AMERICAN UNIVERSITY OF BEIRUT

OVERVIEW OF THE GUT MICROFLORA IN WILD-CAUGHT FISH AT THE LEBANESE SHORE

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science to the Department of Biology of the Faculty of Arts and Sciences at the American University of Beirut

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AN ABSTRACT OF THE THESIS OF

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Microflora refers to the collection of live microscopic organisms that flourish inside the organs of living creatures including fishes. Bacteria living on the inner intestinal walls play an important role in digesting food, absorbing nutrients and defending the host from outer pathogens. All living organisms including fish interact with and are affected by bacteria. However, the gut flora of wild fish remains poorly characterized. The aim of this work is to provide an overview of the bacteria colonizing the gut of wild-caught fish. After isolation of cultivable bacteria, 16s ribosomal DNA sequencing was performed for identification purposes. R program was then used to compare sequences corresponding to bacteria from 15 different fish species divided into three categories based on their habitat, diet and origin. The potential pathogenicity of some bacteria was also investigated using the model organisms Danio rerio and Drosophila melanogaster. Serratia and Aeromonas salmonicida were lethal to Drosophila while all Danio rerio fish didn't show any distress symptoms when exposed to these bacteria. This study paves the way to a more complete project including the identification of the uncultivable bacteria that reside in the gut, the examination of several specimens per fish species and the extension of the analysis to a larger number of fish species.

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ABBREVIATIONS

%	Percent
/	Per
~	Almost equal
PH	Power of Hydrogen
G	Grams
CFU	Colony-forming unit
°C	Degrees Celsius
PCR	Polymerase Chain Reaction
μl	Microliter
MgCl ₂	Magnesium Chloride
mM	Millimolar
dNTPs	Deoxynucleotide Tri Phosphate
rRNA	Ribosomal ribonucleic acid
16s rRNA F	Forward primer
16s rRNA R	Reverse primer
DNA	Deoxyribonucleic acid
min	Minutes
ml	Milliliter
TBE	Tris/Borate/EDTA buffer
mg	Milligram
UV	Ultra-Violet
V	Volts
g	G-Force
M	Molar
ng	Nanogram
NaCl	Sodium Chloride
EDTA	Ethylenediaminetetraacetic acid
SDS	Sodium dodecyl sulfate
STE	Nacl/Tris/EDTA buffer
p-GEM-T	Promega vector
CaCl ₂	Calcium chloride
DH5a	Competent E. coli cells
LB	Luria Bertani broth
AMP	Ampicillin
Solution I	Tris/EDTA/Glucose
Solution II	NaOH/SDS
Solution III	KOH/Acetic acid/ H ₂ O
EcoRI	Endonuclease enzyme
X-Gal	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside
IPTG	Isopropyl β-D-galactoside
L	Liter
OD	Optical Density
Hrs	Hours
nl	Nanoliter
PBS	Phosphate buffered saline

CHAPTER I

INTRODUCTION

A. The Mediterranean and modes of fish introduction:

It is widely accepted that the Mediterranean Sea is populated by organisms from the Atlantic Ocean when connection was reestablished after the Messinian period (McKenzie, 1987; Por and Dimentman, 1989). Nowadays, the Mediterranean is a semienclosed sea connected to surrounding water bodies by the Strait of Gibraltar, the Dardanelles and the Suez Canal.

Hundreds of non-indigenous marine species thrive in the Mediterranean Sea. They were introduced by several means such as ballast waters, aquaculture or as fouling organisms (Coll et al., 2010). However, the most important source of introduction is undoubtedly by crossing the Suez Canal. The connection between the Red Sea and the Mediterranean allowed a massive flow of organisms from the Indo-Pacific realm. This process was termed Lessepsian Migration (Por, 1978; 2010). Today, the number of bony fish species of Indo-Pacific origin has exceeded 89 species and this number is expected to increase further in the future (CIESM, 2016).

B. Microflora and modes of ingestion:

Microflora refers to the living microscopic organisms that flourish inside and on the surface of living creatures, including fishes. These organisms are usually found on the skin, tissues and inside guts (Cahill, 1990; Austin, 2006). Bacteria living on the inner intestinal walls play an important role in digestion, absorption and in defense against pathogens (Cahill, 1990; Austin, 2006).

The composition of microbial communities within fish guts differs significantly from those living in the surrounding environment in both diversity and specificity (Austin and Austin, 1987; Cahill, 1990; Ringø et al., 1995). Some of these bacteria permanently occur within the microflora while others appear to be transient (Cahill, 1990). These bacteria reach the inside of the organisms through different means. While some are ingested during the larval stage and may establish in the guts of juvenile fishes others may result from the intimate contact of egg chorions with bacteria in the aqueous environment (Hansen and Olafsen, 1999). Adapting to gut environmental conditions like nutrient availability, pH and digestive enzymes remain the key factor for those bacterial communities to proliferate and thus persist within the intestines (Hansen and Olafsen, 1999). The composition of the gut microflora changes in response to a variety of factors affecting the host, such as feeding strategies, developmental stages, digestive physiology and changing environmental conditions (Yoshimizu and Kimura, 1976; MacFarlane et al., 1986; Cahill, 1990; Vemer-Jeffreys et al., 2003; Romero and Navarrete, 2006; Uchii et al., 2006).

C. Interactions between microbial communities and their hosts:

Microbial communities are known to help in the digestion. They interact with their hosts in several ways, affecting nutrition, growth, reproduction and vulnerability of the host to diseases. In addition, they have an impact on the overall population dynamics (MacFarlane et al., 1986). As such, *Bacteroides* and *Clostridium* spp. were shown to enhance nutrition by providing essential fatty acids and vitamins (Ringø et al.,

1995). *Lactobacillus* spp. may be more or less important components of microflora communities promoting fish health and preventing the establishment and growth of potential pathogens (Strøm, 1988; Izvekova et al., 2007).

D. Composition of microflora:

Different studies showed that the composition of gut microflora varies among different fish species. However, differences and inconsistencies among methods do not allow direct comparisons between species. Studies used culture-based techniques to identify bacteria providing valuable insights into the composition of microbial communities (Newman et al., 1972; MacFarlane et al., 1986; Spanggaard et al., 2000; Aschfalk and Muller, 2002; Verner-Jeffreys et al., 2003; Al-Harbi and Naim Uddin, 2004; Martin-Antonio et al., 2007; Skrodenytė-Arbaĉiauskienė, 2007). However, these studies were mostly focused on fish grown in farms and they provided biased assessments of the microbial community composition since typically less than one percent of microflora cells produce colonies when cultured on solid media (Ferguson et al., 1984; Head et al., 1998). Gamma-Proteobacteria such as Aeromonas spp., Escherichia coli, Photobacterium spp., Pseudomonas spp. and Vibrio spp., dominated the gut microbiome of most fishes (Newman et al., 1972; MacFarlane et al., 1986; Ringø, 1993b; Ringø and Strøm, 1994; Spanggaard et al., 2000; Verner-Jeffreys et al., 2003; Al-Harbi and Naim Uddin, 2004; Bates et al., 2006; Romero and Navarrete, 2006; Skrodenyte-Arbaciauskiene et al., 2006; Martin-Antonio et al., 2007; Skrodenytė-Arbaĉiauskienė, 2007; Ransom, 2008; Ward et al., 2009). It was estimated that some bacterial populations reach 10^8 aerobic bacteria and 10^5 anaerobic bacteria per gram of gut content with different abundances within the gut of the same fish (Austin, 2006).

E. Environmental stressors and alteration of the composition of gut microflora:

The composition of gut microflora is altered with the changing environmental conditions and stressors where the composition of the transient microflora is reported to be affected by environmental factors, by bacteria presence in the water column and with a changing diet (Hansen and Olafsen, 1999; Nayak, 2010). Since stressors such as temperature, low oxygen concentration and pollutants may weaken the immune system of the host, the presence of pathogens in the surrounding environment may affect the fish health negatively as these opportunistic cells may colonize the host's gut (Hansen and Olafsen, 1999).

