmission from the surrounding water (Sylvain and Derome, 2017). However, during the fry stage of discus, vertical transmission prevails because parents feed their skin mucus to their offspring (Sylvain and Derome, 2017). Interestingly, in pipefish (*Syngnathus typhle*), specific bacteria from both parents shape the microbiota of the embryo because eggs are transferred from mother to paternal pouches (Beemelmanns et al., 2019). The little skate (*Leucoraja erinacea*) egg capsule holds a variety of bacteria, which will be transferred to offspring (Mika et al., 2021). To our knowledge there are no publications on the microbial transmission from a mouthbrooder to their offspring. Hence, we used Nile tilapia (*Oreochromis niloticus*) females as a model to understand the bacterial transfer from mother to offspring, and across generations employing the 16S rRNA amplicon sequencing technology, based on the presence of bacteria in different samples.

MATERIALS AND METHODS

Ethics Statement

This study was performed under a license from the Norwegian Animal Research Authority (FOTS ID 10427). The experiment was conducted according to the guidelines for research using experimental animals; 3Rs principle, fish welfare and respect toward animals were given weightage while balancing between experimental procedures and benefits from the results.

Experimental Fish

Nile tilapia for this study were produced from fertilized eggs that were being incubated by wild mouthbrooders, caught from Nile River, Luxor, Egypt (location GPS: 25°39′56″ N, 32°37′07″ E). The eggs were kept in a 60-L tank for 2 weeks. Water in these tanks was replaced with sterilized water every 2 days. The eggs hatched on day 5–6 after fertilization, and the larvae were transported to the research station of Nord University, Bodø, Norway. Juveniles obtained from different wild females were tagged and assigned as the base population or the first generation (F0). The second (F1) and third (F2) generations were obtained from the F0 generation. All fish generations were reared in a common garden in a recirculating aquaculture system for 8 months to avoid the influence of environmental confounding factors. Rearing conditions were: pH 7.6, oxygen saturation 100%, temperature 28°C, and photoperiod 11:13 dark:light. The experimental fish were fed *ad libitum* (0.15–0.8 mm) Amber Neptun pellets, Skretting, Norway (Konstantinidis et al., 2021). **Figure 1** illustrates the breeding strategy to produce the experimental fish.

In the present study, we first examined the microbiota of the wild mouthbrooders (caught from River Nile, Egypt) from which their offspring (F0, maintained in Norway) were produced. To investigate the microbial transfer across generations, we employed two generations (F0 and F2) -from parent 1: F059 (F0), F2C1 (F2, fish from same parents); from parent 2: F072 (F0), F2S2 (F2, fish from same parents). Furthermore, to investigate differences in the microbial composition in F2, we compared the microbiota of two families (within F2; F2S1, F2S2 from F072 vs. F2C1 from F059) from the above mentioned F0 parents. The F1 generation [data published in Abdelhafiz et al. (2021a)] was not included in this study because we did not employ any microbial enrichment kits (Abdelhafiz et al., 2021a), which limits comparability between datasets.

Sample Collection

Buccal cavity mucus and posterior intestine samples were collected from the wild-caught fish (n = 3) and transferred to cryotubes containing DNA/RNA shield (ZYMO Research Corp, Irvine, CA, United States). As for the samples from the fish reared in controlled conditions, they were collected from fish that were starved for 48 h. Buccal cavity and intestine samples from 20 fish (five fish in each group) were collected for the microbiota studies. Before collecting the samples, the fish were sacrificed by exposing them to an emulsion containing 12 mL of clove oil (Sigma Aldrich, MO, United States), 96% ethanol (1:10 v/v), and 10 L of water (Podgorniak et al., 2019). Mucus samples from the buccal cavity were taken using swabs (Copan Italia, Brescia, Italy), which were transferred to cryotubes and immediately frozen in liquid nitrogen. In addition, posterior intestine mucus samples were collected as described previously (Abdelhafiz et al., 2021a). The collected samples were stored at −80°C until further use.

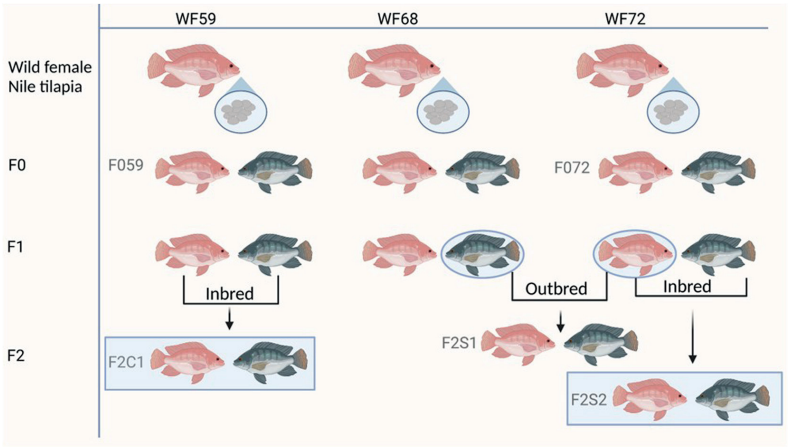

FIGURE 1 | Breeding plan of the different generations of Nile tilapia. F0 generation: F059 and F072; F2 generation: F2C1, F2S1, and F2S2. Inbred groups (F2C1 and F2S2) and outbred (F2S1). WF59, W68, and WF72 are the wild mothers. The pink color represents female and gray male fish.

Microbial DNA Extraction and Library Preparation

DNA was extracted from both the mouth and posterior intestine using QIAamp DNA stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The collected samples were transferred to a 5 ml tube that contained 1.4 mm zirconium oxide beads (Cayman chemical, Ann Arbor, MI, United States). Then, 2 ml of InhibitEX buffer (Qiagen) was added to the tube. The extracted DNA was eluted in 75 μl ATE buffer. Thereafter, the quality and quantity of the extracted DNA were checked with NanoDrop spectrophotometer ND-8000 (Thermo Fisher Scientific Inc., Waltham, MA, United States).

Prior to library preparation, the extracted DNA from each sample was treated with REPLI-G kit (Qiagen) to enrich the microbial DNA. Library preparation and sequencing were performed as described previously (Abdelhafiz et al., 2021a).

Data Processing and Analyses

Paired-end reads were truncated at 270 bp using VSEARCH (Rognes et al., 2016), and then processed using MICCA pipeline, V1.7.2 (Albanese et al., 2015). Sequences of paired-end reads with a minimum overlap length of 60 bp and a maximum mismatch of 20 bp were merged. Next, forward and reverse primers from the merged reads were trimmed off and the reads that did not contain the primers were discarded. The sequences with an expected error rate >0.75 were filtered out (Edgar and Flyvbjerg, 2015). Then the obtained reads were denoised using the "*de novo* unoise" method implemented in MICCA, which utilizes the UNOISE3 algorithm (Edgar, 2016). Thereafter, RDP classifier (Lan et al., 2012) was used to assign the taxonomic names of the representative bacterial amplicon sequence variants (ASVs). The sequences were aligned using the NAST (DeSantis et al., 2006) multiple sequence aligner, and a phylogenetic tree was prepared using the FastTree software available in the MICCA pipeline, as described previously (Abdelhafiz et al., 2021a,b). The downstream analyses were performed using the phyloseq package in R (McMurdie and Holmes, 2013).

Statistical Analysis

To understand the differences in richness, evenness, and dominance of the bacterial communities across generations, we performed α-diversity analysis by calculating the Chao1 species richness, Shannon and Simpson diversities using estimate_richness function in *phyloseq* R package (version 1.38.0). Kruskal-Wallis rank sum test (R package *stats* version 4.1.2) and Dunn's test (R package *rstatix* version 0.7.0) were used to check the differences between the study groups. On the other hand, to understand the dissimilarities in bacterial compositions, we performed β-diversity analysis using unweighted and weighted UniFrac distances (Lozupone and Knight, 2005). The differences were visualized by principal coordinates analysis (PCoA). After checking the dispersions within the data set of each generation using the beta.disper function in the R package *vegan* version 2.5-7, statistically significant differences between the groups were assessed using Permutational Multivariate Analysis of Variance Using Distance Matrices (Anderson, 2001), i.e., employing the adonis function implemented in the *vegan* R package version 2.5-7 (Oksanen et al., 2013). Furthermore, *post-hoc* test in *RVAideMemoire* R package (version 0.9-81) was employed to understand the differences between the groups. To detect the differentially abundant ASVs in the unrarefied data (Weiss et al., 2017), we used the R package *DESeq2* version 1.34.0 (Love et al., 2014). The core microbiome analysis was performed using *microbiome* (version 1.16.0) and *microbiomeutilities* (version 1.00.16) packages, at a detection level of 0.1% and prevalence level of 0.75%. Euler diagrams were

generated for core microbiomes present in different generations; using the R package *Eulerr* package version 6.1.1 (Larsson, 2018). The differences in core bacterial communities across generations were analyzed by performing PERMANOVA on weighted and unweighted UniFrac distances.

