The Generation of a Zebrafish Microbial Culture Collection to Facilitate Mechanistic Understanding of Host-Microbe Interactions

by
Zoe VanderHoek

A THESIS

submitted to

Oregon State University

Honors College

in partial fulfillment of
the requirements for the
degree of

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(Honors Scholar)

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Abstract approved:____

Thomas Sharpton

A rapidly expanding body of evidence suggests that relationships exist between the microbiome and vertebrate health and disease. The zebrafish is a frequently utilized model organism to support this claim. Specifically, its gut microbiome is an advantageous resource for the study of microbiome health and diversity. While it is an important model for understanding host-microbe interactions, its utility is mitigated by the limited number of cultured isolates made publicly available for zebrafish model systems. Establishment of a culture collection would enhance our ability to conduct empirical assessments of host microbiome interactions in a high-throughput manner. The resulting objective was to generate a foundational zebrafish microbial culture collection and quantify the proportion of zebrafish culturable gut microbial diversity. Gut microbes were cultured from 5D-line zebrafish fecal samples obtained at 5 different time points over a 6-month period. For each isolate we characterized morphological characteristics and oxygen tolerance abilities. To quantify taxonomic diversity of the culture collection we PCR amplified and Sanger Sequenced the 16S rRNA gene of each isolate. In total, 108 zebrafish gut microbes were isolated and preserved, and we were able to assign taxonomy to 78. Together, these represented approximately 50% of the typical zebrafish gut genera.

Key Words: Microbiome, Zebrafish, Culture Collection, Reductive Experimentation, Taxonomy, Diversity

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I understand that my project will become part of the permanent collection of Oregon State University, Honors College. My signature below authorizes release of my project to any reader upon request.

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Zoe VanderHoek, Author

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Introduction

A growing body of evidence associates gut microbial diversity with vertebrate health (Cooper et al., 2019). Specifically, the gut microbiota, the collection of bacteria, viruses, and eukaryotes that live in association with the gastrointestinal tract, form a number of beneficial symbioses with their hosts including, but not limited to: metabolism of complex carbohydrates, vitamin metabolism and synthesis, and pathogen resistance (Cooper et al., 2019; Rowland et al., 2018). Specifically, many microbes are natural synthesizers of many important B-vitamins (LeBlanc et al., 2011). This vitamin synthesis is merely supplemental to the primary dietary source of B-vitamins, but may reduce vulnerability to B-vitamin deficiencies if the host gut is adequately colonized with these microbes (LeBlanc et al., 2011).

In addition to beneficial relationships, there also exist many diseases that have been associated with microbiome composition and function. Importantly, disruptions of the existing homeostatic relationships within the gut and other areas of the human body have been associated with type 2 diabetes, obesity, inflammatory bowel disease, Crohn’s disease, *Clostridium difficle* infections, colon cancer and many other ailments (NIH Human Microbiome Portfolio Analysis Team, 2019). In many cases, the microbiome composition can even be used to explain phenotypic variation beyond genetic factors; for example, microbiome composition has been used to accurately predict obesity with up to 90% accuracy (Gilbert et al., 2018; Goldsmith & Sartor, 2014).

Despite increasing evidence suggesting relationships between the microbiome and health, we lack a mechanistic understanding of the observed associations. Elucidating the inherent

complexity of the mechanisms underlying these relationships is critical for continued progress in microbiome research. Highly controlled reductive experimentation is needed to facilitate this understanding, which necessitates the use of well-developed animal model systems. Direct human experimentation presents several ethical questions which call for the utilization of these animal models.

The zebrafish model is widely used for toxicology and developmental biology research and, more recently, has emerged as an important animal model in microbiome investigations (Bryson-Richardson et al., 2007; Gaulke et al., 2019; Roeselers et al., 2011). The zebrafish is an ideal model organism for microbiome research, as zebrafish have similar nervous and endocrine systems to humans, are inexpensive to maintain, have a fully-sequenced genome that shares significant homology with the human genome (Barbazuk et al., 2000; Ericsson, 2019) and also enable high-throughput experimental study designs (Ericsson, 2019) which result in increased statistical power. In addition, during development, zebrafish embryos are transparent, which allows for clear visualization of developmental stages and direct observation of how microbes colonize (Ericsson, 2019). These embryos can be sterilized using disinfectants and antibiotic cocktails (Ericsson, 2019) to yield germ-free populations for use in reductive experimentation to allow for a mechanistic understanding of host-microbe interactions. Finally, the microbiota of zebrafish and mammals, including humans, share substantial genetic homology, suggesting these microbiomes likely execute similar functions (Gaulke et al., 2020).

Despite the various advantages of the zebrafish model, we lack critical tools and data resources that will maximize its utility. Notably, an established in vitro bacteria culture collection that

encompasses the diversity found in the zebrafish gut microbiome is lacking. Culture collections not only allow scientists to perform experiments revealing causal relationships, but also allow for the discovery of the extent of existing biodiversity and the mechanisms behind observable phenomena (Liu et al., 2020). Additionally, specific taxa may be identified from culture collections that encode molecular pathways that influence host health. The analyses of the mechanistic relationships and pathways between microbiota and gut health will potentially allow for the discovery of natural products. These natural products can provide therapeutic targets for future research and medical innovation.

Existing culture collections have proven very useful for the investigation of host-microbe interactions and mechanistic relationships. A variety of studies have explored colonization resistance, which is the resistance to the enteric colonization of pathogenic microbes. Though the mechanistic understanding is still lacking, it is believed that microbiome-host relationships play a significant role in colonization resistance. For example, in one study, germ-free mice were inoculated with 12 bacterial phyla typically found in the mouse microbiome to identify whether they offered protection from colonization by the pathogenic bacteria *Salmonella enterica* within the host’s intestines (Brugiroux et al., 2016). It was found that the presence of microbes typically found in the gut offered at least partial protection from colonization of pathogenic bacteria. This is likely due to the occupation of various nutritional niches by a complex network of host-associated bacteria that prevent the efficient utilization of such resources (Brugiroux et al., 2016).

Additionally, probiotics used to improve host-microbe interactions in a zebrafish model were shown to promote growth performance, possibly via the biosynthesis of vitamin molecules and nutrients by the host's microbes, as well as increased resistance to pathogenic organisms (Qin et al., 2018). The results of this study suggest that the microbiome may produce antibiotic compounds in addition to the maintenance of epithelial barriers in the host digestive tract that allow for immunomodulation and protection against pathogenic colonization (Qin et al., 2018).