Variations in salinity and temperature also play a major role in the composition microflora communities in fishes. Yoshimizu and Kimura (1976) and MacFarlane et al. (1986) documented shifts in the composition of fish gut microflora coinciding with salinity variations encountered in estuarine environments. Other studies showed that many freshwater fishes contain *Aeromonas* sp. within their guts while *Vibrio* sp. was documented in estuarine and marine species (Cahill, 1990; Ringø et al., 1995; Ringø and Birkbeck, 1999). This suggests that any change in the salinity of water due to global warming or raining patterns may lead to an alteration in the microflora community in fish's gut. The potentially pathogenic *Vibrio vulnificus* was detected in the sheepshead (*Archosargus probactocephalus*, Sparidae) sampled from the Gulf of Mexico (DePaola et al., 1994; 1997). The presence and abundance of this bacterium is closely linked to increased water temperature, with highest densities occurring when water temperatures range between 20 and 30°C (Kelly, 1982; DePaola et al., 2003; Tantillo et al., 2004). Although this bacterium is naturally present within the sheepshead gut, it was recorded that its increased abundance is correlated with warmer water temperatures where

densities were 2-3 orders of magnitudes lower in March and December (colder period) compared to those in May and September (warmer period) (DePaola et al., 1997).

F. Pathogens and seafood illness:

Human pathogens can be found in the fish gut microflora and play a major role in seafood-associated bacterial illness and mortality. Vibrio parahaemolyticus and V. *vulnificus* are leading causes of human and marine mammals casualties although several members of this genus are nonpathogenic and are found to be the dominant bacteria in and on marine fishes (Iwamoto et al., 2010). They have been commonly reported as members of the gut microflora in both farmed and wild fishes (MacFarlane et al., 1986; Cahill, 1990; Sakata, 1990; Blanch et al., 1997; Martin-Antonio et al., 2007; Ward et al., 2009; Iwamoto et al., 2010). Most infections involving these two bacteria are through consumption of raw or undercooked seafood, while others results from wound infections leading to gastroenteritis, septicemia and V. vulnificus septicemia (Constantin de Magny et al., 2009). Infections with V. parahaemolyticus are the leading cause of bacterial illnesses from seafood consumption in the United States with 22.5% hospitalization and 0.9% mortality rates (Iwamoto et al., 2010; Scallan et al., 2011). However, V. vulnificus infections are rare but they are the leading cause of seafoodrelated deaths with a 91.3% hospitalization and 34.8% mortality among all foodborne pathogens (Iwamoto et al., 2010; Scallan et al., 2011).

Photobacterium damselae, which is a virulent strain, can also impact fish and humans causing septicemia and internal hemorrhage in fish and septicemia and wound infections in humans (Shin et al., 1996; Fouz et al., 2000). *Photobacterium damselae* subsp. *piscida,* which is a subspecies of *P. damselae,* is not a human pathogen but it is a

serious fish pathogen leading to high levels of mortality (Thyssen et al., 1998; Fouz et al., 2000). *Streptococcus inae, Aeromonas hydrophilia, Edwardsiella tarda, E. rhusopathiae, Mycobacterium marimum* and other *Vibrio* spp. are additional pathogens leading to human diseases (Zlotkin et al., 1998; Lehane and Rawlin, 2000; Colorni et al., 2002).

Transmission of pathogens may also occur via infections and open wounds if gut microflora can persist in water. Cahill (1990) showed that *Aeromonas* spp.'s population increased in seawater aquarium when fishes stayed for longer times (181 days). But, since *Aeromonas* spp. were not isolated from seawater, it was suggested that this population is a result of accumulation of fish feces in the water of the aquarium (Cahill, 1990). Water quality in areas with dense fish populations, in shallow waters, in areas of decreased water flushing and in areas with increased residency may be affected by persisting gut microflora. Also, fish inhabiting water polluted by human sewage may become a carrier or vector of human diseases representing a great public health threat (Janssen and Meyers, 1968).

Pathogenic bacteria may be also transferred through fish to new hosts like humans and other marine animals. This risk of transmission depends on the interaction between the organism, the physiology of the infected person and other environmental factors (Strom and Paranjpye, 2000; Oliver, 2006). Consumption of raw and undercooked shellfish and fish containing pathogens increases the risk of infection where *V. vulnificus* can move directly into edible portions of the fish from intestines in addition to *E. coli* and *Salmonella* spp. which can move from stomach to blood and fish muscles. Other infections by pathogens can take place during filleting and direct contact and handling of some species (Buras et al., 1985; DePaola et al., 1994).

Fish may be a key link in pathogen cycling between fishes, the water column, sediments and other marine organisms. This theory refers to the point that most pathogens require nutrient rich environments like the gut for growth and survival. These pathogens are then expelled with fecal matter and represent a seed population that can colonize the surrounding environment. Therefore, fishes may play an important role in the epidemiology of illnesses and deaths arising from oyster and shellfish consumption since they harbor potential pathogenic bacteria within their intestines and affect fishes, oceans and human health (Ruby and Nealson, 1978; Givens, 2012).

G. Infections of ectoparasites and nestedness:

Several studies analyzing infections of fish with ectoparasites found nested patterns of parasite occurrence (Gonzalez and Poulin, 2005; Gonzalez and Oliva, 2009; Rohde et al., 1998; Worthen and Rohde, 1996; Poulin and Guègan, 2000). Werner et al. (2009) defined nested patterns as "those in which the species composition of small assemblages is a nested subset of larger assemblages". Different colonization probabilities and dominance of infection of ectoparasites are the main reasons of a nested distribution (Worthen and Rohde, 1996). Any changes in patterns may be due to the interactions with different host fish species, intermediate hosts, host densities and environmental conditions (Gonzalez and Poulin, 2005; Gonzalez and Oliva, 2009).

H. Aims and significance of the project:

The bacterial presence in the guts of wild fishes has rarely been investigated and remains poorly characterized. Furthermore, it is also not known whether introduced fish species acquire a flora similar to that of indigenous species, when sharing a similar

habitat and diet, or whether they retain a different gut flora, similar to that of the original habitat. The aim of this work was to provide an overview of the bacteria colonizing the gut of wild-caught native and introduced fishes from off the coast of Beirut (Lebanon) and to compare them according to their habitat, diet and origin. We also attempted to determine whether bacterial species distribution over host fish displayed nestedness and investigated the potential pathogenicity of some bacteria using the model organisms *Danio rerio* (Fish) and *Drosphila melanogaster* (Fruit fly).

CHAPTER II

Materials and Methods:

A. Sampling Design

Gut samples were collected from fish species that were caught in coastal waters around Beirut. 15 different species of fish belonging to different families were collected from Ozaii Port. All fishes were kept on ice when transported to the lab and dissected directly.

B. Polymerase Chain Reaction PCR:

Each PCR mix consisting of 28µl distilled water, 4µl 10X buffer, 3.5µl Mgcl₂ (25mM), 0.75µl dNTPs (2mM), 0.75µl 16sF, 0.75µl 16sR and 0.25µl Taq polymerase was added to 2µl DNA template. The PCR program was as follows: Step 1: 95°C for 5 min, Step 2: 95°C for 30 seconds, Step 3: 53°C for 30 seconds, Step 4: 72°C for 2 min, Step 5: repeat steps 2-4 30 times, Step 6: 72°C for 5 min and Step 7: 4°C forever.

C. Gel Electrophoresis:

0.5g of agarose were dissolved in 50ml 1X TBE to obtain a 1% agarose suspension. This mix was heated until no solid particles were found. Then 2µl of Ethidium Bromide (4mg/ml) were added and the mix was poured in a comb holding tray. After the gel solidified, combs were gently removed. 10µl of PCR product were mixed with 2µl 5X loading buffer and then loaded into the wells. The ladder was loaded in the first well of each row. After checking the electrodes and their proper positions the gel ran at 90V for 40 min. The results were checked using UV light.

D. DNA Purification:

PCR product was diluted in water to reach 100µl volume, then samples were mixed with 1 volume of Tris-Saturated Phenol and 1 volume of chloroform. This mix was centrifuged at 15000g for 5 min at room temperature. 90µl of the upper aqueous phase was then transferred to a new tube where an equal volume of chloroform was added and vortexed. The new mix was centrifuged at 15000g for 5 min at room temperature. The upper aqueous phase was transferred to a new tube where 1/10 the volume of 3M sodium acetate solution and 2.5 volumes of ethanol were added to precipitate the DNA. The mix was incubated for 30 min at -80°C then centrifuged for 15 min at 20000g. The supernatant was discarded and the pellet was rinsed with 70% cold ethanol then centrifuged again for 5 min at 20000g. The pellet was left to air dry after discarding the supernatant and resuspended in 12µl of nuclease free water.