RESULTS

The constructed amplicon 16S rRNA gene libraries generated 8,323,440 high-quality reads with an average coverage of 138,724 reads per sample. The reads were rarefied to 14,000 reads per sample (without replacement). Out of the 58 samples, one library with a number of reads below the cut-off was discarded. After normalization, we obtained 9535 ASVs, distributed among 27 phyla and 383 genera.

We first determined the relative abundance of the most abundant phyla and genera in the buccal cavity mucus and posterior intestine of the wild fish. Thereafter, to understand the microbial transfer across generations, we describe the differences in composition between two F0 families and the corresponding inbred F2, and between F0 and one outbred family (**Figure 1**). Later we disclose the differences in the bacteria between fish groups within F2.

Microbial Composition in the Wild Parents, F0, and F2 Generations

Relative Abundance of Bacteria in Wild Fish

The dominant phyla in the buccal cavity and posterior intestine of the wild fish were *Actinobacteria, Proteobacteria*, and *Firmicutes*. However, their abundance was different across samples (**Figure 2A**). The most abundant genera in the buccal cavity and posterior intestine were *Nocardioides*, *Propionibacterium*, *Paenibacillus*, and *Methylobacterium* (**Figure 2B**).

Relative Abundance of Bacteria in F0 and F2 Generations From Different Mothers

The most abundant phyla in the buccal cavity mucus in F0 and F2 generations were *Actinobacteria* followed by *Firmicutes*, *Proteobacteria,* and *Bacteriodetes* (**Figure 3A**). At the genus level, the buccal cavity was mostly dominated by *Propionibacterium* and *Nocardioides* (**Figure 3B**). However, other genera such as *Paenibacillus*, *Sphingomonas*, *Corynebacterium*, and *Enhydrobacter* were also common but in lower abundance compared to *Propionibacterium* and *Nocardioides* (**Figure 3B**). The most dominant phyla in the posterior intestine of the F0 and F2 generations were *Actinobacteria, Firmicutes*, and *Proteobacteria* (**Figure 4A**) while the dominant genera were *Propionibacterium*, *Nocardioides*, and *Solirubrobacter* (mostly in F2), *Corynebacterium*, *Enhydrobacter*, and *Paracoccus* (**Figure 4B**).

Alpha and Beta Diversity and Differential Abundance Across Generations

To delineate the alpha diversity of the bacterial communities in the mucus of the buccal cavity and posterior intestine of the F0 and F2 generations, we employed three different ecological diversity measures. Species richness (Chao1), effective number of common (Shannon diversity), and dominant bacteria (Simpson diversity) in the mucus of buccal cavity as well as posterior intestine were not significantly different between F0 and F2 generations (**Figures 5A,B**). Weighted and unweighted UniFrac distances-based beta diversity analysis also did not reveal any difference between the mucus bacterial communities (both from the buccal cavity and posterior intestine) of the different generations (**Figures 5C,D**). As for the posterior intestine of the fish families, we observed a statistical trend that indicated the difference in the weighted and unweighted UniFrac distances of the microbial communities [**Figure 5D**: unweighted UniFrac (F059, F2C1, F072, and F2S2); R^2 = 0.86, P = 0.05, **Figure 5D**: weighted UniFrac (F059, F2C1, F072, and F2S2); R^2 = 0.23, P = 0.09]. The *post-hoc* test revealed differences in the microbial communities in the posterior intestine between F059 (F0) and F2C1 (F2) (unweighted UniFrac; P = 0.04). Although F059 and F2C1 are statistically significant, DESeq2 did not find any ASV that is significantly different between the two fish groups.

Core Microbiome in the Wild Fish and Two Generations Bred in Captivity

To investigate the microbial transfer across generations, first we identified the core microbiome/microbes (present in 80% of the samples) in the wild fish. We presume that these microbes are essential for the host and therefore are transferred from one generation to another or common between generations. Hence, we also identified the shared core microbiome that is found in both F0 and F2 generations. At the genus level, *Nocardioides* and *Propionibacterium* were the most abundant core microbiome members in the mucus from the buccal cavity and posterior intestine of the wild fish (WF59, WF68, and WF72) (**Figure 6A**). Moreover, *Sphingomonas* and *Corynebacterium* were also core microbiome members in both the mouth and posterior intestine (**Figure 6A**). The buccal cavity mucus of F0 and F2 generations also had both *Nocardioides* and *Propionibacterium* as the most abundant members of the core microbiome (**Figure 6B**). Furthermore, *Sphingomonas* and *Enhydrobacter* were also abundant in some fish from F0 and F2 generations (**Figure 6B**). In addition, we found *Rhodococcus* in low abundance in the F2 generation. Furthermore, one ASV which is classified as *Propionibacterium* was common in both F0 and F2 generations, while three ASVs that also belong to the genus *Propionibacterium* were common only in the F0 generation (F059 and F072). ASVs of *Actinomycetales* were also shared in the F0 generation. Moreover, two ASVs of the genus *Nocardioides* were common in F0 and F2 generations (**Figure 6D** and **Supplementary Table 1**). In the posterior intestine of all the samples from both lineages (F059, F2C1, and F072, F2S2), *Nocardioides* and *Propionibacterium* were the most abundant bacteria (**Figure 6C**). Furthermore, in F0 (F059 and F072) *Sphingomonas* was also observed as the prominent genus, but not detected as frequently as *Nocardioides* and *Propionibacterium*. Moreover, ASVs of *Nocardioides* (*DENOVO* 2) and *Propionibacterium* (*DENOVO* 1) were present in different generations (**Supplementary Table 2** and **Figure 6E**). However,

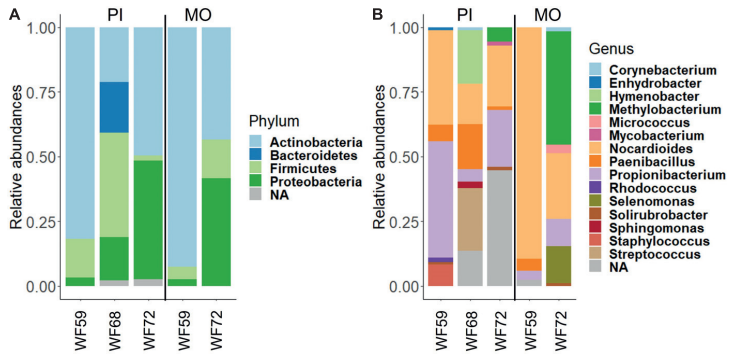

FIGURE 2 | Relative abundance of bacteria found in the buccal cavity mucus and posterior intestine of wild Nile tilapia. **(A)** Phylum level. **(B)** Genus level. Three samples from the posterior intestine of three wild fish (WF59, WF68, and WF72). However, for the buccal cavity we employed the samples from only two wild fish (WF59 and WF72). MO, buccal cavity; PI, posterior intestine; NA, Unclassified.