Zebrafish and other animal models have been used to generate microbial culture collections to study the interactions between specific bacterial strains and their hosts. This year, a mouse model culture collection was generated using 16s RNA sequencing that yielded 154 bacterial taxa to be used for downstream experimentation. Expanding the coverage of cultured bacteria is crucial for performing experiments that seek to explain microbe-host interactions (Liu et al., 2020).

For this reason, the goal of the work to be conducted is to generate a foundational zebrafish microbial culture collection utilizing multiple media types with varying oxygen levels to quantify the proportion of zebrafish gut microbial diversity that is culturable. We hypothesize that we will successfully culture 77% of the microbial genera in the zebrafish using standard aerobic culturing techniques based on data from studies with human microbiomes (Lagier et al., 2016). The culture collection to be generated will be the *Zebrafish Culture Collection* (ZFCC). Prior studies of the human gut microbiome have applied related culture-based procedures, namely the use of a variety of media types and growth conditions, to recover a diverse array of taxa (Lagier et al., 2016). Correspondingly, we posit that a similar level of diversity will be recovered when

using these approaches to culture the zebrafish gut microbiome to be used in downstream reductional experimentation for investigations into host-microbe relationships.

Materials and Methods

Zebrafish Facilities and Husbandry

A quantity of 30 5D line zebrafish of mixed sex were held in a 9-liter single pass, flow-through tank upon approval and with a permit from the Institutional Animal Care and Use Committee (permit number: 4800) in Dr. Michael Kent's laboratory at Oregon State University. The fish originated from the Sinnhuber Aquatic Research Laboratory at Oregon State University, which is pathogen-free for important pathogens of zebrafish (Barton et al., 2016). All of the tanks in this system were held at 28°C with a pH of 7.50, conductivity of about 115-150 microsiemens. The fecal samples were obtained from each of these tanks on 5 different occasions and subsequently homogenized using syringe homogenization.

Streak Plating

Freshly collected zebrafish fecal samples were homogenized using syringe homogenization and diluted in Gibco® phosphate-buffered saline. These samples were then streaked onto selective and non-selective media of varying concentrations (10% and 100%) including Brain Heart Infusion (BHI) media, and Tryptic Soy Agar (TSA).

Morphology and Oxygen Tolerance

It remains unclear how oxygen tolerant the zebrafish gut microbes are. To account for this uncertainty, the microbes were cultured for 48h under anaerobic, aerobic, and microaerophilic conditions at 27℃ to select for different levels of oxygen tolerance on multiple media types, including Brain-Heart Infusion Agar and Trypic Soy Agar. The temperature 27˚C was selected as this is the temperature at which zebrafish are maintained in this facility. Distinct colonies selected from the dilution plates were isolated by re-streaking individual colonies a total of 2 times after allowing for subsequent growth of the cultured organisms. Colony morphology characteristics were observed prior to oxygen tolerance and causative metabolic mechanisms being analyzed.

To obtain more precision in determining the oxygen tolerance of specific colonies, they were tested and subsequently analyzed via the inoculation of stab tubes containing brain-heart infusion and thioglycolate medium indicator for determining the degree of oxygenation of the media. Each isolate was classified as aerobic, facultative anaerobic, anaerobic, or microaerophilic (Prescott et al., 2005) by visual inspection of the resulting cultures and patterns of growth within the stab tube medium.

Cryopreservation & DNA Extraction

Once isolated and classified according to their oxygen tolerance, individual strains were cryopreserved in 25% glycerol and stored at -80℃ in triplicate. A separate aliquot of each culture was spun at 10,000 x g and the resulting pellet stored at -80℃ until DNA extraction. Microbial DNA was extracted using the DNeasy® Ultraclean® Microbial kit in accordance with the manufacturer's instructions. A 16S rRNA gene amplicon was generated using the 27f 1492r

primer set. 16S rRNA gene amplicons were purified using the QIAquick PCR Purification Kit in accordance with the manufacturer's instructions and their concentration quantified using the Qubit dsDNA HS Assay Kit DNA quantification system.

Sequencing

The resulting PCR amplicons were sequenced on an ABI 3730 capillary sequencer at the Oregon State University Center for Genome Research and Biocomputing. The resulting sequences were aligned against the National Center for Biotechnology Information bacterial 16S rRNA targeted loci database using blastn (v2.10.0). The best hit ($e < 1$) for each isolate was used to assign a genus taxonomic label.

Culture collection diversity

A previously generated 16S rRNA amplicon dataset of 252 5D line zebrafish fecal samples obtained from the same facility fecal samples used for culture were derived from was taxonomically annotated using DADA2 and the SILVA 16 rRNA gene database. The prevalence of each genus was calculated by dividing the number of samples with a non-zero abundance by the total number of samples. The percent of genera that were present in both the amplicon dataset and the culture collection was calculated by dividing the number of genera in both datasets by the total number of genera present in the amplicon dataset. The percent genera shared was calculated at prevalence > 0, 25, 50, 75, and 90%.

Results:

Developing a foundational zebrafish microbial culture collection

To identify the culturable diversity of the zebrafish fecal microbiome individual bacterial colonies were derived and identified from five individual fecal samplings from a combined collection of the feces from the tank following fecal collection, syringe homogenization, DNA extraction, PCR amplification and Sanger Sequencing. From these samplings we identified 108 bacterial isolates representing 18 different known genera. Of these isolates, 68 were cultured utilizing 100% brain heart infusion media and 40 were cultured utilizing 100% TSA media. Of the 68 utilizing 100% BHI media, 56 (~82%) were facultative anaerobes, 4 (~6%) were aerobes, 2 (~3%) were microaerophilic, and 2 (~3%) were aerotolerant. Of the 40 utilizing 100% TSA media, 36 (90%) were aerobic, while 4 (10%) remained unclassified.

The aerobicity of the zebrafish gut microbes remains largely unknown. To account for this information, microbes were cultured under various oxygen levels to select for different levels of oxygen tolerance and expand the potentially obtainable diversity of gut taxa. Most of these isolates had some degree of oxygen tolerance, and of the 108 total isolates obtained representing multiple media types, 56 (~52%) were found to be facultative anaerobes, 40 (~37%) were aerobes, 2 (~2%) were aerotolerant, 2 (~2%) were microaerophilic and 8 (~7.5%) remain unclassified (see *Figure 1*).