E. Preparation of DNA for Sequencing:

After measuring DNA concentration using a Nanodrop machine, it was diluted to 80ng/µl. Then, 8.5µl of DNA was mixed with 1.5µl of 16sF or 16sR primers and 6µl of distilled water.

F. Identification of Species and BLAST (<u>www.ncbi.nlm.nih.gov</u>):

We used the NCBI nucleotide blast facility to compare the 16s sequences we obtained to database sequences.

G. STE DNA extraction:

Intestines were shredded using blades and STE was added proportional to the intestines' sizes (0.5g: 1ml). Tubes were incubated at 95°C for 10 min then centrifuged at 20000g for 10 min. Supernatant was transferred to a fresh tube and 100µl of sodium acetate were added. After a gentle mix, the tubes were incubated on ice for 20 min. Then 630µl of isopropanol were added and tubes were manually mixed by inverting several times. This mix was centrifuged at 20000g at 4°C for 15 min. Supernatant was discarded and the pellet was rinsed with 70% ethanol then centrifuged for 2 min at 20000g. After discarding the supernatant, the pellet was partially air dried (5 min) and resuspended in 100µl distilled water. To make sure all DNA was dissolved, the eppendorf was incubated at 50°C for 30 seconds and vortexed. The dissolved DNA was stored at -20°C or directly used for PCR.

H. Livak DNA Extraction:

Gut samples were homogenized with a sterile pestle in 500µl of pre-heated Livak grind buffer (80mM NaCl, 0.16M sucrose, 130mM Tris Base, 50.8mM EDTA ph= 8.0 and 5mM SDS) in 1.5ml microcentrifuge tubes. The mix was incubated at 65°C for 30 min. Then, 70µl of potassium acetate were added to obtain a 1.0M solution. This solution was mixed gently and incubated on ice for 30 min. Eppendorfs were centrifuged at 4°C at 20000g for 20 min and the resulting supernatant was added to 1000µl of 100% ice cold ethanol and mixed. The tubes were centrifuged at 4°C at 20000g for 15 min to pellet the DNA. Then, the pellet was rinsed with 500µl of cold 70% ethanol. After discarding the supernatant, the pellet was kept to air dry for 5 min, then resuspended in 50µl of distilled water and incubated at 65°C for 10 min.

I. Bacterial Genomic DNA Preparation:

1.5ml of each bacterial culture were transferred to eppendorfs then centrifuged at 6000g for 5 min at 4°C. Supernatant was discarded and the pellet was resuspended in 300 μ l STE buffer then incubated at 95°C for 15 min. This mix was centrifuged at 12000g for 10 min at 4°C. Then, 200 μ l of the supernatant were transferred to a clean eppendorf. Tubes were placed on ice and 20 μ l of 3M sodium acetate were added and mixed gently. Eppendorfs were centrifuged at 20000g for 15 min at 4°C after the addition of 2V of 100% cold ethanol. Supernatant was discarded and the pellet was then washed with 500 μ l of 70% cold ethanol and centrifuged at 20000g for 5 min at 4°C. Supernatant was then discarded and any residual liquid was removed without dislodging the pellet. This pellet was then left to air dry at room temperature for 5-10 min. Then 50-100 μ l of distilled water were added and the pellet was resuspended by flicking and heating at 60°C for 5 min before being stored at -20°C.

J. DNA Ligation and Transformation:

3 different reactions were prepared according to the manufacturer protocol (Promega). The standard reaction was made up of 5µl of 2x Rapid Ligation Buffer, 1µl of pGEM-T Easy Vector (50ng), 3µl of PCR Product and 1µl of T4 DNA Ligase (3 units/µl). The background control was made up of the same mix but the PCR product was replaced by 3µl of distilled water. The positive control was made up of 5µl of 2x Rapid Ligation Buffer, 1µl of pGEM-T Easy Vector(50ng), 2µl of Control Insert DNA, 1µl of T4 DNA Ligase (3 units/µl) and 1µl of distilled water. Reactions were kept at room temperature for 2 hours then they were mixed and heated at 65°C for 5 min. 50µl of CaCl₂ Competent cells (*E. coli* DH5α) were added to clean pre-chilled eppendorfs

containing 2µl of ligation mix. These eppendorfs were left on ice for 30 min then heat shocked for 2 min at 42°C and returned back on ice. 350µl of LB were added to each eppendorf and 100µl of the mix were plated on LB agar + AMP plates and kept at 37°C overnight. Using a tip, single colonies were inoculated in a 13ml falcon tube containing 3ml of LB+AMP mix and grew overnight at 37°C in a shaker.

K. Plasmidic DNA Mini Preps:

Eppendorfs containing 1.5ml of bacterial culture were centrifuged at 6000g for 5 min at 4°C. After discarding supernatant, 200µl of Solution I were added to resuspend pellet. Then 200µl of Solution II were added and mixed by inverting the eppendorf 2-3 times and left for 5 min at room temperature. 200µl of Solution III were added and mixed by inversion. Tubes were centrifuged at 17000g at 4°C and 500µl of supernatant were transferred into new eppendorfs. Then 350µl of Isopropanol were added and mixed and eppendorfs were centrifuged at 20000g for 15 min. The supernatant was discarded and the pellet was washed with 200µl of 70% ethanol and spun for 5 min at 20000g. Supernatant was discarded and pellet was left to air dry then resuspended in 50µl distilled water. After resuspension, DNA concentration was measured using NanoDrop device.

L. Digestion and Gel Electrophoresis:

A mix containing 5µl of purified DNA, 0.5µl of EcoRI enzyme, 2µl of Buffer and 12.5µl of distilled water was kept at 37°C for 2 hours. Then, this mix was run on a gel to check DNA bands.

M. Plate culturing:

3 small slices from different places of the intestine were cut and ground in 200µl of LB and diluted to reach a volume of 1ml. Plate 50 and 100µl of each mix on different LB plates and keep to solidify at room temperature until colonies were observed.

N. Streaking of Individual Colonies:

Bacterial colonies were checked for their colors and patterns and individual colonies were isolated in proximity to a bunsen burner using a loop. Dishes were kept overnight at room temperature for colonies to grow.

O. Liquid Culture:

In presence of a flame, single colonies were taken using a tip to inoculate a 13ml falcon tube containing 3ml of liquid LB. They were left to grow overnight in a shaker at 25°C.

P. Preparation of Solid Media Plates:

1. Preparation of LB Agar + AMP plates:

200ml of LB Agar were autoclaved then kept at 50°C for 1 hour. 200 μ l of Ampicillin (100mg/mL) were added and plates were poured next to a flame. As the plates solidified, they were turned upside down and kept at room temperature overnight. Then they were stored at 4°C.

2. Preparation of LB Agar + AMP + X-Gal + IPTG plates:

500ml of LB Agar was autoclaved then kept at 50°C for 1 hour. 500µl of Ampicillin (100mg/ mL), 500µl of 3% X-Gal and 50µl of IPTG (100mM) were added

and plates were poured next to a flame. The plates then were covered with aluminum foil.

3. Preparation of LB plates (Luria Bertani Broth):

12.5g of LB and 500ml of distilled water were mixed in a 1000ml flask and autoclaved at 121°C for 20 min. After cooling down at 55°C, LB was poured in plates.

Q. Bacterial Exposure Experiment:

6 containers were prepared by adding 2 liters of tap water, 1 liter of aquarium water and bubblers. 50µl of bacterial mix (OD \approx 50) were added to 6 small cups containing 100ml of water each and 3 zebra fish were added and left for 45 min. The fish were transferred to 2 liters containers. Different time points were set to determine any change in the behavior of fish (3hrs, 6hrs, 9hrs, 12hrs, 24hrs, 36hrs, 48hrs, 60hrs and 72hrs).

R. Drosophila Injection:

32nl bacterial suspension (OD= 0.15) were injected into the thorax of wild type *Drosophila melanogaster* using a Nanoject II apparatus. Each experiment was performed using 15 flies in fresh vials and the survival was monitored by counting the flies at regular intervals after injection.