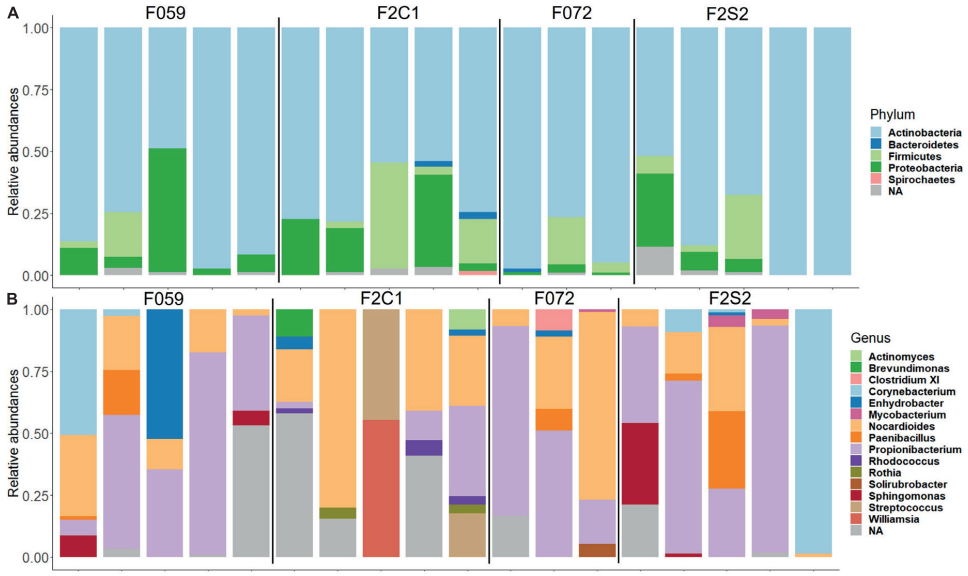

FIGURE 3 | Relative abundance of bacteria found in the buccal cavity mucus of Nile tilapia bred in captivity. **(A)** Phylum level. **(B)** Genus level. F0 generation: F059, F072 and F2 generation: F2C1, F2S2.

one ASV belonging to *Nocardioides* (*DENOVO* 2) was not found in F2S1 generation.

Comparison of the Microbial Composition Among Second Generation Families of Nile Tilapia

The differences/similarities in the buccal cavity and posterior intestine mucus of families F2C1, F2S1, and F2S2 from the second generation of Nile tilapia were studied. In the buccal cavity of F2C1 and F2S2, the most dominant phyla were *Actinobacteria, Proteobacteria, Firmicutes*, *Bacteroidetes*, and *Spirochaetes*; latter two only in some samples (**Figure 3A**). At the genus level, *Propionibacterium*, *Nocardioides*, and *Corynebacterium* (in some samples) were the most abundant bacteria in both families (**Figure 3B**). Furthermore, in the F2C1 family, *Rhodococcus* and *Enhydrobacter* were also abundant in some samples. On the other hand, in F2S2 family, *Sphingomonas*

appeared in some of the samples. In the posterior intestine, *Actinobacteria, Firmicutes, Proteobacteria*, and *Bacteroidetes* were the most dominant phyla in both families (**Figure 4A**). In the F2S2 family, in addition to *Propionibacterium, Nocardioides* and *Corynebacterium, Paenibacillus* (in some samples), and *Pediococcus* (in some samples) also belonged to the most dominant genera (**Figure 4**). On the other hand, the buccal cavity of F2C1 and F2S1 were mostly dominated by *Actinobacteria, Firmicutes, Proteobacteria, Bacteroidetes, Nitrospirae* (F2S1, one sample), and *Spirochaetes* (F2C1, one sample). Bacteria belonging to *Nitrospirae* were more dominant in F2S1, while *Spirochaetes* were higher in F2C1 (**Supplementary Figure 1A**). The most abundant genera in the buccal cavity were *Propionibacterium* and *Nocardioides*. The F2S1 family was mostly dominated by *Propionibacterium*. In addition, *Nocardioides, Sphingomonas, Spirosoma*, and *Nitrospira* were also abundant in some samples of F2S1 (**Supplementary Figure 1B**). In the posterior intestine of F2C1 and F2S1, *Actinobacteria, Firmicutes, Proteobacteria*, and *Bacteroidetes* were dominant (**Supplementary Figure 2A**). At the genus level, F2C1 was mostly dominated by *Propionibacterium, Nocardioides, Paracoccus*, and *Solirubrobacter*. The F2S1 family was dominated by *Propionibacterium* (**Supplementary Figure 2B**) but *Sphingomonas, Geobacillus, Paenibacillus*, and *Alloiococcus* were also abundant.

Alpha and Beta Diversity and Differentially Abundant Amplicon Sequence Variants in the F2 Families From the Second Generation of Nile Tilapia

Statistically significant differences were not detected for the alpha diversity measures of the mucus bacterial communities (in the buccal cavity as well as posterior intestine) between F2C1 and F2S2 families (**Figures 5A,B**). Similarly, beta diversity analysis did not reveal any statistically significant differences in both unweighted and weighted UniFrac distances (**Figures 5C,D**). Statistically significant differences were also not found in alpha diversity values of the F2C1 and F2S1 families (**Supplementary Figures 3A,B**). However, statistically significant differences were detected for the weighted UniFrac distances; for both the buccal cavity and the posterior intestine microbiota ($R^2 = 0.67$, $P = 0.01$; $R^2 = 0.30$, $P = 0.02$, respectively; **Supplementary Figures 3C,D**).

We performed differential abundance analyses to understand the differences between the buccal cavity bacteria of inbred families (F2S2 vs. F2C1) and between outbred and inbred (F2S1 vs. F2S2) to investigate the influence of breeding strategy on the buccal cavity microbial composition. The result revealed that one ASV had significantly lower abundance in F2S2 compared to the F2C1: *Kocuria* with a log2foldchange (LFC) of −35 (**Supplementary Figure 4A**). On the other hand, in the buccal cavity of F2S1, *Corynebacterium*, *Propionibacterium*, and *Staphylococcus* had higher abundance (more than 5 LFC) compared to the F2C1 family (**Supplementary Figure 4B**). While the abundance of *Nocardioides* and *Rothia* were lower (5 LFC) in F2S1 compared to F2C1 (**Supplementary Figure 4B**). In the case of the posterior intestine, *Corynebacterium* had lower abundance (25 LFC) in F2S1 compared to F2C1. Furthermore, *Brevibacillus, Geobacillus, Paenibacillus*, and *Sphingomonas* had higher abundance (10 LFC) in the F2S1 family compared to F2C1 (**Supplementary Figure 4C**).

DISCUSSION

Microbial transmission from different body sites of parents to offspring is extensively studied in humans compared to other animals. The gathered data on microbial transmission have provided insights into the beneficial as well as disease-causing microbes that are passed on to generations. A recent study has reported that bacterial transmission is dependent on both relationship and cohabitation (Valles-Colomer et al., 2022). Transfer of bacteria from mother to infant shapes the microbial composition in infants. It should be noted that delivery mode (cesarean section) can disrupt the normal assemblage of microbes in infants, and this issue can make the offspring susceptible to diseases such as celiac disease, asthma, and obesity (Mueller et al., 2015). These facts indicate the importance of normal microbial community at the early stage of organism development. Only a few studies have reported microbe transfer in aquatic animals. These studies showed evidence of vertical microbial transmission (Stephens et al., 2016; Sylvain and Derome, 2017). Here we present the first report of bacterial transmission across generations of Nile tilapia, a mouthbrooder fish species.

Our results revealed the dominance of the phyla, *Actinobacteria, Proteobacteria*, and *Firmicutes* in the buccal cavity and posterior intestine of the wild Nile tilapia individuals. These phyla are known to be dominant in the gut of wild Nile tilapia (Bereded et al., 2020; Bereded et al., 2021) and many other fish species (Legrand et al., 2020; Yukgehnaish et al., 2020). On the other hand, the buccal cavity microbiome of wild Nile tilapia has not been previously reported. The microbial composition in the buccal cavity and the posterior intestine in F0 and F2 generations was also dominated by the aforementioned phyla. Furthermore, *Actinobacteria, Proteobacteria*, and *Firmicutes* were found as the dominant phyla in breast tissue and human milk (Togo et al., 2019). In our study, at the genus level, the most abundant bacteria were *Propionibacterium* and *Nocardioides*; in both the buccal cavity and the posterior intestine. We found that these genera are also dominant in the wild fish samples though their abundance was different across individuals.