Enterococcus, Cetobacterium and *Streptococcus* genera*,* as well as all but one *Plesiomonas* isolate were only found on 100% BHI Media. *Pseudomonas, Acineobacter, Shewanella, Fictibacillus, Staphylococcus, Chryseobacterium, Mycolicibacterium, Bosea* and *Bacillus* bacterial genera were only found on 100% TSA media. Members from the genera *Aeromonas,*

Ensifer, Microbacterium, and *Flavobacterium* were found on both 100% BHI and 100% TSA media types.

Culturable Diversity

From the culture collection that was generated from 5 different zebrafish fecal samples, 18 total taxonomic genera were obtained. The primary taxonomic genus recovered was *Aeromonas,* representing 34 of the isolates taxonomically classified. The genera *Plesiomonas* and *Pseudomonas* were also discovered in the cultured gut microbiota from the zebrafish fecal samples. The presence of these three taxa is consistent with studies conducted that examined the composition of gut and surface bacteria of freshwater fish in different parts of the world (Hänninen et al., 1997; Janda et al., 2016; N M E Eissa et al., 2010). These genera accounted for ~40.1% of taxa, representing 44 individual isolates. *Aeromonas*, *Plesiomonas* and *Pseudomonas* represented 34, 8 and 2 isolates, respectively.

A major challenge in building a culture collection is capturing the totality of microbial diversity for the environment under investigation. Culturing efforts from the microbiome have reportedly captured in excess of 75% of the total estimated diversity but only after exhaustive culturing efforts analyzing over 900 fecal samples (Lagier et al., 2016). Evaluation of the true microbial diversity is often accomplished by comparing taxonomic distribution of a culture collection to those found in high throughput 16S sequence libraries. In its entirety, the *ZFCC* culture collection contains 108 isolates that constitute great diversity in taxonomic classification, representing 18 different genera. The isolates with known identities include representatives from 18 taxonomic genera. To quantify the proportion of microbial diversity encapsulated by our

culture collection we evaluated the overlap in genera present in our collection and in a 252 sample 16S rRNA gene survey derived from animals housed in the same facility. The degree of overlap depended heavily on the prevalence of the genus in the 16S survey (rho=1, $P = 0.02$; figure 3) with the lowest prevalence (>0%) corresponding to the lowest percent overlap (~2%) and the highest (≥90%) corresponding to highest percent overlap (75%). When only genera present in ≥ 50% of samples were considered, ~53% of the genera were represented by at least one culture isolate.

Discussion

We used limited techniques to culture and isolate different bacteria from the zebrafish gut and obtained over 100 isolates that can be used in downstream experimentation. Perhaps mimicking the methodology of Liu et al. would yield additional culturable isolates that represented greater microbial diversity (Liu et al., 2020). As noted above, prior investigations have applied related techniques to recover upwards of 77% of the genus-level microbial diversity from other human gut systems, which greatly assists in expanding the known cultured diversity of microbiome systems (Lagier et al., 2016). A similar level of diversity may have been able to be recovered from the zebrafish gut microbiome using our proposed approaches if our methods had been more comprehensive and all-encompassing.

Among the 18 total genera that were recovered from the culture collection we generated, *Aeromonas* was the most prevalent taxon observed, representing 34 of the organisms collected and isolated. This is consistent with other studies of freshwater fish, fish eggs, shrimp and freshwater samples that examined *Aeromonas* contamination; a study conducted in 1997 observed 93% contamination of the fish sampled (Hänninen et al., 1997). Zebrafish are important

model organisms for the study of *Aeromonas* virulence factors due to the fact that they don't suffer mortalities upon inoculation with pathogenic *Aeromonas spp.* with the same frequency as some animal models (Romero et al., 2016).

Additionally, bacteria of the *Pseudomonas* genus were present. *Pseudomonas* have been found in approximately 30% of sampled freshwater tilapia fish in one study (Eissa et al., 2010). Some species of *Pseudomonas* are pathogenic and do not elicit a host response, while others are associated with upwards of 85% mortality observed in reductive experimentation (Eissa et al., 2010). *Pseudomonas* bacteria are frequent inhabitants of aquatic environments as well as the guts of healthy fish (N M E Eissa et al., 2010). By contrast, only 2 isolates of 78 identified taxa (~ 2.5%) in our culture collection represented the *Pseudomonas* genus. This may have been due to the relatively small sample size of isolates obtained or inherent differences between natural aquatic systems and laboratory aquatic environments. Additionally, *Pseudomonas* has been shown to be altered upon disturbance in zebrafish, and for this reason many consider it to be an indicator taxon for other disturbed environments (Gaulke et al., 2016). Specifically, *Pseudomonas'* relative abundance increases upon zebrafish exposure to the antibiotic triclosan, while other genera decrease in relative abundance (Gaulke et al., 2016). These results were found to be consistent upon adjustment of triclosan exposure level, but it is unclear if *Pseudomonas'* changes in abundance are a cause or a consequence of other disturbances (Gaulke et al., 2016). Similarly, *Pseudomonas* has been observed to increase in relative abundance upon the establishment of a high parasite burden within the zebrafish gut (Gaulke et al., 2020). Further studies are necessary to elucidate this relationship. While the presence of this genus is consistent with previous data from other studies, its relative abundance in our culture collection is sharply

different being that this genus only represented 2 of 108 cultured organisms (Gaulke et al., 2016).

Plesiomonas bacteria were also found in relatively high abundance within our culture collection and are typically present in freshwater aquatic environments, especially those in Africa and Southeast Asia (Janda et al., 2016). *Plesiomonas* bacteria has some potential pathogenic effects that have been associated with several human diseases such as dysentery, cholera-like diarrhea, fatal gastrointestinal infections, and others. This bacterial genus also possesses a plasmid for cellular invasion (Janda et al., 2016). Unlike *Pseudomonas, Plesiomonas* taxa generally decrease in relative abundance upon exposure to triclosan (Gaulke et al., 2016). *Pseudomonas spp.* often demonstrate antibiotic resistance to many classes of antibiotics (Lambert, 2002). By contrast, zebrafish that have demonstrated a high relative frequency of *Plesiomonas* in their gut microbiome generally exhibited a lower vulnerability to the parasitic nematode *Pseudocapillaria tomentosa*, as shown in previous studies (Gaulke et al., 2020). These implications are valuable for further experimentation on the value of *Plesiomonas* as an indicator taxon that can be used for further exploration of host-microbiome associations and relationships under different environmental conditions. These types of experiments are important for identifying whether *Plesiomonas* bacteria abundance is the source or the consequence of low parasitic burden (Gaulke et al., 2020). Having a culture collection containing these bacterial genera allows for the necessary experimentation to occur.