S. OD Measurement:

Bacterial cultures were centrifuged for 5 min at 6000g. Supernatant was removed and the pellet was vortexed in the residual volume of LB and transferred to an

eppendorf. 10µl of bacterial culture was added to 990µl of water, PBS or 0.9% saline solution in a clean cuvette. After mixing, OD was measured using a spectrophotometer.

T. Data Analysis:

The statistical programming language R (R Core Team 2015) was used for data analysis.

1. Defining Operational Taxonomic Units

An alignment of 16s nucleotide sequences was used to define bacterial operational taxonomic units (OTUs). The proportion of nucleotides that differ between 16s sequences was calculated for all sequence pairs and a graph was constructed treating 16s sequences as vertices and connecting all vertices that differ by less than 5% of the nucleotide positions. The maximal cliques (i.e. fully connected subgraphs) were determined for this graph using the function *clique* in the R package *optpart*. Vertices that belonged to multiple cliques were arbitrarily assigned to one of the cliques they belong to. The cliques were considered OTUs. This procedure ensured that all members of the same OTU differ by not more than 5% of their nucleotides. However, some members of different OTUs could differ by 5% or less.

2. Analysis of the Community Matrix

A binary community matrix was created to indicate which bacterial OTU occurred within which fish species. This community matrix had one row per bacterial OTU and one column per fish species and contained a one if the respective OTU was present in the fish species and a zero otherwise. The nestedness of this community matrix was analyzed using the function *nestedtemp* in the *vegan* package. Furthermore, the community matrix was used to calculate pairwise Euclidian distances between

OTUs and between fish species. For each set of pairwise distances (one for the OTUs and one for the fish species) multidimensional scaling was applied to represent the species as points in two-dimensional space (function *cmdscale* in *stats* package). These points were plotted to determine by visual inspection whether either fish species or bacterial OTUs form distinct clusters.

CHAPTER III

Results:

A. Fish collection and bacteria cultivation:

Fifteen different fish species were collected from Beirut seashore (Figure 1). Fishes were identified and categorized according to their diet, origin and swimming mode (Bariche, 2012). Dissected guts were ground with their contents in LB, spread on agar plates and incubated at room temperature to allow the growth of bacterial cells. Different colonies were removed and streaked on different plates after noting their appearance, relative abundance and color. Identification of fishes and shapes of bacterial colonies are summarized in Table 1 and Table 2.



Figure 1. 15 species of wild-caught fishes. A: Sargocentron rubrum, B: Pagellus acarne, C: Pomadasys incisus, D: Siganus rivulatus, E: Dentex macrophthalmus, F: Diplodus vulgaris, G: Dicologlossa cuneate, H: Oblada melanura, I: Pagellus erythrenus, J: Pomadasys stridens, K: Plotosus lineatus, L: Pempheris mangula, M: Sardinella maderensis, N: Lithognathus mormyrus, O: Liza aurata

Species	Common	Fish	Carnivore	Pelagic	Lessepsian	Description of	Number of different
	Name	ID	vs.	vs.	vs. Native	colonies on	colonies picked
			Herbivore	Benthic		petri dish	
Sargocentrom	soldier	1	C	Р	L	carpet +	grey carpet, white,
rubrum	fish					individual,	pink, black only 2
D 11		2	C	D	N	colonies on top	1 1 1 (
Pagellus acarne	sea	2	C	Р	IN	a lot but not a	bright orange, grey,
	bream					carpet, annost	white pink
Pomadasys	bastard	3	C	B	N	a lot but not a	grev carpet bright
incisus	grunt	5	C	D	1	carpet, almost	orange(a lot), black.
	8						white, yellow, pink,
							yeast like
							(white/cream-pink)
Siganus	rabbit	4	Н	Р	L	individual	very small yellow,
rivulatus	fish					colonies	big cream-pink,
							white, pearl white,
							yeast white,
Dontor	large eve	5	C	В	N	carpet grev⊥	white/grey_cream_
macrophthalmus	dentex	5	C	Б	1	colonies on ton	nink bright nink
maerophinaimus	uentex					colonies on top	pearl white, bright
							orange
Diplodus	two	6	С	В	N	carpet, grey +	white, carpet,
vulgaris	banded					colonies on top	white/cream-pink,
	sea						bright orange, 1-2
	bream						small yellow,
							black(center of the
Dicologlossa	sole	7	C	В	N	carpet grev⊥	grey carpet white
cuneate	3010	'	C	Б	1	colonies on ton	white/cream-pink
cuncure						colonies on top	bright and small
							orange
Oblada	saddled	8	С	Р	N	carpet, grey +	pink, white/pink,
melanura	sea					colonies on top	carpet, white
	bream						irregular shape
			~				(yeast like)
Pagellus	pandora	9	С	В	Ν	no carpet,	yellow, cream-
erythrinus						colonies	big white round
Pomadasys	striped	10	C	р	T	carpet	carpet_white/cream-
stridens	piggy	10	C	1	L	+individual	pink
Plotosus	cat fish	11	С	В	L	carpet	carpet, white/pink,
lineatus						+individual	1-2 bright orange
Pempheris	sweeper	12	C	Р	L	almost no	bright orange,
mangula						carpet,	yellow, white,
		10				individual	white/cream-pink
Sardinella	sardine	13	C	Р	N	carpet on big	pink, carpet, white
maderensis						plate,	
Lithognathus	sand	14	C	р	N	carpet on big	nink 2-3 vellow
mormvrus	steenbras	17		· ·	11	plate.	white, white/cream-
						individual	pink
Liza aurata	mullet	15	С	Р	N	no carpet,	small pink, yellow,
						individual	irregular(yeast like)
						colonies	cream-pink

Table 1. Fish species

B. Identification of cultivable bacteria:

In total, seventy different colonies were isolated on LB agar plates. Based on the colony morphology, we suspected two isolates to be yeast colonies and 16s ribosomal RNA gene amplification was not successful with their DNA. Seven bacterial colonies were not able to grow in liquid culture media and were subsequently discarded. The remaining sixty-one bacterial colonies were lysed to prepare genomic DNA from each. Then, 16s gene was amplified and sequenced for identification purposes. The results were as follows:

- Three bacterial species were cultured from *Sargocentrom rubrum* (*Staphylococcus hominus, Shewanella baltica* and *Psychrobacter faecalis*).
- Four bacterial species were cultured from *Pagellus acarne (Psychrobacter species, Psychrobacter species, Shewanella species* and *Aeromonas salmonicida*).
- Seven bacterial species were cultured from *Pomadasys incisus (Psychrobacter faecalis, Planococcus species, Psychrobacter faecalis, Shewanella putrefaciens, Planococcus species, Psychrobacter species* and *Arthrobacter species).*
- Three bacterial species were cultured from *Siganus rivulatus* (*Shewanella species, Kocuria rhizophila* and *Psychrobacter species*).
- Four bacterial species were cultured from *Dentex macrophthalmus* (*Psychrobacter maritimus, Planococcus species, Shewanella baltica* and *Psychrobacter species*).

- Five bacterial species were cultured from *Diplodus vulgaris* (*Psychrobacter cibarius, Psychrobacter faecalis, Psychrobacter species, Arthrobacter species* and *Psychrobacter species*).
- Three bacterial species were cultured from *Dicologlossa cuneate* (Shewanella species, Shewanella baltica and Psychrobacter species).
- Four bacterial species were cultured from *Oblada melanura (Psychrobacter maritimus, Shewanella baltica, Psychrobacter faecalis* and *Psychrobacter putrefaciens)*.
- Three bacterial species were cultured from *Pagellus erythrinus (Shewanella putrefacien, Vibrio metschnikovii* and *Arthrobacter species)*.
- Two bacterial species were cultured from *Pomadasys stridens (Psychrobacter psychrophilus* and *Aeromonas species)*.
- Two bacterial species were cultured from *Plotosus lineatus* (*Enterobacteriaceae* and *Aeromonas salmonicida*).
- Seven bacterial species were cultured from *Pempheris mangola* (Shewanella baltica, Psychrobacter species, Arthrobacter species, Planococcus species, Planococcus species).
- Three bacterial species were cultured from *Sardinella maderensis* (*Shewanella baltica*, *Shewanella baltica* and *Psychrobacter species*).
- Four bacterial species were cultured from *Lithognathus mormyrus* (Shewanella baltica, Psychrobacter species, Psychrobacter cryohalolentis and Arthrobacter arilaitensis).