Various body sites of fishes harbor microbes and different factors such as diet, environment, and host pressure may help in the establishment of a balanced healthy microbiota which is known as normobiosis (Johny et al., 2021). From an ecological point of view, niche- and neutral- processes lead to well-established host microbial communities (Liao et al., 2016). The niche-based theory indicates the deterministic effects of factors such as environmental conditions, among which rearing systems can influence the gut microbiome assemblage during the development of Nile tilapia larvae. Shared OTUs of the rearing water and gut bacterial communities of Nile tilapia larvae points to the niche selection of the water bacteria (Giatsis et al., 2015). Distinct core gut microbiota in zebrafish was

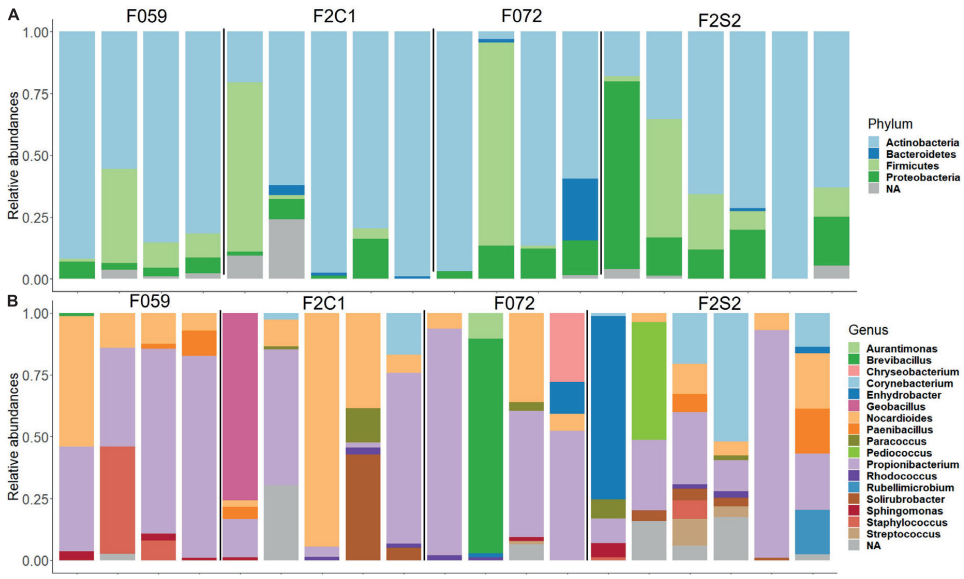

FIGURE 4 | Relative abundance of bacteria found in the mucus of the posterior intestine from Nile tilapia bred in captivity. **(A)** Phylum level. **(B)** Genus level. F0 generation: F059, F072 and F2 generation: F2C1 (Inbred), F2S2 (Inbred). NA, Unclassified.

suggested to be due to host selective pressure or a niche selection based on certain bacteria in the rearing water (Roeselers et al., 2011). A study reported differences in the gut of larvae reared in two different rearing systems. However, when they were moved to a common recirculating aquaculture system (RAS), the gut microbial diversity and composition was similar in the individuals (Giatsis et al., 2014; Deng et al., 2021). It is also known that as fishes grow host pressure overtakes the environmental factors in deciding the microbial profile (Talwar et al., 2018). In the present study, we did not find any difference in the microbial richness and evenness in F0 and F2 generations that were reared in a common garden. This may indicate that the oral microbiome is colonized by similar microbial communities. However, in the case of the posterior intestine, we found a statistical trend, probably indicating a difference between the microbial communities in F0 and F2 generations. We speculate that the differences are due to breeding/genetic effects in F059 (F0) and F2C1 (F2) families (Abdelhafiz et al., 2021a). To understand this fact, we analyzed the microbial composition in two families of the F2 generation; these results also did not reveal any differences in the diversities of the microbial communities in F2C1 and F2S2, which are both inbred groups. On the other hand, we found dissimilarities between the microbial community compositions of the buccal cavity and the posterior intestine in F2C1 (inbred) and F2S1 (outbred), based on the weighted UniFrac distance of the inbred and outbred groups. Furthermore, the differential expression analysis of ASVs revealed significant differences between ASVs in both the buccal cavity and the posterior intestine in all the F2 family comparisons. When the buccal cavity communities of the two inbred groups (F2C1 and F2S2) were compared, *Kocuria* was noted to be the less abundant bacteria in F2S2 compared to F2C1. On the other hand, when we compared the buccal cavity communities of an inbred group with those of an outbred group (F2C1 and F2S1, respectively) we found differences in the microbial taxa. Furthermore, one of the ASVs of the core microbiome belonged to *Nocardioides* (*DENOVO* 2), which was not found in the outbred group (F2S1). The differences between the microbial communities, in this case, are likely due to the breeding strategy (Abdelhafiz et al., 2021a).

The core microbiome is known to be present across any population of a particular host organism, and this community plays an essential role in the host biological functions (Risely, 2020). Although it is well known that many factors modulate the microbiome composition in a host, the presence of the core microbial community may not be disrupted (Salonen et al., 2012; Henderson et al., 2015; Deng et al., 2021). In our previous study, we reported the lower microbial inter-individual variability amongst the intestine bacteria of the inbred Nile tilapia (Abdelhafiz et al., 2021a). However, in the present study, our analysis showed inter-individual variation across the buccal cavity and posterior intestine samples of the F2 and F0 generation. In fish, microbial inter-individual variation is common. This was observed even in individuals (cod and bluefin tuna larvae) reared in the same tank (Fjellheim et al., 2012; Gatesoupe et al., 2013). Furthermore, when the microbial interactions between core microbiome members and

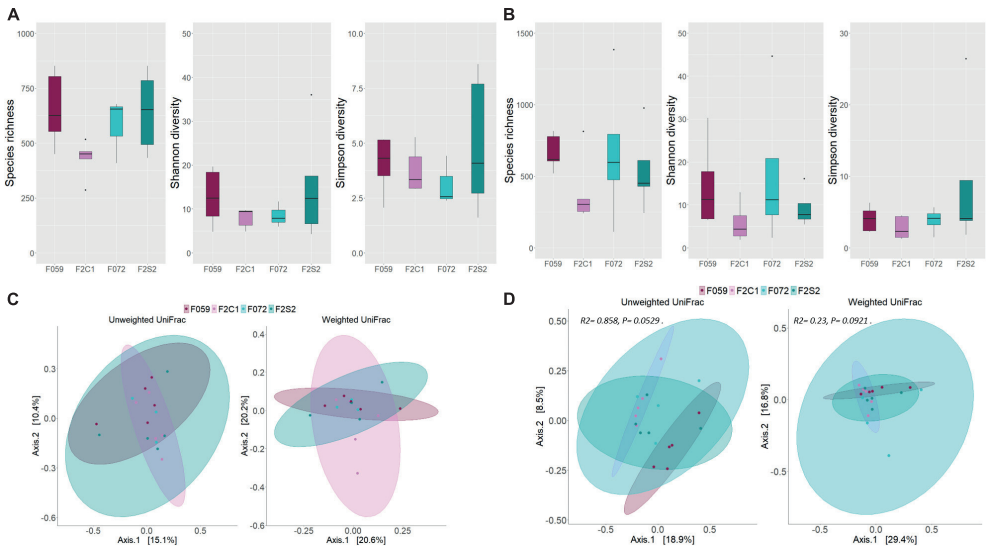

FIGURE 5 | Differences in microbial diversity and composition of mucus bacteria from the buccal cavity and posterior intestine of Nile tilapia from F0 and F2 generations. Chao1, Shannon and Simpson diversities of **(A)** buccal cavity bacteria, **(B)** intestine bacteria. PCoA plots of the unweighted and weighted distances associated with **(C)** buccal cavity bacteria, **(D)** posterior intestine bacteria. Note that in panel **(C)**, the ellipse is not drawn for F072 because there were only three samples for this group. F0 generation: F059, F072 and F2 generation: F2C1, F2S2.