The establishment of a culture collection containing this degree of genetic- and species-level diversity coverage will be valuable for future downstream reductive experimentation in that it

provides a source of common, and potentially uncommon, microbiome bacteria from which we can examine functional relationships. For example, this culture collection affords investigators to empirically determine the biological functions of specific groups of gut microbes like *Plesiomonas* and *Pseudomonas* that have been linked to certain physiological processes and pathogenic effects in 16S sequence-based investigations of the zebrafish gut.

Conclusions and Future Directions

Many correlational evidences exist suggesting the relationship between microbiome composition and host health. However, causal relationships between microbes and their hosts remain largely unexplored due to the absence of a necessity for established microbiome culture collections with adequate taxonomic diversity to as to represent the organism of interest. Additionally, parallels between human and zebrafish genomes, endocrine and nervous systems, and gut microbial composition indicate the potential for zebrafish models to help explain various phenomena associated with the human microbiome and its associated conditions.

For this reason, the goal of the work conducted was to generate a foundational zebrafish microbial culture collection and accurately quantify the proportion of zebrafish gut microbial diversity that is culturable. Only by establishing this culture collection can further progression of microbiome research be obtained so as to elucidate and continue to explore the complexities of microbe-host relationships and colonization patterns. In total, 108 zebrafish gut microbes were isolated and preserved, and we were able to assign taxonomy to 78. Together, these represented approximately 50% of the typical zebrafish gut genera. Though this was less than the anticipated microbial representation, 18 genera are still represented in a collection of just 108 isolates. This

research demonstrates the diversity of coverage that can be easily obtained utilizing limited methods and media types in a zebrafish microbial culture collection in a relatively short time period of 6 months.

Although 18 genera were observed, many were found only on a single media type, including all but one isolate of a major taxa identified in the established culture collection. Other media types could be incorporated to potentially yield a greater diversity of taxa that are only viable using other media compositions. Additionally, selective media may be used to identify organisms resistant to antibiotics, representing a particular cell wall composition or performing a particular metabolic function. All of these are necessary to explore and more clearly explain the more complete composition of culturable zebrafish gut bacteria.

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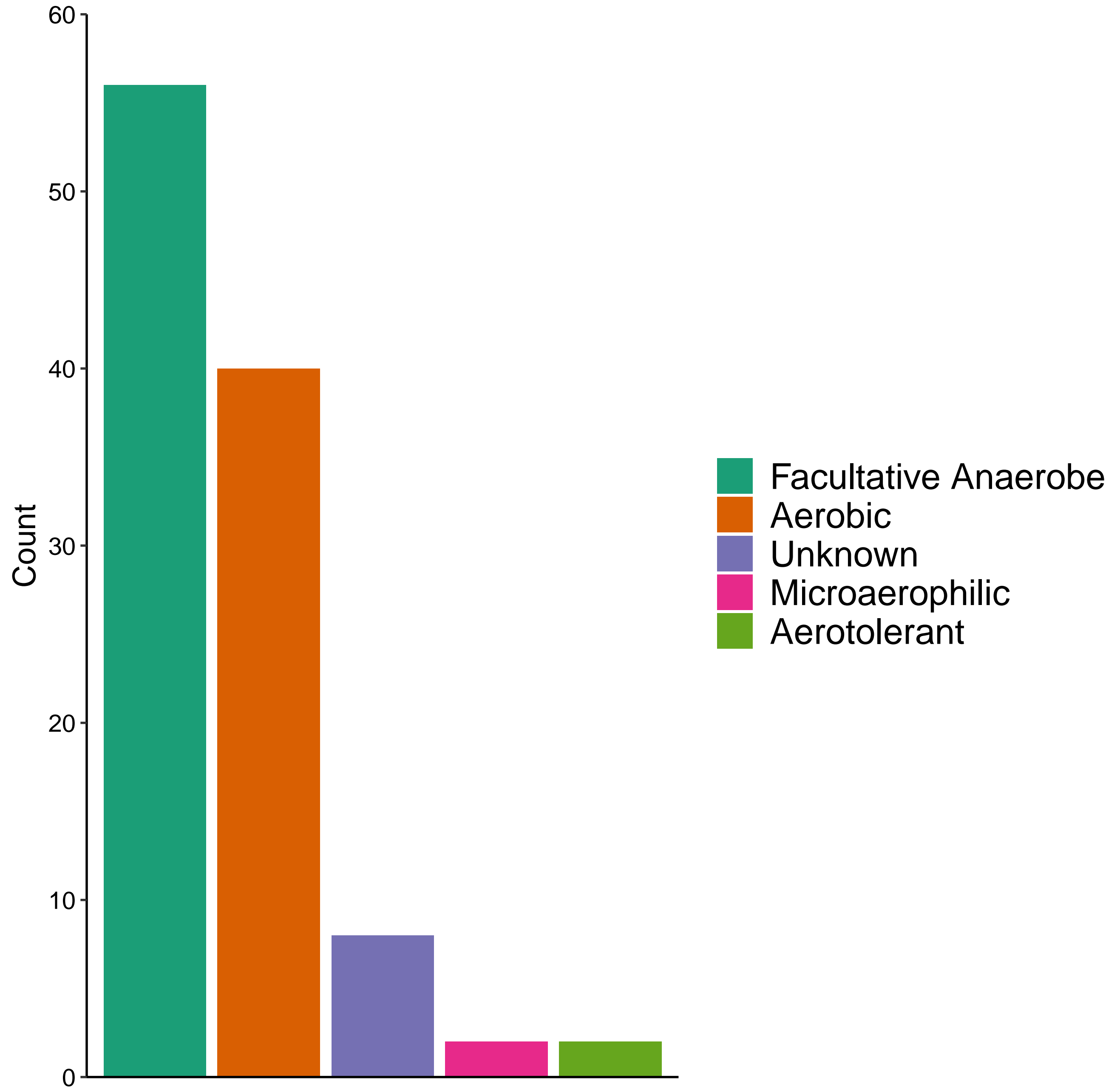

Figure 1. **Oxygen Tolerance across zebrafish culture collection isolates** This figure represents the oxygen tolerance of different isolates derived from zebrafish feces.