• Seven bacterial species were cultured from *Liza aurata (Kocuria species,*

Kocuria palustris, Exiquobacterium species, Chryseobacterium, Psychrobacter faecalis, Psychrobacter species and Rothia species).

Bacterial	Bacterial Species	Blast-Match	Appearance	Relative
ID		percentage %		Abundance
1a	Staphylococcus hominus	99	White	+
1b	Shewanella baltica	98	cream-pink, jelly	++
1c	Psychrobacter faecalis	99	Cream	+++
2a	Psychrobacter sp.	94	Cream	++
2b	Psychrobacter sp.	99	Cream	++
2d	Shewanella sp.	99	cream-pink, jelly	++
2e	Aeromonas salmonicida	95	white beige	++
3a	Psychrobacter faecalis	99	Cream	+++
3b	Planococcus sp.	88	Orange	+
3c	Psychrobacter faecalis	99	Cream	+++
3d	Shewanella putrefaciens	96	cream-pink, jelly	++
3e	Planococcus sp.	99	Orange	+
3f	Psychrobacter sp.	97	Cream	+++
3g	Arthrobacter sp.	98	yellow, bright	+
4a	Shewanella sp./ baltica	97	cream-pink, jelly	++
4c	Kocuria rhizophila	88	yellow, bright	+
4e	Psychrobacter sp.	92	Cream	++
5a	Psychrobacter maritimus/ sp.	97	Cream	++
5b	Planococcus sp.	98	Orange	+
5c	Shewanella baltica	96	cream-pink, jelly	++
5e	Psychrobacter sp.	97	Cream	++
ба	Psychrobacter cibarius/ immobilis	90	Cream	++
6b	Psychrobacter faecalis/ pulmonis	97	Cream	++
6c	Psychrobacter sp.	79	Cream	++
6d	Arthrobacter sp.	95	Yellow	+
6e	Psychrobacter sp.	91	yellow, bright	+
7b	Shewanella sp.	97	cream-pink, jelly	++
7c	Shewanella sp./ baltica	98	cream-pink, jelly	++
7d	Psychrobacter sp.	96	Cream	++
8a	Psychrobacter maritimus	97	cream, jelly	++
8b	Shewanella baltica	99	cream-pink,	++

			jelly	
8c	Psychrobacter faecalis/ pulmonis	99	Cream	++
8d	Psychrobacter faecalis/ pulmonis	99	Cream	++
9a	Shewanella putrefacien	97	cream-pink, jelly	++
9b	Vibrio metschnikovii	91	cream, rough	++
9c	Arthrobacter sp.	96	Yellow	++
10a	Psychrobacter psychrophilus	97	Cream	+++
10b	Aeromonas sp.	98	cream, jelly	++
11a	Serratia/ Enterobacteriaceae	83	Cream	++
11b	Aeromonas salmonicida	99	Cream	++
12a	Shewanella baltica	95	cream, jelly	++
12c	Psychrobacter sp.	98	cream, jelly	++
12d	Arthrobacter sp.	97	yellow, bright	++
12e	Planococcus sp.	95	Orange	+
12f	Planococcus sp.	98	Orange	+
12g	Psychrobacter sp.	99	cream, jelly	++
12h	Bacillus sp.	99	Whitish	++
13a	Shewanella baltica	95	cream, jelly	++
13b	Shewanella baltica	97	cream, jelly	++
13c	Psychrobacter sp.	95	cream, jelly	++
14a	Shewanella baltica	87	cream, jelly	++
14b	Psychrobacter sp.	95	Cream	++
14c	Psychrobacter cryohalolentis	96	Cream	++
14d	Arthrobacter arilaitensis	97	yellow, bright	+
15a	Kocuria sp.	96	Yellow	+
15b	Kocuria palustris	95	Yellow	+
15c	Exiquobacterium sp.	96	Orange	+
15d	Chryseobacterium/ Flavobacteriaceae	97/94	mustard orange	+
15e	Psychrobacter faecalis	96	Cream	++
15g	Psychrobacter sp.	92	Cream	++
15h	Rothia sp.	99	mustard, light	+

Table 2. Bacterial species

C. Identification of non-cultivable bacteria:

After comparing both STE and Livak DNA extraction methods, we decided to use the latter one since it gave better results. After extraction, DNA was amplified then cloned using pGEM-T Easy vectors. Individual colonies that grew on plates were amplified again but unfortunately, this technique gave false positive results due to contamination of bacterial DNA from air.

D. Data Analysis:

R was used to show the frequency distribution of bacterial OTU's abundance and diversity among fish species. 38 different bacterial strains were unique (found in 1 fish only) while 1 bacterial strain was found in 3 and another in 7 different fish species respectively (Figure 2).



Figure 2. Frequency distribution of bacterial OTU abundance among wild caught fishes.

9 different fish specimens contained 2 bacterial strains, 2 fish specimens contained 3 bacterial strains, 2 fish specimen contained 5 bacterial strains, one fish specimen contained 4 bacterial strains and one fish specimen contained 6 bacterial strains (Figure 3).



Figure 3. Frequency distribution of bacterial OTU diversity within fish species.

R was also used to detect whether bacteria obtained have a nested distribution pattern. Figure 4 shows that bacterial distribution is not nested and most of bacteria obtained are considered rare. Principal component analysis shows no strict clusters that coincided with any of the three fish categories (diet, swimming mode and origin). PCA shows that *Plotosus lineatus* and *Pomadasys stridens* and *Pagellus acarne* and *Pagellus erythrinus* have the same clustering position respectively. Furthermore, PCA shows that the 4 native fish species *Pagellus acarne*, *Pagellus erythrinus*, *Oblada melanura* and *Dentex macrophthalmus* form a cluster (Figure 6). There is no pattern describing the relationship between fish and their swimming mode (Figure 7). For example, *Liza aurata* and *Sardinella maderensis* are far away from each other. One reason for this might be that they were caught from different places where they might be eating different food.



Figure 4. Nestedness analysis. Bacterial species are nestdess if the rarest strain is found in the most diverse fish. Bacterial strains are distributed from the most common to the rarest moving from the top of the figure to the bottom. Fish species however are distributed from the most diverse to the less diverse moving from the left to the right of the figure. The most diverse fish is *Liza aurata* containing 7 rare species while the most common bacteria is *Shewanella baltica* which is found in 8 fish species. Also, common bacterial species should be located on the left of the isocline. This analysis shows that most of the bacteria are rare. Thus, bacterial strains are not nested.



Figure 5. Multidimensional scaling of fish species. Species are colored according to their diet. Blue dots represent carnivorous species while the green dot represents herbivorous species. The green dot here representing *Siganus rivulatus* is masked with the blue dot of *Dicologlossa cuneate*.



Figure 6. Multidimensional scaling of fish species. Species are colored according to their origin. The red dots represent native species while the yellow dots represent Lessepsian species. There is a relationship between 4 native fish species including *Pagellus acarne*, *Pagellus erythrinus*, *Oblada melanura* and *Dentex* macrophthalmus where they form a cluster.



Figure 7. Multidimensional scaling of fish species. Species are colored according to their swimming mode. The yellow dots represent species that swim on the surface. The blue dots represent species that swim near the bottom.

E. Bacterial exposure:

To test the capacity of bacteria to colonize the gut of zebra fish (*Danio rerio*), we used a selection of the isolated bacteria that includes the following bacterial strains: *Aeromonas salmonicida, Planococcus species, Vibrio metschnikovii, Enterobacteriaceae, Kocuria palustris, Psychrobacter faecalis, Shewanella baltica, Arthrobacter species, Aeromonas salmonicida, Bacillus species, Exiquobacterium, Rothia species, Shewanella putrefacien, Planococcus species, Shewanella baltica, Psychrobacter cryohalolentis, Arthrobacter ariliatensis, Chryseobacterium, Psychrobacter faecalis, Shewanella putrefacien, Arthrobacter species, Psychrobacter species, Psychrobacter maritimus, Aeromonas species, Kocuria species, Planococcus species, Kocuria* *rhizophila, Psychrobacter cibarius, Shewanella baltica, Psychrobacter psychrophilus* and *Aeromonas salmonicida*. After exposure to bacteria (see methods), we monitored the behavior of the fish. No lethality was observed. However, fish treated with *Kocuria palustris, Psychrobacter faecalis* and *Kocuria species* showed distress symptoms (abnormal swimming, rapid respiration) for 9 hours but they recovered afterwards.