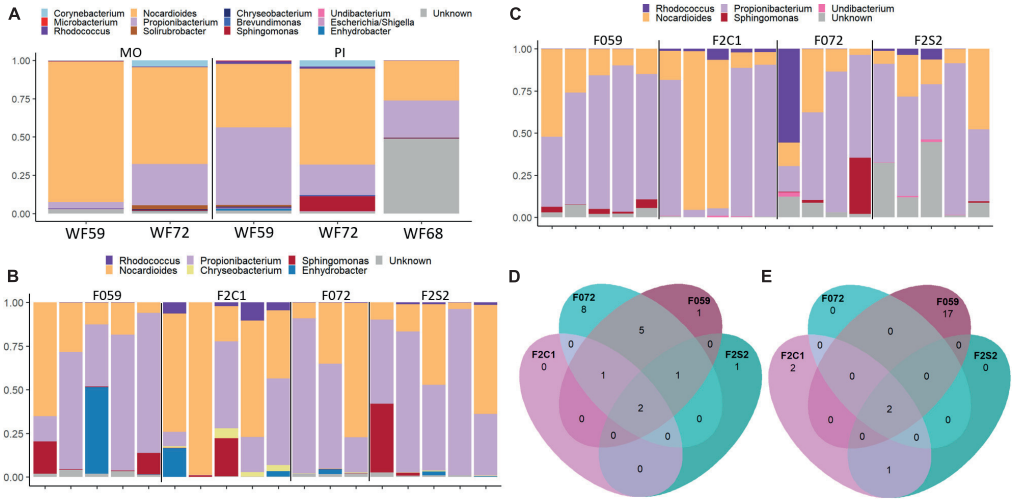

FIGURE 6 | Core and shared microbiome in the mucus from the buccal cavity and posterior intestine of Nile tilapia from wild, F0, and F2 generations. **(A)** Core microbiome in the mouth mucus and posterior intestine of wild Nile tilapia. Core microbiome in the **(B)** buccal cavity and **(C)** intestine of F0 and F2 generations. Shared core microbiome in the **(D)** buccal cavity and **(E)** intestine of F0 and F2 generations. In panel **(D)**, five ASVs of *Propionibacterium* were common in both F059 and F072 and one ASV was common in both F0 and F2 generations. MO, buccal cavity; PI, posterior intestine. F0 generation: F059, F072 and F2 generation: F2C1, F2S2.

other microbes are positive, competition between the microbes will be less (Jones et al., 2018), allowing host genetic/selection or ecological pressure to shape the microbial compositional variation (Shan and Cordero, 2020). Moreover, the inter- and intra-individual compositional variations in humans are regarded stable over time (Jones et al., 2018).

In Nile tilapia larvae, the core microbiome was not affected by the early life environment (Deng et al., 2021) and these bacteria had high abundance (Wu et al., 2020; Deng et al., 2021). In the current study, the abundance of the members of the core microbiome was high in the buccal cavity and the intestine of the wild as well as F0 and F2 generations, mostly dominated by *Nocardioides, Propionibacterium, Sphingomonas*, and *Enhydrobacter*. However, the core microbiome in the intestine of wild Nile tilapia from Lake Awassa and Chamo in Ethiopia was reported by Bereded et al. (2020). At the phylum level, the core microbiome in the fishes from these two lakes was similar to wild tilapia (in the current study) from the Nile river in Egypt. The core microbiome was mostly dominated by *Actinobacteria, Firmicutes*, and *Proteobacteria*. However, at the genus level, the core microbiome in our study and that of Bereded et al. (2020) were different. In Awassa and Chamo lakes, the most abundant genera were *Clostridium_XI, GPXI, Cetobacterium*, and *Turicibacter*. In the current study, the core microbiome was mostly dominated by *Nocardioides, Propionibacterium, Sphingomonas*, and *Corynebacterium*. In our previous study, we observed *Cetobacterium* as a core member in the mouth and intestine of Nile tilapia from the F1 generation (Abdelhafiz et al., 2021a). These differences in the core microbiome could be attributed to the microbial functional groups. It was reported that microbes with similar metabolic functions can be combined into functional groups which are controlled by various ecological pressures (Shan and Cordero, 2020). Furthermore, in blue tilapia (*Oreochromis aureus*) maternal cold-tolerant genetic components were reported to be transferred to offspring (Nitzan et al., 2016). In addition, Kokou et al. (2018) reported host-microbe selection of cold-tolerant microbes in the gut of blue tilapia. Therefore, we also speculate that the difference in the core microbiome between wild Nile tilapia from Egypt and Ethiopia could be due to a genetic pressure directed toward environmental factors.

The core microbiome that is vertically transmitted across generations (Funkhouser and Bordenstein, 2013; Sylvain and Derome, 2017; Lee et al., 2019; Jorge et al., 2020) has conserved functions (Ramos et al., 2021). In the current study, we observed a presumed vertical transmission of the core microbiome from the wild Nile tilapia to the subsequent generations (F0 and F2). The core microbiome in the buccal cavity was mostly dominated by different ASVs of *Nocardioides, Propionibacterium, Sphingomonas*, and *Enhydrobacter*. These core members were also abundant in the posterior intestine, the exception was *Enhydrobacter*. Breast milk microbiome of humans is dominated by nine genera, and among them are *Propionibacterium* and *Sphingomonas* (Mueller et al., 2015). In infants, breastfeeding promotes the colonization and maturation of the infant gut microbiome in addition to the vertically transmitted microbes from different body sites of mothers (Mueller et al., 2015). However, microbes transmitted from the mother's skin and vagina are transient microbes that facilitate the early colonization of other microbes also. Maternal gut microbes that are known to have better ecological adaptation capacity were found to be more persistent in the infant gut (Ferretti et al., 2018). In discus fish, maternal skin microbiome that is vertically transmitted to offspring shapes the gut microbial community of the fry (Sylvain and Derome, 2017). In our study, we found that microbes from both maternal mouth and gut shape the microbiome in offspring. For example, in wild fish, *Sphingomonas* was a member of the core microbiome of only posterior intestine samples. Nevertheless, we detected bacteria belonging to this genus in the mouth of F0 and F2 generations. The egg capsule of little skate was reported to have a high microbial richness and core microbiome that are essential for embryonic development (Mika et al., 2021). Therefore, we speculate that *Nocardioides, Propionibacterium*, and *Sphingomonas* may have a role in facilitating the colonization of other microbes in the buccal cavity and gut of Nile tilapia. Furthermore, the incubation of eggs in the buccal cavity of Nile tilapia could be the route for vertical transmission of microbes to the eggs. *Propionibacterium* have been found in human skin microbiome, raw milk, soil, silage, and anaerobic digesters (Gautier, 2014). Moreover, members of *Propionibacterium* were reported to break down urea and release ammonia (Gautier, 2014). It was reported that carp and zebrafish gill nitrogen-cycle microbes can detoxify ammonia (van Kessel et al., 2016). In European seabass, *Propionibacterium* was noted to be dominant in digesta and mucosa (Serra et al., 2021). Furthermore, *Propionibacterium* species are known for their unique metabolism to convert lactate to propionic acid and acetic acid by fermentation (Ciani et al., 2013). In addition, *Propionibacterium* sp. have immunomodulatory effects in the mice intestine. It was reported that propionate, which is produced by *Propionibacterium*, prevents acute colitis in mice (Plé et al., 2015). It is also known that *Propionibacterium* surface proteins interact with human epithelial cell surface and improve barrier functions in the intestine, and probably act against inflammatory bowel diseases (do Carmo et al., 2017). *Nocardioides*, the other abundant member of the core microbiome in Nile tilapia, are known to produce the anti-tumor antibiotics, sandramycin, and they can modify complex compounds chemically and enzymatically. Moreover, *Nocardioides* spp. have antimicrobial and antifungal activities (Lee et al., 2012), and they belong to healthy microbiota, as reported in the case of feces from healthy cottontail rabbits (Zhang et al., 2015). Furthermore, bacteria from this genus were found enriched in mice intestine after *Lactobacillus plantarum* administration (Xie et al., 2016). *Nocardioides* was also reported as gut microbiome member in Malaysian population (Chua et al., 2019). Other articles have also indicated the presence of *Nocardioides* and *Sphingomonas* in other fishes and fish rearing facilities; *Nocardioides* in the intestine of Korean spotted sleeper (*Odontobutis interrupta*) and leopard mandarin fish (*Siniperca scherzeri*) (Hyun et al., 2021), and *Sphingomonas* in fish ponds (Chen et al., 2016) and fish intestine (Hyun et al., 2021).

Sphingomonas species produce poly-β-hydroxybutyrate, in situations where carbon is available, but when there

is a limited nutrient source (Dedkova and Blatter, 2014; Chen et al., 2016). Furthermore, *Sphingomonas* species can grow and survive in a wide range of environments that other bacteria do not tolerate (Kuehn et al., 2013). *Sphingomonas* was found as a core microbiome member in healthy human milk (Hunt et al., 2011), in Cyprus donkey milk (Papademas et al., 2021) and in bovine milk (Kuehn et al., 2013). In humans, feces microbiome of infants are different between breastfed and formula-fed infants, which indicates the transfer of microbes from milk to infant gut and/or involvement of milk prebiotics in the proliferation of specific microbes (Moossavi et al., 2018). Disturbance of the transfer process of the microbes from human milk to the infant was associated with many diseases (Hunt et al., 2011). *Sphingomonas* protect maternal breast tissue against breast cancer, and the abundance of *Sphingomonadaceae* family was higher in the nipple aspirate fluid of healthy women (Xuan et al., 2014; Chan et al., 2016). Thus, *Propionibacterium, Nocardioides*, and *Sphingomonas* that are transmitted vertically from the wild fish and possess the aforementioned functional potential are likely beneficial members in the core microbiome of Nile tilapia.