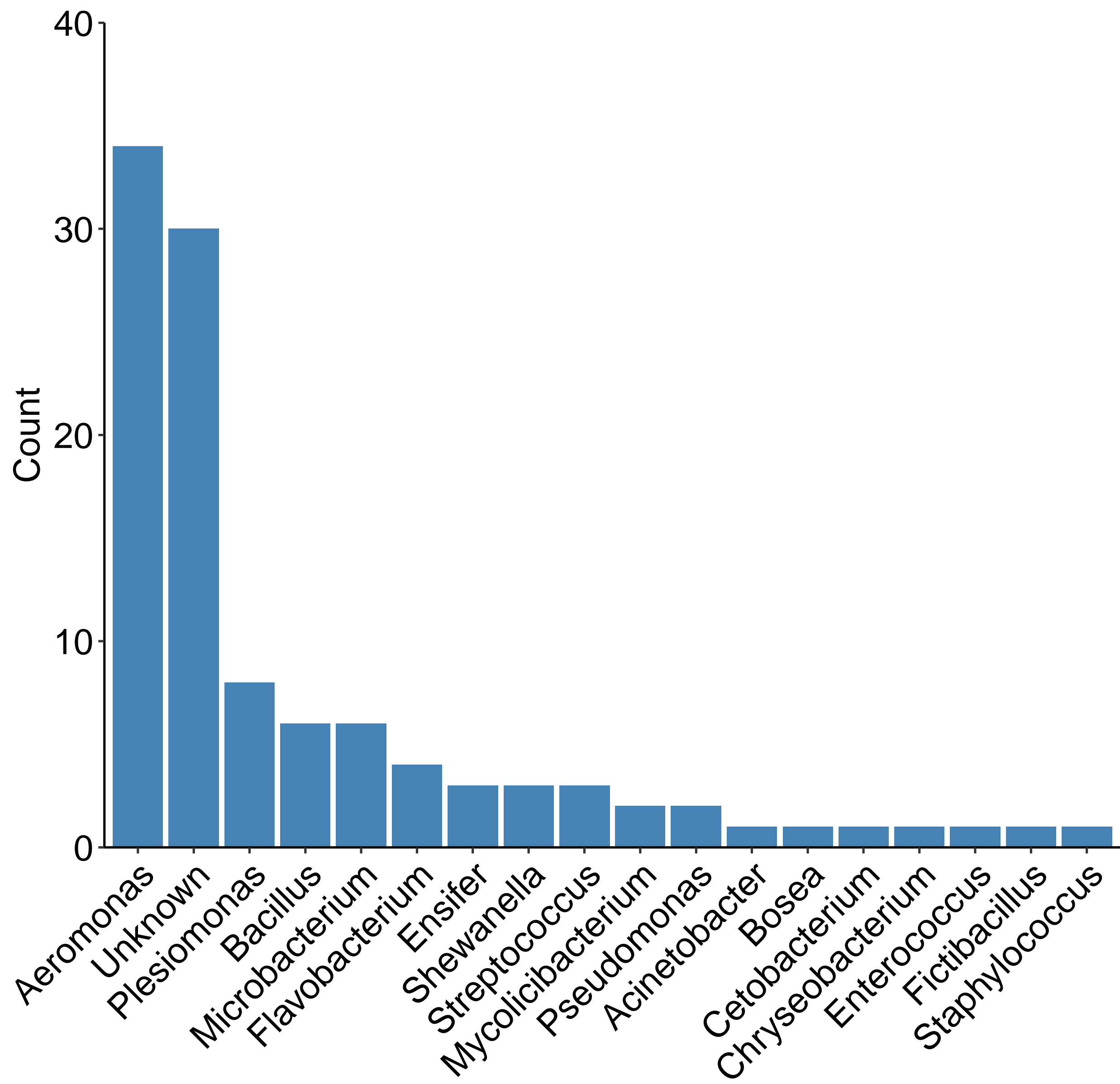

Figure 2. **Taxonomic composition of the zebrafish culture collection.** This figure demonstrates the number of top blast hits that correspond to different genera.