Out of the 29 exposure experiments, we selected 10 bacterial strains to check whether they were able to resist host immunity and attach to the intestinal lining of zebra fish. Bacterial strains selected were: *Psychrobacter species, Shewanella species, Planococcus species, Psychrobacter species, Shewanella species, Psychrobacter species, Aeromonas species, Shewanella species, Psychrobacter species* and *Arthrobacter species.* We dissected one fish exposed to each bacterial species to isolate bacteria from its gut, sequence the obtained bacterial DNA and identify it using Blast technique. Four out of ten bacterial strains were able to colonize the intestinal lining of *Danio rerio* (Table 3).

Bacterial strain	Ability of bacterial strain to persist
Psychrobacter species	-
Shewanella species	+
Planococcus species	-
Psychrobacter species	-
Shewanella species	+
Psychrobacter species	-
Aeromonas species	-
Shewanella species	+
Psychrobacter species	-
Arthrobacter species	+

Table 3. Bacterial strains that resisted host's immunity. (+: resisted, -: didn't resist)

F. Drosophila Infection:

We wanted to assess the pathogenicity of these bacteria on the well-established *Drosophila* immunity model. For this, we injected a bacterial suspension of a known OD into the thorax of wild-type flies. Ability to cause lethality to the flies is summarized in Table 4.

Bacterial Species	Ability to kill injected flies
Staphylococcus hominus	-
Shewanella baltica	-
Psychrobacter faecalis	-
Psychrobacter sp.	-
Psychrobacter sp.	-
Psychrobacter faecalis	-
Planococcus sp.	-
Psychrobacter faecalis	-
Shewanella putrefaciens	-
Planococcus sp.	-
Psychrobacter sp.	-
Arthrobacter sp.	-
Kocuria rhizophila	-
Psychrobacter sp.	-
Psychrobacter maritimus/ sp.	-
Planococcus sp.	-
Psychrobacter sp.	-
Psychrobacter cibarius/ immobilis	-
Psychrobacter faecalis/ pulmonis	-
Psychrobacter sp.	-
Arthrobacter sp.	-
Psychrobacter sp.	-
Shewanella sp./ baltica	-
Psychrobacter sp.	-
Psychrobacter maritimus	-
Psychrobacter faecalis/ pulmonis	-
Psychrobacter faecalis/ pulmonis	-
Shewanella putrefacien	-
Vibrio metschnikovii	-
Arthrobacter sp.	-
Psychrobacter psychrophilus	-
Serratia/ Enterobacteriaceae	+
Aeromonas salmonicida	+/-
Psychrobacter sp.	-
Arthrobacter sp.	-
Planococcus sp.	-
Planococcus sp.	-
Psychrobacter sp.	-
Bacillus sp.	-
Psychrobacter sp.	-
Psychrobacter sp.	-
Psychrobacter cryohalolentis	-
Arthrobacter arilaitensis	-
Kocuria sp.	-
Kocuria palustris	-
Exiquobacterium sp.	-
Chryseobacterium/ Flavobacteriaceae	-
Psychrobacter faecalis	-
Psychrobacter sp.	-
Rothia sp.	-

 Table 4. Ability to cause lethality to the injected flies (-: less than 30%, +/-: 50-90%, +: 100% death)

CHAPTER IV Discussion

We have collected bacteria from the guts of 15 different fish species. After isolation of cultivable bacteria, we identified 61 bacterial species by sequencing their 16s ribosomal DNA and comparing the obtained sequences to bacterial genomic databases (NCBI nucleotide BLAST). We used statistical analysis in an attempt to determine whether the association of certain bacterial strains with their host-fish species were correlated to the habitat (pelagic versus benthic), diet (carnivorous, herbivorous or omnivorous) or origin (indigenous versus introduced) of the fish. A selection of the identified bacteria was used to assess the effects of these strains on two laboratory model organisms, *Danio rerio* (zebrafish) and *Drosophila melanogaster*. In agreement with previous reports (Nehme *et al.*, 2007), *Serratia*, was highly pathogenic when injected into *Drosophila melanogaster*. We were also able to show that in 4 out of 10 cases the isolated bacteria was capable of colonizing the guts of zebrafish in laboratory conditions. We finally tried to identify the uncultivable bacterial species that are found in the fish guts by direct amplification of 16s DNA from guts content but this approach wasn't pursued due to technical difficulties and lack of time.

Sample size and limitations:

In this pilot study, the number of fish species analysed was small (n=15) and only one specimen for each fish species was collected. In addition, although all the specimens were collected from the same fishing port, we can't be certain that they were caught from the same location. This is a limitation that hinders us from linking with confidence a given bacterial species to the fish life style and its environment.

Bacterial isolation:

Another important limitation was that this study focused only on the cultivable bacteria present in the fish guts. In addition, it is very likely that relying on visual differences in the colours and shapes for the isolation of bacterial colonies resulted in the non-selection of several bacterial species that appeared similar to the naked eye. This is due to our sampling/isolating technique since we took only one representative colony from each phenotype per plate to avoid picking several isolate of the same bacterial species from each fish specimen.

Possible improvements and perspectives:

Several facets of this work can be improved: one evident thing to do is to increase the sample size of wild-caught fish and to study more than one specimen for each fish species. Another added value would be to obtain several fish samples per species from different locations and at different seasons to determine whether these two parameters will be reflected on variations in the microflora. Finally, the results obtained could be compared to samples of the same fish species caught from different areas such as the Red sea.

Most of the bacteria that thrive in the digestive system of fish don't grow on solid or liquid (artificial) media. Therefore, to have a better and more representative picture of gut flora, bacteria should be identified by the direct extraction of bacterial DNA from guts contents followed by 16s amplification and sequencing.

The exposure experiment showed that the isolated bacteria weren't accidentally present in the wild-caught fishes' guts since four of these isolates successfully colonized the gut of aquarium kept zebrafish. This is also an indication that these bacteria are adapted to live in the gut independently of whether the host is a freshwater or a seawater fish species.

Altogether, these preliminary results give a small overview of the bacterial species found in the guts of wild type fish living in Beirut seashore and can be considered a pilot or a feasibility study for a large scale project. With the above-mentioned improvements and additions, this analysis could consist a starting point to some experiments aimed at testing a potential utilisation of certain isolated bacteria in fish farming, since some may have a good impact on fish health by boosting growth and preventing harmful or pathogenic bacteria from colonizing the guts of farm raised fish species and ultimately increasing productivity. Another possible application would be the identification of certain bacterial species that can be used as indicator of polluted or poor water quality (residential sewage, garbage, oil spills etc...).

APPENDIX

I. Supplementary Tables:

Species	Common Name	Fish ID	Bacterial ID	Bacterial Species	Weight (g)	Length (cm)	Weight of gut for plating (g)	Description of colonies on petri dish	Relative Abundance	Number of different colonies picked
Sargocentrom rubrum	soldier fish	1	1a	Staphylococcus hominus	67.05	15.5	0.86	carpet + individual, colonies on top	+	grey carpet, white, pink, black only 2
			1b	Shewanella baltica					++	
			1c	Psychrobacter faecalis					+++	
Pagellus acarne	sea bream	2	2a	Psychrobacter sp.	47.64	14.9	1.56	a lot but not a carpet, almost	++	bright orange, grey, yellow, white, black white, pink
			2b	Psychrobacter sp.					++	
			2d	Shewanella sp.					++	
			2e	Aeromonas salmonicida					++	
Pomadasys incises	bastard grunt	3	3a	Psychrobacter faecalis	30.96	12.5	0.6	a lot but not a carpet, almost	+++	grey carpet, bright orange(a lot), black, white, yellow, pink, yeast like (white/cream- pink)
			3b	Planococcus sp.					+	
			3с	Psychrobacter faecalis					+++	
			3d	Shewanella putrefaciens					++	
			3e	Planococcus sp.					+	
			3f	Psychrobacter sp.					+++	
			3g	Arthrobacter sp.					+	
Siganus rivulatus	rabbit fish	4	4a	Shewanella sp./ baltica	65.47	17.1	3.5	individual colonies	++	very small yellow, big cream-pink, white, pearl white, yeast white, transparent
			4c	Kocuria rhizophila					+	
			4e	Psychrobacter sp.					++	
Dentex macrophthalmus	large eye dentex	5	5a	Psychrobacter maritimus/ sp.	30.61	13	0.74	carpet, grey + colonies on top	++	white/grey, cream-pink, bright pink, pearl white, bright orange
			5b	Planococcus sp.					+	
			5c	Shewanella baltica					++	
			5e	Psychrobacter sp.					++	