CONCLUSION

Here we report for the first time a presumed vertical transmission (based on similar ASVs in different generations) of buccal cavity and intestine microbial communities across generations in a mouthbrooder species. To our knowledge, the buccal cavity microbiomes in wild Nile tilapia has not been previously reported. We presume that the buccal cavity and the intestine core microbiome facilitate the colonization of other gut microbiome across generations. Furthermore, we suggest that the route of vertical transmission is through the mouth when eggs are incubated in the buccal cavity of Nile tilapia. Based on the literature, we believe that the core microbiome members that were likely vertically transmitted from the wild tilapia are beneficial bacteria and could play an essential role in the development of the offspring.

DATA AVAILABILITY STATEMENT

The data presented in this study are deposited in the repository, Sequence Read Archive (SRA) with accession number PRJNA808265.

ETHICS STATEMENT

The animal study was reviewed and approved by this study was performed under a license from the Norwegian Animal Research Authority (FOTS ID 10427).

AUTHOR CONTRIBUTIONS

VK, JF, and YA designed the study. YA carried out the sampling, lab work, analyzed the data, and wrote the manuscript. CD also analyzed the data. MP performed sequencing and data generation. YA, JF, CD, and VK interpreted the data. VK, JF, and CD reviewed and edited the manuscript. All authors approved the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.879990/full#supplementary-material

REFERENCES

Abdelhafiz, Y., Fernandes, J. M. O., Larger, S., Albanese, D., Donati, C., Jafari, O., et al. (2021a). Breeding strategy shapes the composition of bacterial communities in female Nile tilapia reared in a recirculating aquaculture system. *Front. Microbiol.* 12:709611. doi: 10.3389/fmicb.2021.709611

Abdelhafiz, Y., Fernandes, J. M. O., Stefani, E., Albanese, D., Donati, C., and Kiron, V. (2021b). Power play of commensal bacteria in the buccal cavity of female Nile tilapia. *Front. Microbiol.* 12:773351. doi: 10.3389/fmicb.2021.773351

Albanese, D., Fontana, P., De Filippo, C., Cavalieri, D., and Donati, C. (2015). MICCA: a complete and accurate software for taxonomic profiling of metagenomic data. *Sci. Rep.* 5:9743.

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Aust. Ecol.* 26, 32–46. doi: 10.1111/j.1442-9993.2001.01070.pp.x

Beemelmanns, A., Poirier, M., Bayer, T., Kuenzel, S., and Roth, O. (2019). Microbial embryonal colonization during pipefish male pregnancy. *Sci. Rep.* 9:3. doi: 10.1038/s41598-018-37026-3

Bereded, N. K., Abebe, G. B., Fanta, S. W., Curto, M., Waidbacher, H., Meimberg, H., et al. (2021). The impact of sampling season and catching site (wild and aquaculture) on gut microbiota composition and diversity of Nile tilapia (*Oreochromis niloticus*). *Biology* 10:180. doi: 10.3390/biology10030180

Bereded, N. K., Curto, M., Domig, K. J., Abebe, G. B., Fanta, S. W., Waidbacher, H., et al. (2020). Metabarcoding analyses of gut microbiota of Nile tilapia

(*Oreochromis niloticus*) from Lake Awassa and Lake Chamo, Ethiopia. *Microorganisms* 8:1040. doi: 10.3390/microorganisms8071040

Berg, G., Rybakova, D., Fischer, D., Cernava, T., Vergès, M.-C. C., Charles, T., et al. (2020). Microbiome definition re-visited: old concepts and new challenges. *Microbiome* 8:103. doi: 10.1186/s40168-020-00875-0

Chai, J. N., Zhou, Y. W., and Hsieh, C.-S. (2014). T cells and intestinal commensal bacteria-ignorance, rejection, and acceptance. *FEBS Lett.* 588, 4167–4175. doi: 10.1016/j.febslet.2014.06.040

Chan, A. A., Bashir, M., Rivas, M. N., Duvall, K., Sieling, P. A., Pieber, T. R., et al. (2016). Characterization of the microbiome of nipple aspirate fluid of breast cancer survivors. *Sci. Rep.* 6:28061. doi: 10.1038/srep28061

Chen, W.-M., Li, Y.-S., and Sheu, S.-Y. (2016). *Sphingomonas piscinae* sp. nov., isolated from a fish pond. *Int. J. Syst. Evol. Microbiol.* 66, 5301–5308. doi: 10.1099/ijsem.0.001511

Cholewińska, P., Górniak, W., and Wojnarowski, K. (2021). Impact of selected environmental factors on microbiome of the digestive tract of ruminants. *BMC Vet. Res.* 17:25. doi: 10.1186/s12917-021-02742-y

Chua, E. G., Loke, M. F., Gunaletchumy, S. P., Gan, H. M., Thevakumar, K., Tay, C. Y., et al. (2019). The influence of modernization and disease on the gastric microbiome of Orang Asli, Myanmars and modern Malaysians. *Microorganisms* 7:174. doi: 10.3390/microorganisms7060174

Ciani, M., Comitini, F., and Mannazzu, I. (2013). "Fermentation," in *Encyclopedia of Ecology*, 2nd Edn, ed. B. Fath (Oxford: Elsevier), 310–321.

Dedkova, E. N., and Blatter, L. A. (2014). Role of β-hydroxybutyrate, its polymer poly-β-hydroxybutyrate and inorganic polyphosphate in mammalian health and disease. *Front. Physiol.* 5:260. doi: 10.3389/fphys.2014.00260

Deng, Y., Kokou, F., Eding, E. H., and Verdegem, M. C. J. (2021). Impact of early-life rearing history on gut microbiome succession and performance of Nile tilapia. *Anim. Microbiome* 3:81. doi: 10.1186/s42523-021-00145-w

DeSantis, T., Hugenholtz, P., Keller, K., Brodie, E., Larsen, N., Piceno, Y., et al. (2006). NAST: a multiple sequence alignment server for comparative analysis of 16S rRNA genes. *Nucleic Acids Res.* 34, W394–W399. doi: 10.1093/nar/gkl244

do Carmo, F. L. R., Rabah, H., Huang, S., Gaucher, F., Deplanche, M., Dutertre, S., et al. (2017). *Propionibacterium freudenreichii* surface protein SlpB is involved in adhesion to intestinal HT-29 Cells. *Front. Microbiol.* 8:1033. doi: 10.3389/fmicb.2017.01033

Edgar, R. C. (2016). UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. *bioRxiv* [preprint]. doi: 10.1101/081257

Edgar, R. C., and Flyvbjerg, H. (2015). Error filtering, pair assembly and error correction for next-generation sequencing reads. *Bioinformatics* 31, 3476–3482. doi: 10.1093/bioinformatics/btv401

Estellé, J. (2019). Benefits from the joint analysis of host genomes and metagenomes: select the holobiont. *J. Anim. Breed. Genet.* 136, 75–76. doi: 10.1111/jbg.12383

Ferretti, P., Pasolli, E., Tett, A., Asnicar, F., Gorfer, V., Fedi, S., et al. (2018). Mother-to-infant microbial transmission from different body sites shapes the developing infant gut microbiome. *Cell Host Microbe* 24, 133–145.e135. doi: 10.1016/j.chom.2018.06.005

Fjellheim, A. J., Playfoot, K. J., Skjermo, J., and Vadstein, O. (2012). Inter-individual variation in the dominant intestinal microbiota of reared Atlantic cod (*Gadus morhua L.*) larvae. *Aquac. Res.* 43, 1499–1508. doi: 10.1111/j.1365-2109.2011.02952.x

Funkhouser, L. J., and Bordenstein, S. R. (2013). Mom knows best: the universality of maternal microbial transmission. *PLoS Biol.* 11:e1001631. doi: 10.1371/journal.pbio.1001631

García-Mantrana, I., Calatayud, M., Romo-Vaquero, M., Espín, J. C., Selma, M. V., and Collado, M. C. (2019). Urolithin metabotypes can determine the modulation of gut microbiota in healthy individuals by tracking walnuts consumption over three days. *Nutrients* 11:2483. doi: 10.3390/nu11102483

Gatesoupe, F. J., Covès, D., Ortega, A., Papandroulakis, N., Vadstein, O., and De La Gandara, F. (2013). A spatiotemporal study of bacterial community profiles associated with Atlantic bluefin tuna larvae, *T hunnus thynnus L.*, in three Mediterranean hatcheries. *Aquac. Res.* 44, 1511–1523. doi: 10.1111/j.1365-2109.2012.03158.x

Gautier, M. (2014). "Propionibacterium," in *Encyclopedia of Food Microbiology*, 2nd Edn, eds C. A. Batt and M. L. Tortorello (Oxford: Academic Press), 232–237.