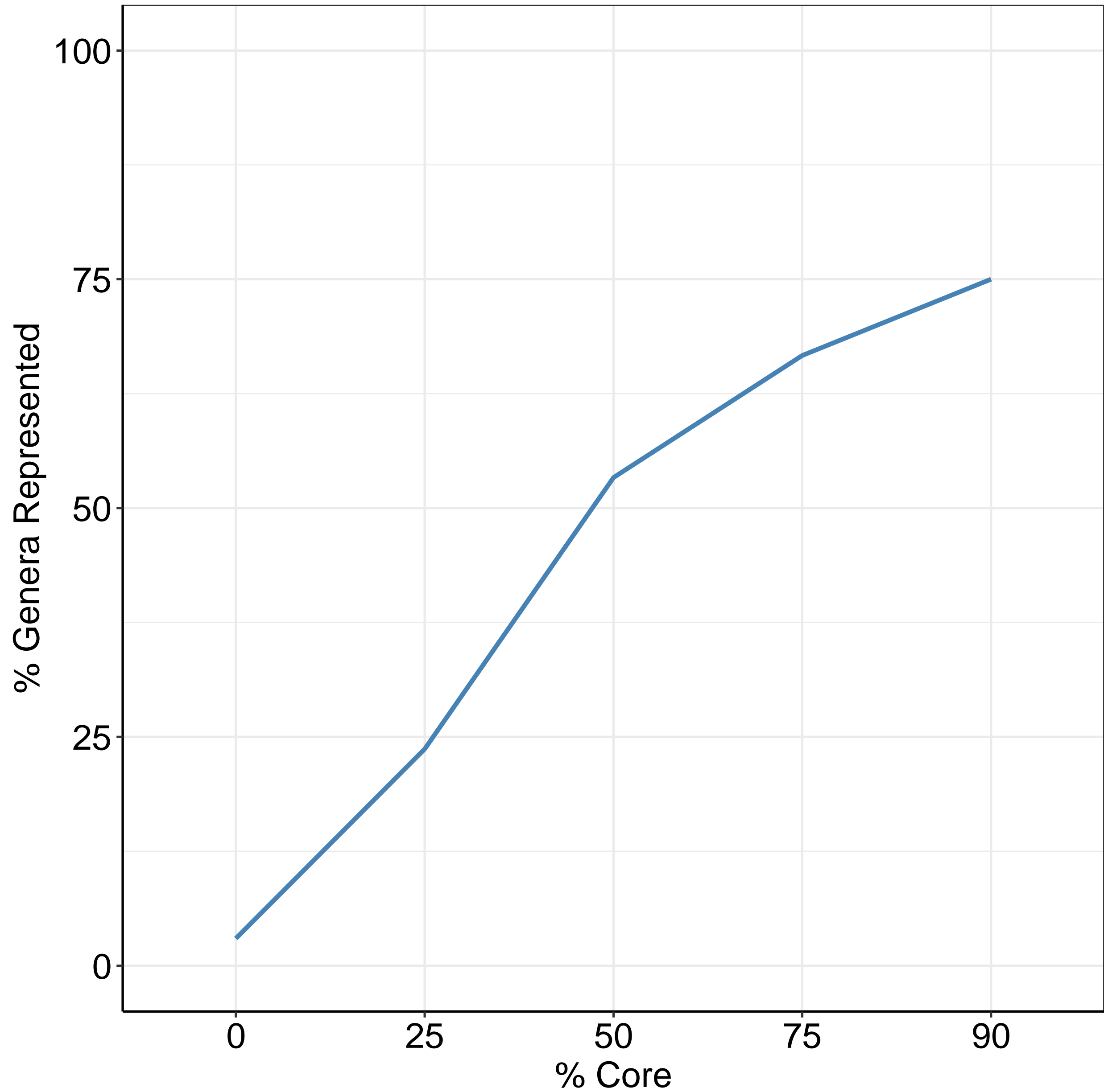

Figure 3. The taxonomic overlap between cultured microbial diversity and 16S rRNA gene taxonomic diversity. A line graph representing the percent of genera identified in a large 16S rRNA screen of zebrafish that are represented by at least one isolate in the zebrafish culture collection. The percent overlap is presented for various levels of prevalence (%core) in the population level screen.

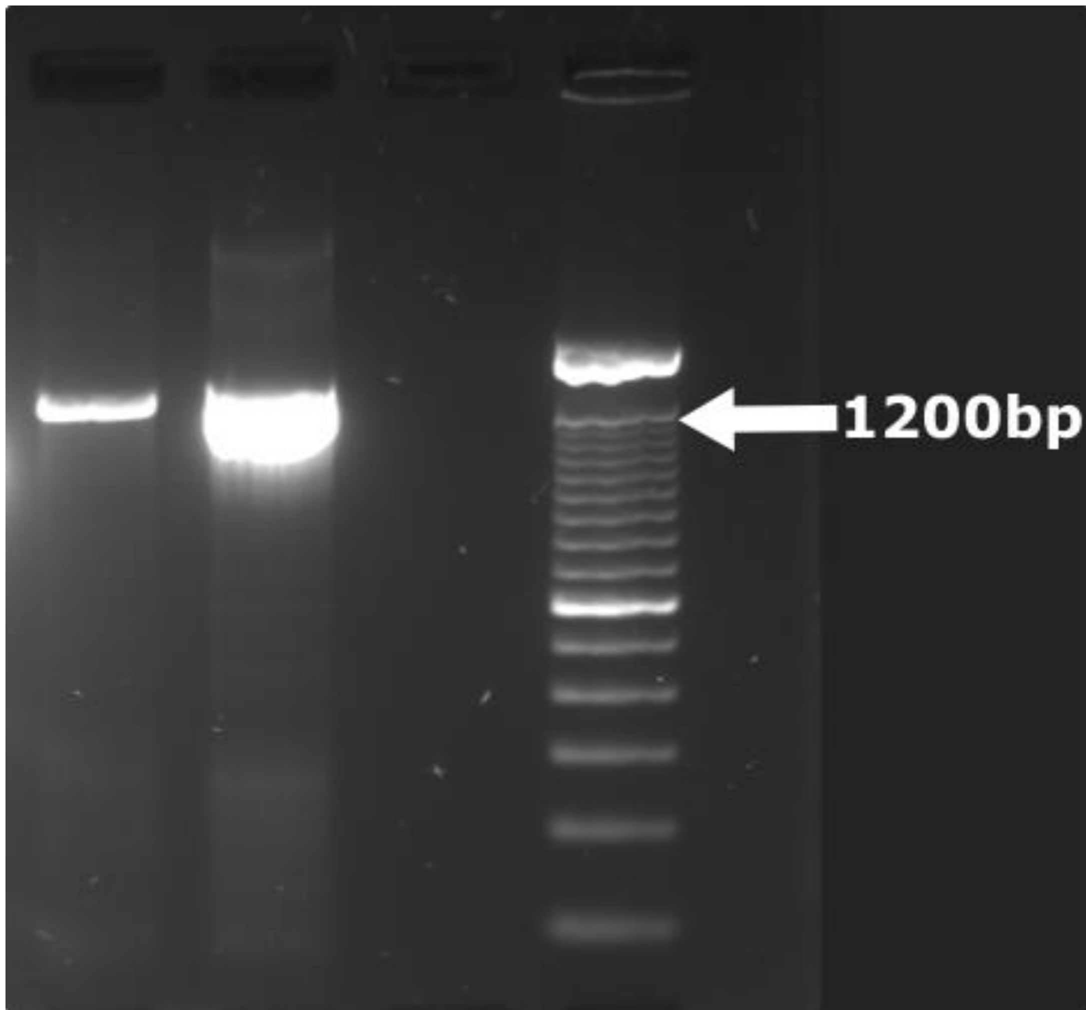

Figure 4. **Amplification of 16s ribosomal RNA gene.** Representative image of PCR amplicons generated from culture collection isolates. The arrow indicates a band of approximately 1200 base pairs

Appendix 1. **Microbial culture collection characteristics.** Oxygen tolerance indicates the result of the oxygen tolerance test, media type indicates the media that the isolate was cultured on, the microbial taxonomic classification indicates the top blast hit for each isolate based on the 16s rRNA gene.