Giatsis, C., Sipkema, D., Smidt, H., Heilig, H., Benvenuti, G., Verreth, J., et al. (2015). The impact of rearing environment on the development of gut microbiota in tilapia larvae. *Sci. Rep.* 5:18206. doi: 10.1038/srep18206

Giatsis, C., Sipkema, D., Smidt, H., Verreth, J., and Verdegem, M. (2014). The colonization dynamics of the gut microbiota in tilapia larvae. *PLoS One* 9:e103641. doi: 10.1371/journal.pone.0103641

Henderson, G., Cox, F., Ganesh, S., Jonker, A., Young, W., Abecia, L., et al. (2015). Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. *Sci. Rep.* 5:14567. doi: 10.1038/srep14567

Hunt, K. M., Foster, J. A., Forney, L. J., Schütte, U. M. E., Beck, D. L., Abdo, Z., et al. (2011). Characterization of the diversity and temporal stability of bacterial communities in human milk. *PLoS One* 6:e21313. doi: 10.1371/journal.pone.0021313

Hyun, D. W., Jeong, Y. S., Lee, J. Y., Sung, H., Lee, S. Y., Choi, J. W., et al. (2021). Description of *Nocardioides piscis* sp. nov., *Sphingomonas piscis* sp. nov. and *Sphingomonas sinipercae* sp. nov., isolated from the intestine of fish species *Odontobutis interrupta* (Korean spotted sleeper) and *Siniperca scherzeri* (leopard mandarin fish). *J. Microbiol.* 59, 552–562. doi: 10.1007/s12275-021-1036-5

Johny, T. K., Puthusseri, R. M., and Bhat, S. G. (2021). A primer on metagenomics and next-generation sequencing in fish gut microbiome research. *Aquac. Res.* 52, 4574–4600.

Jones, R. B., Zhu, X., Moan, E., Murff, H. J., Ness, R. M., Seidner, D. L., et al. (2018). Inter-niche and inter-individual variation in gut microbial community assessment using stool, rectal swab, and mucosal samples. *Sci. Rep.* 8:4139. doi: 10.1038/s41598-018-22408-4

Jorge, F., Dheilly, N. M., and Poulin, R. (2020). Persistence of a core microbiome through the ontogeny of a multi-host parasite. *Front. Microbiol.* 11:954. doi: 10.3389/fmicb.2020.00954

Kokou, F., Sasson, G., Nitzan, T., Doron-Faigenboim, A., Harpaz, S., Cnaani, A., et al. (2018). Host genetic selection for cold tolerance shapes microbiome composition and modulates its response to temperature. *eLife* 7:e36398. doi: 10.7554/eLife.36398

Konstantinidis, I., Anastasiadi, D., Sætrom, P., Nedoluzhko, A. V., Mjelle, R., Podgorniak, T., et al. (2021). Epigenetic mapping of the somatotropic axis in Nile tilapia reveals differential DNA hydroxymethylation marks associated with growth. *Genomics* 113, 2953–2964. doi: 10.1016/j.ygeno.2021.06.037

Kostopoulos, I., Elzinga, J., Ottman, N., Klievink, J. T., Blijenberg, B., Aalvink, S., et al. (2020). *Akkermansia muciniphila* uses human milk oligosaccharides to thrive in the early life conditions *in vitro*. *Sci. Rep.* 10:14330. doi: 10.1038/s41598-020-71113-8

Kuehn, J. S., Gorden, P. J., Munro, D., Rong, R., Dong, Q., Plummer, P. J., et al. (2013). Bacterial community profiling of milk samples as a means to understand culture-negative bovine clinical mastitis. *PLoS One* 8:e61959. doi: 10.1371/journal.pone.0061959

Lan, Y., Wang, Q., Cole, J. R., and Rosen, G. L. (2012). Using the RDP classifier to predict taxonomic novelty and reduce the search space for finding novel organisms. *PLoS One* 7:e32491. doi: 10.1371/journal.pone.0032491

Larsson, J. (2018). *eulerr: Area-Proportional Euler Diagrams with Ellipses*. Bachelor's thesis. Lund: Lund University.

Lee, L.-H., Cheah, Y.-K., Mohd Sidik, S., Ab Mutalib, N.-S., Tang, Y.-L., Lin, H.-P., et al. (2012). Molecular characterization of Antarctic *actinobacteria* and screening for antimicrobial metabolite production. *World J. Microbiol. Biotechnol.* 28, 2125–2137. doi: 10.1007/s11274-012-1018-1

Lee, S., La, T.-M., Lee, H.-J., Choi, I.-S., Song, C.-S., Park, S.-Y., et al. (2019). Characterization of microbial communities in the chicken oviduct and the origin of chicken embryo gut microbiota. *Sci. Rep.* 9:6838. doi: 10.1038/s41598-019-43280-w

Leftwich, P. T., Edgington, M. P., and Chapman, T. (2020). Transmission efficiency drives host–microbe associations. *Proc. R. Soc. B* 287:20200820. doi: 10.1098/rspb.2020.0820

Legrand, T. P., Wynne, J. W., Weyrich, L. S., and Oxley, A. P. (2020). A microbial sea of possibilities: current knowledge and prospects for an improved understanding of the fish microbiome. *Rev. Aquac.* 12, 1101–1134. doi: 10.1111/raq.12375

Liao, J., Cao, X., Zhao, L., Wang, J., Gao, Z., Wang, M. C., et al. (2016). The importance of neutral and niche processes for bacterial community assembly differs between habitat generalists and specialists. *FEMS Microbiol. Ecol.* 92:fiw174. doi: 10.1093/femsec/fiw174

Llewellyn, M. S., Boutin, S., Hoseinifar, S. H., and Derome, N. (2014). Teleost microbiomes: the state of the art in their characterization, manipulation and

importance in aquaculture and fisheries. *Front. Microbiol.* 5:207. doi: 10.3389/fmicb.2014.00207

Lokesh, J., Kiron, V., Sipkema, D., Fernandes, J. M. O., and Moum, T. (2019). Succession of embryonic and the intestinal bacterial communities of Atlantic salmon (*Salmo salar*) reveals stage-specific microbial signatures. *MicrobiologyOpen* 8:e00672. doi: 10.1002/mbo3.672

Love, M. I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-Seq data with DESeq2. *Genome Biol.* 15:550. doi: 10.1186/s13059-014-0550-8

Lozupone, C., and Knight, R. (2005). UniFrac: a new phylogenetic method for comparing microbial communities. *Appl. Environ. Microbiol.* 71, 8228–8235. doi: 10.1128/aem.71.12.8228-8235.2005

McGrath-Blaser, S., Steffen, M., Grafe, T. U., Torres-Sánchez, M., Mcleod, D. S., and Muletz-Wolz, C. R. (2021). Early life skin microbial trajectory as a function of vertical and environmental transmission in Bornean foam-nesting frogs. *Anim. Microbiome* 3:83. doi: 10.1186/s42523-021-00147-8

McMurdie, P. J., and Holmes, S. (2013). phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* 8:e61217. doi: 10.1371/journal.pone.0061217

Mika, K., Okamoto, A. S., Shubin, N. H., and Mark Welch, D. B. (2021). Bacterial community dynamics during embryonic development of the little skate (*Leucoraja erinacea*). *Anim. Microbiome* 3:72. doi: 10.1186/s42523-021-00136-x

Moossavi, S., Miliku, K., Sepehri, S., Khafipour, E., and Azad, M. B. (2018). The prebiotic and probiotic properties of human milk: implications for infant immune development and pediatric asthma. *Front. Pediatr.* 6:197. doi: 10.3389/fped.2018.00197

Mueller, N. T., Bakacs, E., Combellick, J., Grigoryan, Z., and Dominguez-Bello, M. G. (2015). The infant microbiome development: mom matters. *Trends Mol. Med.* 21, 109–117. doi: 10.1016/j.molmed.2014.12.002

Nagy-Szakal, D., Ross, M. C., Dowd, S. E., Mir, S. a. V., Schaible, T. D., Petrosino, J. F., et al. (2012). Maternal micronutrients can modify colonic mucosal microbiota maturation in murine offspring. *Gut Microbes* 3, 426–433. doi: 10.4161/gmic.20697

Nitzan, T., Slosman, T., Gutkovich, D., Weller, J. I., Hulata, G., Zak, T., et al. (2016). Maternal effects in the inheritance of cold tolerance in blue tilapia (*Oreochromis aureus*). *Environ. Biol. Fishes* 99, 975–981. doi: 10.1007/s10641-016-0539-0

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P., O'hara, R., et al. (2013). *Community Ecology Package. R Package Version*, 2.0-2.