Isolate Identification #	Oxygen Tolerance	Media Type	Microbial Taxonomic Classification
ZFCC_0000	Aerobic	TSA 100%	Bacillus aryabhattai B8W22 16S ribosomal RNA, partial sequence
ZFCC_0001	Aerobic	TSA 100%	Flavobacterium tistrianum strain GB 56.1 16S ribosomal RNA, partial sequence
ZFCC_0002	Aerobic	TSA 100%	Bosea robiniae strain R-46070 16S ribosomal RNA, partial sequence
ZFCC_0003	Aerobic	TSA 100%	Staphylococcus saprophyticus subsp. saprophyticus ATCC 15305 16S ribosomal RNA, complete sequence
ZFCC_0004	Aerobic	TSA 100%	Microbacterium foliorum strain P 333/02 16S ribosomal RNA, partial sequence
ZFCC_0005	Aerobic	TSA 100%	Microbacterium foliorum strain P 333/02 16S ribosomal RNA, partial sequence
ZFCC_0006	Aerobic	TSA 100%	Aeromonas veronii strain JCM 7375 16S ribosomal RNA, partial sequence
ZFCC_0007	Aerobic	TSA 100%	Aeromonas veronii strain JCM 7375 16S ribosomal RNA, partial sequence
ZFCC_0008	Aerobic	TSA 100%	Acinetobacter modestus strain NIPH 236 16S ribosomal RNA, partial sequence
ZFCC_0009	Aerobic	TSA 100%	Microbacterium foliorum strain P 333/02 16S ribosomal RNA, partial sequence
ZFCC_0010	Aerobic	TSA 100%	Microbacterium hydrocarbonoxydans strain BNP48 16S ribosomal RNA, partial sequence
ZFCC_0011	Aerobic	TSA 100%	Aeromonas jandaei strain CDC0787-80 16S ribosomal RNA, partial sequence
ZFCC_0012	Aerobic	TSA 100%	Bacillus mycoides strain DSM 11821 16S ribosomal RNA, partial sequence
ZFCC_0013	Aerobic	TSA 100%	Bacillus mycoides strain DSM 11821 16S ribosomal RNA, partial sequence
ZFCC_0014	Aerobic	TSA 100%	Mycolicibacterium neworleansense strain ATCC 49404 16S ribosomal RNA, partial sequence
ZFCC_0015	Aerobic	TSA 100%	Mycolicibacterium neworleansense strain ATCC 49404 16S ribosomal RNA, partial sequence
ZFCC_0016	Aerobic	TSA 100%	Unknown
ZFCC_0017	Aerobic	TSA 100%	Chryseobacterium zeae strain JM-1085 16S ribosomal RNA, partial sequence

ZFCC_0018	Aerobic	TSA 100%	Pseudomonas mosselii strain CFML 90-83 16S ribosomal RNA, partial sequence
ZFCC_0019	Aerobic	TSA 100%	Mycolicibacterium neworleansense strain ATCC 49404 16S ribosomal RNA, partial sequence
ZFCC_0020	Aerobic	TSA 100%	Aeromonas hydrophila strain CCM 7232 16S ribosomal RNA, partial sequence
ZFCC_0021	Aerobic	TSA 100%	Unknown
ZFCC_0022	Aerobic	TSA 100%	Fictibacillus nanhaiensis strain JSM 082006 16S ribosomal RNA, partial sequence
ZFCC_0023	Aerobic	TSA 100%	Bacillus wiedmannii strain FSL W8-0169 16S ribosomal RNA, partial sequence
ZFCC_0024	Aerobic	TSA 100%	Bacillus megaterium NBRC 15308 = ATCC 14581 16S ribosomal RNA, partial sequence
ZFCC_0025	Aerobic	TSA 100%	Bacillus horikoshii strain DSM 8719 16S ribosomal RNA, partial sequence
ZFCC_0026	Aerobic	TSA 100%	Bacillus horikoshii strain DSM 8719 16S ribosomal RNA, partial sequence
ZFCC_0027	Aerobic	TSA 100%	Shewanella oneidensis strain MR-1 16S ribosomal RNA, partial sequence
ZFCC_0028	Aerobic	TSA 100%	Aeromonas veronii strain JCM 7375 16S ribosomal RNA, partial sequence
ZFCC_0029	Aerobic	TSA 100%	Aeromonas veronii strain JCM 7375 16S ribosomal RNA, partial sequence
ZFCC_0030	Aerobic	TSA 100%	Microbacterium azadirachtae strain AI-S262 16S ribosomal RNA, partial sequence
ZFCC_0031	Aerobic	TSA 100%	Flavobacterium tistrianum strain GB 56.1 16S ribosomal RNA, partial sequence
ZFCC_0032	Aerobic	TSA 100%	Shewanella putrefaciens strain Hammer 95 16S ribosomal RNA, partial sequence
ZFCC_0033	Aerobic	TSA 100%	Aeromonas veronii strain JCM 7375 16S ribosomal RNA, partial sequence
ZFCC_0034	Aerobic	TSA 100%	Ensifer sesbaniae strain CCBAU 65729 16S ribosomal RNA, partial sequence
ZFCC_0035	Aerobic	TSA 100%	Ensifer adhaerens strain LMG 20216 16S ribosomal RNA, partial sequence
ZFCC_0044	Facultative Anaerobe	BHI 100%	Ensifer adhaerens strain LMG 20216 16S ribosomal RNA, partial sequence
ZFCC_0045	Unknown	BHI 100%	Aeromonas veronii strain JCM 7375 16S ribosomal RNA, partial sequence
ZFCC_0046	Unknown	BHI 100%	Aeromonas tecta strain F518 16S ribosomal RNA, partial sequence
ZFCC_0047	Unknown	BHI 100%	Enterococcus casseliflavus strain NCIMB 11449 16S ribosomal RNA, partial sequence
ZFCC_0048	Unknown	BHI 100%	Aeromonas veronii strain JCM 7375 16S ribosomal RNA, partial sequence
ZFCC_0049	Unknown	TSA 100%	Shewanella putrefaciens strain Hammer 95 16S ribosomal RNA, partial sequence
ZFCC_0050	Unknown	TSA 100%	Unknown
ZFCC_0051	Unknown	TSA 100%	Plesiomonas shigelloides strain DSM 8224 16S ribosomal RNA, partial sequence
ZFCC_0052	Unknown	TSA 100%	Unknown
ZFCC_0053	Facultative Anaerobe	BHI 100%	Plesiomonas shigelloides strain DSM 8224 16S ribosomal RNA, partial sequence
ZFCC_0054	Facultative Anaerobe	BHI 100%	Plesiomonas shigelloides strain NCIMB 9242 16S ribosomal RNA, partial sequence
ZFCC_0055	Aerobic	BHI 100%	Plesiomonas shigelloides strain DSM 8224 16S ribosomal RNA, partial sequence
ZFCC_0056	Facultative Anaerobe	BHI 100%	Unknown