Papademas, P., Kamilari, E., Aspri, M., Anagnostopoulos, D. A., Mousikos, P., Kamilaris, A., et al. (2021). Investigation of donkey milk bacterial diversity by 16S rDNA high-throughput sequencing on a Cyprus donkey farm. *J. Dairy Sci.* 104, 167–178. doi: 10.3168/jds.2020-19242

Plé, C., Richoux, R., Jardin, J., Nurdin, M., Briard-Bion, V., Parayre, S., et al. (2015). Single-strain starter experimental cheese reveals anti-inflammatory effect of *Propionibacterium freudenreichii* CIRM BIA 129 in TNBS-colitis model. *J. Funct. Foods* 18, 575–585. doi: 10.1038/s41467-020-18983-8

Podgorniak, T., Brockmann, S., Konstantinidis, I., and Fernandes, J. M. O. (2019). Differences in the fast muscle methylome provide insight into sex-specific epigenetic regulation of growth in Nile tilapia during early stages of domestication. *Epigenetics* 14, 818–836. doi: 10.1080/15592294.2019.1618164

Rackaityte, E., and Lynch, S. V. (2020). The human microbiome in the 21st century. *Nat. Commun.* 11:5256.

Ramos, C., Calus, M., and Schokker, D. (2021). Persistence of functional microbiota composition across generations. *Sci. Rep.* 11:19007. doi: 10.1038/s41598-021-98097-3

Risely, A. (2020). Applying the core microbiome to understand host–microbe systems. *J. Anim. Ecol.* 89, 1549–1558. doi: 10.1111/1365-2656.13229

Roeselers, G., Mittge, E. K., Stephens, W. Z., Parichy, D. M., Cavanaugh, C. M., Guillemin, K., et al. (2011). Evidence for a core gut microbiota in the zebrafish. *ISME J.* 5, 1595–1608. doi: 10.1038/ismej.2011.38

Rognes, T., Flouri, T., Nichols, B., Quince, C., and Mahé, F. (2016). VSEARCH: a versatile open source tool for metagenomics. *PeerJ* 4:e2584. doi: 10.7717/peerj.2584

Salonen, A., Salojärvi, J., Lahti, L., and De Vos, W. M. (2012). The adult intestinal core microbiota is determined by analysis depth and health status. *Clin. Microbiol. Infect.* 18, 16–20. doi: 10.1111/j.1469-0691.2012.03855.x

Serra, C. R., Oliva-Teles, A., Enes, P., and Tavares, F. (2021). Gut microbiota dynamics in carnivorous European seabass (*Dicentrarchus labrax*) fed plant-based diets. *Sci. Rep.* 11:447. doi: 10.1038/s41598-020-80138-y

Shan, X., and Cordero, O. X. (2020). A fundamental structure-function mapping in the ocean microbiome. *bioRxiv* [Preprint]. doi: 10.1101/2020.03.12.989426

Silva, Y. P., Bernardi, A., and Frozza, R. L. (2020). The role of short-chain fatty acids from gut microbiota in gut-brain communication. *Front. Endocrinol.* 11:25. doi: 10.3389/fendo.2020.00025

Stephens, W. Z., Burns, A. R., Stagaman, K., Wong, S., Rawls, J. F., Guillemin, K., et al. (2016). The composition of the zebrafish intestinal microbial community varies across development. *ISME J.* 10, 644–654. doi: 10.1038/ismej.2015.140

Sylvain, F. -É, and Derome, N. (2017). Vertically and horizontally transmitted microbial symbionts shape the gut microbiota ontogenesis of a skin-mucus feeding discus fish progeny. *Sci. Rep.* 7:5263. doi: 10.1038/s41598-017-05662-w

Talwar, C., Nagar, S., Lal, R., and Negi, R. K. (2018). Fish gut microbiome: current approaches and future perspectives. *Indian J. Microbiol.* 58, 397–414. doi: 10.1007/s12088-018-0760-y

Togo, A., Dufour, J.-C., Lagier, J.-C., Dubourg, G., Raoult, D., and Million, M. (2019). Repertoire of human breast and milk microbiota: a systematic review. *Future Microbiol.* 14, 623–641. doi: 10.2217/fmb-2018-0317

Valles-Colomer, M., Bacigalupe, R., Vieira-Silva, S., Suzuki, S., Darzi, Y., Tito, R. Y., et al. (2022). Variation and transmission of the human gut microbiota across multiple familial generations. *Nat. Microbiol.* 7, 87–96. doi: 10.1038/s41564-021-01021-8

van Kessel, M. a. H. J., Mesman, R. J., Arshad, A., Metz, J. R., Spanings, F. a. T., Van Dalen, S. C. M., et al. (2016). Branchial nitrogen cycle symbionts can remove ammonia in fish gills. *Environ. Microbiol. Rep.* 8, 590–594. doi: 10.1111/1758-2229.12407

Weiss, S., Xu, Z. Z., Peddada, S., Amir, A., Bittinger, K., Gonzalez, A., et al. (2017). Normalization and microbial differential abundance strategies depend upon data characteristics. *Microbiome* 5:27. doi: 10.1186/s40168-017-0237-y

Wu, Z., Wang, S., Zhang, Q., Hao, J., Lin, Y., Zhang, J., et al. (2020). Assessing the intestinal bacterial community of farmed Nile tilapia (*Oreochromis niloticus*) by high-throughput absolute abundance quantification. *Aquaculture* 529, 735688. doi: 10.1016/j.aquaculture.2020.735688

Xie, Q., Pan, M., Huang, R., Tian, X., Tao, X., Shah, N. P., et al. (2016). Short communication: modulation of the small intestinal microbial community composition over short-term or long-term administration with *Lactobacillus plantarum* ZDY2013. *J. Dairy Sci.* 99, 6913–6921. doi: 10.3168/jds.2016—11141

Xuan, C., Shamonki, J. M., Chung, A., Dinome, M. L., Chung, M., Sieling, P. A., et al. (2014). Microbial dysbiosis Is associated with human breast cancer. *PLoS One* 9:e83744. doi: 10.1371/journal.pone.0083744

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

Zhang, Y., Tan, H., Deng, Q., and Cao, L. (2015). Actinobacterial flora in feces of healthy cottontail rabbits (*Sylvilagus auduboni*). *Probiotics Antimicrob. Proteins* 7, 9–13. doi: 10.1007/s12602-014-9179-x

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Animals are exposed to microbes present in the surrounding environment. In addition, there are various microbes that reside in the mouth, gut and skin of hosts. Some of these could be beneficial and others could be pathogenic organisms. Furthermore, the core microbial members that are transferred to the progeny may have important health implications.

Nile tilapia is the second most farmed fish around the globe. Being a mouthbrooder species, the females of this fish incubate eggs in their mouth. This thesis reveals the bacterial composition in Nile tilapia and compares the communities in males and females, in fishes produced through specific breeding strategies, and in different generations; to understand disease propensity, to reveal the inter-individual variation and to identify the microbes that are transferred across generations. The results revealed that female mouth contains fewer opportunistic bacteria and more beneficial microbes. Moreover, breeding strategy was found to affect the abundance of beneficial bacteria and inter-individual variation in microbial abundance. Interestingly, the core members in the female mouth were found to be similar in wild fish and their progeny. Findings from this thesis provide information to shape the microbial composition to obtain a desired health status in the fish.

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