ZFCC_0057	Facultative Anaerobe	BHI 100%	Streptococcus salivarius strain ATCC 7073 16S ribosomal RNA, partial sequence
ZFCC_0058	Facultative Anaerobe	BHI 100%	Plesiomonas shigelloides strain NCIMB 9242 16S ribosomal RNA, partial sequence
ZFCC_0059	Facultative Anaerobe	BHI 100%	Aeromonas veronii strain JCM 7375 16S ribosomal RNA, partial sequence
ZFCC_0060	Facultative Anaerobe	BHI 100%	Aeromonas veronii strain JCM 7375 16S ribosomal RNA, partial sequence
ZFCC_0061	Microaerophilic	BHI 100%	Microbacterium azadirachtae strain AI-S262 16S ribosomal RNA, partial sequence
ZFCC_0062	Microaerophilic	BHI 100%	Streptococcus salivarius strain ATCC 7073 16S ribosomal RNA, partial sequence
ZFCC_0063	Facultative Anaerobe	BHI 100%	Aeromonas veronii strain JCM 7375 16S ribosomal RNA, partial sequence
ZFCC_0064	Aerobic	BHI 100%	Flavobacterium tistrianum strain GB 56.1 16S ribosomal RNA, partial sequence
ZFCC_0065	Facultative Anaerobe	BHI 100%	Unknown
ZFCC_0066	Facultative Anaerobe	BHI 100%	Unknown
ZFCC_0067	Aerotolerant	BHI 100%	Unknown
ZFCC_0068	Facultative Anaerobe	BHI 100%	Aeromonas veronii strain JCM 7375 16S ribosomal RNA, partial sequence
ZFCC_0069	Aerobic	BHI 100%	Aeromonas tecta strain F518 16S ribosomal RNA, partial sequence
ZFCC_0070	Facultative Anaerobe	BHI 100%	Aeromonas veronii strain JCM 7375 16S ribosomal RNA, partial sequence
ZFCC_0071	Facultative Anaerobe	BHI 100%	Unknown
ZFCC_0072	Facultative Anaerobe	BHI 100%	Aeromonas veronii strain JCM 7375 16S ribosomal RNA, partial sequence
ZFCC_0073	Facultative Anaerobe	BHI 100%	Aeromonas veronii strain JCM 7375 16S ribosomal RNA, partial sequence
ZFCC_0074	Facultative Anaerobe	BHI 100%	Aeromonas veronii strain JCM 7375 16S ribosomal RNA, partial sequence
ZFCC_0075	Facultative Anaerobe	BHI 100%	Aeromonas veronii strain JCM 7375 16S ribosomal RNA, partial sequence
ZFCC_0076	Aerotolerant	BHI 100%	Unknown
ZFCC_0077	Facultative Anaerobe	BHI 100%	Plesiomonas shigelloides strain DSM 8224 16S ribosomal RNA, partial sequence
ZFCC_0078	Facultative Anaerobe	BHI 100%	Unknown
ZFCC_0079	Facultative Anaerobe	BHI 100%	Unknown
ZFCC_0080	Facultative Anaerobe	BHI 100%	Aeromonas veronii strain JCM 7375 16S ribosomal RNA, partial sequence
ZFCC_0081	Facultative Anaerobe	BHI 100%	Aeromonas veronii strain JCM 7375 16S ribosomal RNA, partial sequence
ZFCC_0082	Facultative Anaerobe	BHI 100%	Aeromonas veronii strain JCM 7375 16S ribosomal RNA, partial sequence
ZFCC_0083	Facultative Anaerobe	BHI 100%	Aeromonas veronii strain JCM 7375 16S ribosomal RNA, partial sequence
ZFCC_0084	Facultative Anaerobe	BHI 100%	Unknown
ZFCC_0085	Facultative Anaerobe	BHI 100%	Plesiomonas shigelloides strain DSM 8224 16S ribosomal RNA, partial sequence
ZFCC_0086	Facultative Anaerobe	BHI 100%	Aeromonas veronii strain JCM 7375 16S ribosomal RNA, partial sequence
ZFCC_0087	Facultative Anaerobe	BHI 100%	Streptococcus salivarius strain ATCC 7073 16S ribosomal RNA, partial sequence
ZFCC_0088	Facultative Anaerobe	BHI 100%	Aeromonas veronii strain JCM 7375 16S ribosomal RNA, partial sequence
ZFCC_0089	Facultative Anaerobe	BHI 100%	Aeromonas veronii strain JCM 7375 16S ribosomal RNA, partial sequence

ZFCC_0090	Facultative Anaerobe	BHI 100%	Aeromonas veronii strain JCM 7375 16S ribosomal RNA, partial sequence
ZFCC_0091	Aerobic	BHI 100%	Unknown
ZFCC_0092	Facultative Anaerobe	BHI 100%	Aeromonas veronii strain JCM 7375 16S ribosomal RNA, partial sequence
ZFCC_0093	Facultative Anaerobe	BHI 100%	Unknown
ZFCC_0094	Facultative Anaerobe	BHI 100%	Aeromonas veronii strain JCM 7375 16S ribosomal RNA, partial sequence
ZFCC_0095	Facultative Anaerobe	BHI 100%	Aeromonas veronii strain JCM 7375 16S ribosomal RNA, partial sequence
ZFCC_0096	Facultative Anaerobe	BHI 100%	Unknown
ZFCC_0097	Facultative Anaerobe	BHI 100%	Unknown
ZFCC_0098	Facultative Anaerobe	BHI 100%	Aeromonas veronii strain JCM 7375 16S ribosomal RNA, partial sequence
ZFCC_0099	Facultative Anaerobe	BHI 100%	Unknown
ZFCC_0100	Facultative Anaerobe	BHI 100%	Aeromonas veronii strain JCM 7375 16S ribosomal RNA, partial sequence
ZFCC_0101	Facultative Anaerobe	BHI 100%	Plesiomonas shigelloides strain NCIMB 9242 16S ribosomal RNA, partial sequence
ZFCC_0102	Facultative Anaerobe	BHI 100%	Unknown
ZFCC_0103	Facultative Anaerobe	BHI 100%	Unknown
ZFCC_0104	Facultative Anaerobe	BHI 100%	Aeromonas veronii strain 115/II 16S ribosomal RNA, partial sequence
ZFCC_0105	Facultative Anaerobe	BHI 100%	Cetobacterium somerae strain WAL 14325 16S ribosomal RNA, partial sequence
ZFCC_0106	Facultative Anaerobe	BHI 100%	Unknown
ZFCC_0107	Facultative Anaerobe	BHI 100%	Unknown
ZFCC_0108	Facultative Anaerobe	BHI 100%	Unknown
ZFCC_0109	Facultative Anaerobe	BHI 100%	Unknown
ZFCC_0110	Facultative Anaerobe	BHI 100%	Unknown
ZFCC_0111	Facultative Anaerobe	BHI 100%	Unknown
ZFCC_0112	Facultative Anaerobe	BHI 100%	Unknown
ZFCC_0113	Facultative Anaerobe	BHI 100%	Unknown
ZFCC_0114	Facultative Anaerobe	BHI 100%	Unknown
ZFCC_0115	Facultative Anaerobe	BHI 100%	Unknown