Diplodus vulgaris	two banded sea bream	6	6a	Psychrobacter cibarius/ immobilis	37.97	13.1	1.02	carpet, grey + colonies on top	++	white, carpet, white/cream- pink, bright orange, 1-2 small yellow, black(center of the colony)
			6b	Psychrobacter faecalis/ pulmonis					++	
			бс	Psychrobacter sp.					++	
			6d	Arthrobacter sp.					+	
			6e	Psychrobacter sp.					+	
Dicologlossa cuneate	sole	7	7ь	Shewanella sp.	49.06	16.3	1.99	carpet, grey + colonies on top	++	grey carpet, white, white/cream- pink, bright and small orange
			7c	Shewanella sp./ baltica					++	
			7d	Psychrobacter sp.					++	
Oblada melanura	saddled sea bream	8	8a	Psychrobacter maritimus	91.71	18.9	0.92	carpet, grey + colonies on top	++	pink, white/pink, carpet, white irregular shape (yeast like)
			8b	Shewanella baltica					++	
			8c	Psychrobacter faecalis/ pulmonis					++	
			8d	Psychrobacter faecalis/ pulmonis					++	
Pagellus erythrinus	Pandora	9	9a	Shewanella putrefacien	19.71	10.1	0.5	no carpet, individual colonies	++	yellow, cream- pink/white irregular big, white round
			9Ь	Vibrio metschnikovii					++	
-			9c	Arthrobacter sp.					++	
Pomadasys stridens	striped piggy	10	10a	Psychrobacter psychrophilus	71.71	16.4	1.12	carpet +individual	+++	carpet, white/cream- pink
			10b	Aeromonas sp.					++	
Plotosus lineatus	cat fish	11	11a	Serratia/ Enterobacteriaceae	21.43	14.8	1.34	carpet +individual	++	carpet, white/pink, 1- 2 bright orange
			11b	Aeromonas salmonicida					++	
Pempheris mangola	sweeper	12	12a	Shewanella baltica	49.93	15.5	0.8	almost no carpet, individual	++	bright orange, yellow, white, white/cream- pink
			12c	Psychrobacter sp.					++	
			12d	Arthrobacter sp.					++	
			12e	Planococcus sp.					+	
			12f	Planococcus sp.					+	
			12g	Psychrobacter sp.					++	

			12h	Bacillus sp.					++	
Sardinella maderensis	sardine	13	13a	Shewanella baltica	12.57	11.7	0.34	carpet on big plate, individual	++	pink, carpet, white
			13b	Shewanella baltica					++	
			13c	Psychrobacter sp.					++	
Lithognathus mormyrus	sand Steenbras	14	14a	Shewanella baltica	25.5	11.9	0.45	carpet on big plate, individual	++	pink, 2-3 yellow, white, white/cream- pink
			14b	Psychrobcter sp.					++	
			14c	Psychrobacter cryohalolentis					++	
			14d	Arthrobacter arilaitensis					+	
Liza aurata	mullet	15	15a	Kocuria sp.	171.51	26.8	6.87	no carpet, individual colonies	+	small pink, yellow, irregular(yeast like) cream- pink
			15b	Kocuria palustris					+	
			15c	Exiquobacterium sp.					+	
			15d	Chryseobacterium/ Flavobacteriaceae					+	
			15e	Psychrobacter faecalis					++	
			15g	Psychrobacter sp.					++	
			15h	Rothia sp.					+	

ST 1. First batch of wild-caught fish.

Species	Common Name	Fish ID	Bacterial ID	Weight (g)	Length (cm)	Weight of gut for plating (g)	Description of colonies on petri dish	Relative Abundance	Number of different colonies picked
Pagellus erythrinus	pandora	16	16a	47.03	14.9	1.09	a lot but not a carpet, almost		mixed, white, pinkish
			16b					+	
			16c					+++	
Diplodus vulgaris	two banded sea bream	17	17a	42.64	13.9	0.83	a lot but not a carpet, almost	+	white, pinkish
			17b					+++	
Siganus revulatus	rabbit fish	18	18a	102.58	23.6	5.02	carpet + individual, colonies on top	+++	yellow
Oedalechilus labeo	mullet	19	19a	288.45	31.2	7.9	a lot but not a carpet, almost	+	white, pinkish, orange, whitish, yellow
			19b					+++	
			19c					+	

			104						
			190					++	
			19e					+	
Pagellus erythrinus	pandora	20	20a	52.79	15.3	1.2	carpet + individual, colonies on top	+++	pinkish, pearl white, yellow/orange
			20b					+	
			20c					+	
Diplodus vulgaris	two banded sea bream	21	21a	39.97	13.6	0.35	a lot but not a carpet, almost	+++	pinkish, yellow, white
			21b					+	
			21c					++	
Siganus revulatus	rabbit fish	22	22a	105.64	19.8	7.23	a lot but not a carpet, almost	+++	grey/pink, white
			22b					+++	
Oedalechilus labeo	mullet	23	23a	219.2	28.8	8.83	a lot but not a carpet, almost	+++	pinkish, white, yellow/orange
			23b					+++	
			23c					+	

ST 2. Second batch of wild-caught fish.

Species	Common name	Weight (g)	Intestinal weight (g)
Siganus revulatus	rabbit fish	66.98	12.7
Dentex macrophthalmus	large eye dentex	15.81	0.19
Diplodus vulgaris	two banded sea bream	39.20	0.82
Sparidae	sparid	22.10	0.93
Sparidae	sparid	14.70	0.89
Sparidae	sparid	10.20	0.44
Siganus revulatus	rabbit fish	71.25	5.17

ST 3. Third batch of wild-caught fish.

II. 16s sequences

1a, Staphylococcus hominus:

1b, Shewanella baltica:

1c, Psychrobacter faecalis:

AGAACGCTGASMGGCAGGCTTAACACMTGGRAKYCSAGSGGWAACRRGRGAAGCTTGCTTCYCGSTGACGAGCGGC GGACGGGTGAGTAATACTTAGGAATCTACCTAGTAGTGGGGGGATAGCTCGGGGAAACTCGAATTAATACCGCATACG ACCTACGGGAGAAAGGGGGCARCTTGYTGCTCTCGCTATTAGATGAGCCTAAGTCGGGATTAGCTAGWTGGTGGGGGTA AAGGCCTACCAWGGCGACGATCTGTAGCTGGTCTGAGAGGGGGACGACCCGGGACTGAGACACGGCCCGGA CTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGGGGCAACCCTGATCCAGCCATGCCGCGTGTGTGAAGA AGGCCTTTTGGTTGTAAAGCACTTTAAGCAGTGAAGAAGACTCCATGGTTAATACCCATGGACGATGACATTAGCTG CAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCGGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGG CGTAAAGCGAGCGTAGGTGGCTTRATAAGTCAGATGTGAAAGCCCCGGGCTTAACCTGGGAACGGCATCTGATACTG TTAGGCTAGAGTAGGTGAGAGGAAGGTAGAATTCCAGGTGAAGCGGTGAAATGCGTAGAGATCTGGAGG

2a, Psychrobacter sp.:

2b, Psychrobacter sp.:

2d, Shewanella sp.:

GTGTGARCGMCCCCCCGAAGGTTAAGCTACCCACTTCTTTTGCAGCCCMYTYCCATGGTGTGACGGGCGGTGTGTAC AAGGCCCGGGAACGTATTCACCGTGGCATTCTGATCCACGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAG ACTCCAATCCGGACTACGACGAGGCTTTGTGAGATTAGCTCCACCTCGCGGGCTTTGCAACCCTCGTACTCGCCATTGT AGCACGTGTGTAGCCCTACTCGTAAGGGCCATGATGACTTGACGTCGTCCCCACCTTCCTCCGGGTTTATCACCGGCAG TCTCCCTAGAGTTCCCACCATTACGTGCTGGCAAATAAGGATAGGGGTTGCGCTCGTTGCGGGGACTTAACCCAACATT TCACAACACGAGCTGACGACAGCCATGCAGCACCTGTCTCASAGTTCCCGAAGGCACTAAGCTATCTCTAGCGAATTC TCTGGATGTCAAGAGTAGGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCCCACCGCTTGTGCGGGGCCCCC GTCAATTCATTTGAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTCTACTTAATGCGTTAGCTGAGAGCCCAGTG TTCAAGACACCAAACTCCGAGTAGACATCGTTTACGGCTGGACTACCAGGGTATC

2e, Aeromonas sp.:

3a, *Psychrobacter faecalis*:

3b, Planococcus sp.:

GGGGGGGACGGGSGGGGGGWACAAGGCCCGGRAACGTATTCMCCGKGSMATGCTGATCCACAATTACAASCGATTC CAGCTTCATGCAKGCAAGTTGCAACCTACAATCCGAACTGAAAAGGGTTTTCTGGRATTGSYTCCCCCTCSSGSKTTGS MRACCCTTTGTACCGYCCATTGWASCASGTGTGTASCCCAGGTCATAAGGSGCATGATGATTTGACGTCATCCCCACC TTCCTCCGGTTTGTCACCGGCRGTCACCTTARAGTGCCCAAMTGAATGCTGGSAACTAAAATCAAGGGTTGCGCTCGT TGCGGGACTTAACCCAACATCTCACGACMCSAGCTGACSACCACCATGCACCACCTGTCACCACTGTCCCCGAAGGG AAAAGTGTATCTCTACRCCGGKCASWGSRATGTCAAGACCTGKWAAGGTTCTTCSSGTTGYTYCRAATTAAACCACAT GCTCCACCGCTGGTGCGGSCCCCCGTCAATTCCTTTGAGTTTMASCCTTGCGGCCGTACTCCCCAGGCGGAGWGCTTA ATGCGTTAGCTGCAKCACTAAGTGCGCRGAGACCCCMTMACACTTAKSACTCAKCGTTTAGGYGTGCACTACCAGGA TATCTAATCCTGTTTGCTCCCCACGYTTCRCGCCTCAGCGTCAGTTACAKMMCCAGTARAGT

3c, Psychrobacter faecalis:

3d, Shewanella putrefaciens:

CCGCACCTTACTTTTGAGGGGAAAGGGGGTAARSGYGACGGGGGGGAAAGGCCGGAAGAAAASGRRAGGGCATTCT GATCCACGATWACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGKACTACGACGAGGTTTGTGA GATTAGCTCCACCTCGCGGCTTTGCAACCCTCTGTACTCGCCATTGTAGCACGTGTGTAGCCCTACTCGTAAGGGCCA TGATGACTTGACGTCGTCCCCACCTTCCTCCGGTTTATCACCGGMAGTCTCTCTAGAGTTTCCACCATTACGTGCTGGC AAATAAGGATAGGGGTTGCGCCCGTTGCGGGGACTTAACCCAACATTTCACAACACGAGCTGACGACAGCCAGGCAGC ACCTGTCTCACGGTTCCCGAAGGYACTAAGTTTTCTCTAGCGAATTCCGYGGATGYCAAGAGTAGGTAAGGTTCTTCK CGTTGCATCGAATTAAACCACATGCTCCACCGCTTGTGCGGGGCCCCCGTCAATTCATTTSAGTTCTAACCTTGCGGSCG TACTCCCAGGCGGYCTACTTAATGYTTAGYTTGAA

3e, Planococcus sp.:

GGGTTACCTCACCGACTTCGGGTGTTACWWAYTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATT CACCGTGGCATGCTGATCCACGATTACTAGCGATTCCGGCTTCATGCAGGCGAGTTGCAGCCTACAATCCGAACTGA GAACGGTTTTCTGGGATTGGCTCCCCCTCGCGGGGTTGGCAACCCTTTGTACCGTCCATTGTAGCACGTGTGTAGCCCA GGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCA ACTGAATGCTGGCAACTAARATCAAGGGTTGCGCTCGTTGCGGGGACTTAACCCAACATCTCACGACACGAGCTGACG ACAACCATGCACCACCTGTCACCACTGTCCCCGAAGGGAAAAGTGTATCTCTACACCGGGCAGTGGGATGTCAAGAC CTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGGCCCCCGTCAATTCCTTTGAGT TTCAGCCTTGCGGCCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTGCAGCACTAAGGGGCGGAAACCCCCTAA CACTTAGCACTCATCGTTTACGGCGTGACTACCAGGTATCTAATCCTGTTTGCTCCCCACGCTTCGCGCCCCCACGCTCAGCGTCA GTTACAGACCAG

3f, *Psychrobacter* sp.:

3g, Arthrobacter sp.:

4a, Shewanella sp.:

CMGCTCATGAACCACAAAGTGGTGAGCGCCCCCCGAAGGTTAAGCTACCCACTTCTTTTGCAGCCCACTCCCATGG TGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTGGCATTCTGATCCACGATTACTAGCGATTCCGACT TCATGGAGTCGAGTTGCAGACTCCAATCCGGACTACGACGAGGCTTTGTGAGATTAGCTCCACCTCGCGGCTTTGCAAC CCTCTGTACTCGCCATTGTAGCACGTGTGTAGCCCTACTCGTAAGGGCCATGATGACGTCGACGTCGCCCACCTTCCT CCGGTTTATCACCGGCAGTCTCCCTAGAGTTCCCACCATTACGTGCTGGCAAATAAGGATAGGGGTTGCGCTCGTTGC GGGACTTAACCCAACATTTCACAACACGAGCTGACGACAGCCATGCAGCACCTGTCTCACRGTTCCCGAAGGCACW MMKSTATCTCTASYGRMTTCYGTGGATGTCAAGRGKWRGGKWAAGGTTCTTCGCGTTGCATCGAAATTAAACCACAT GCTCCACCGCTTGTGCGGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTCTACTTAA TGCGTTAGCTTGAGAGCCCAGTGTTCAAGACACCAAACTCCGAGTAGACATCGTTTACGGCGGGGCGCACAGCAGCACC AATCCTGTTTGCTCCCACGCTTCGTGCCTGAGCGTCAGTCTTTGTCCAGGGGGCCGCC

4c, Kocuria rhizophila:

AGGGTTAGGCCACCGGCTTCGGGKGTTAAAACASKSGKGRCTTGACGGGSGGKGKGTACAAGGCCCGGGAACGTATT CACCGCRGCGTKGYTGATCTGCRATWACTAGCRACTCCGAYTTCWCGTGGYCRAKTTGCARACCACRATCCRAAYTG ARACSAGTTTTTTGGRATTAGYTCCMCCTCSCGGYWTCSCAACCCATTGTMCTGGCCWTTGTAGCATGSGKGAAGCC CARGACATARGGGGMATGAKGATTTGACGTCWTCCCCMCCTTCCTCCGAGTTGACCCSGGCRGTCTCCTATGAGTCC CCACCATCASGTGCTGGCWACATAKAACGAGGGTTGCGCTCGTTGCGGKACTTAACCCAWCATCTCACGACACGAGC TGACRACAACCATGCACCACCTGTACACCAGGCCCACAAGGGGGAAAGACCATCTCTGGCCCGGTCCGGTGATGTC AAGCCTTGGTAAGGTTCTTCGCGTTGCATCKAATTAATCCGCATGCTCCGYCGCTTGTGCGSSCCCCCGTCAATTCCTT TGAGTTTTAGSCTTGCGGMCGYACTCCSCAGGCGGGKYACTKAATGCGTTAGCTACGGCCCGGCAAGAMRTGGTMAT GWTCCCCACACCTAGTGCCYAWCGTGTACGGCATGCACTRCTATGATATCTARTCCTGYTCGCTCCYYATGCTTTCGC TCCTCAGCGTTCAGTAACCAGCCCAG

4e, *Psychrobacter* sp.:

CACATGCGTTCCGGGGTAACCTTAACATGGGATCCCGGGGGCTTGGTTCCCGGTTGAMCTCAGGAGTCMARCGCCGS KCGGGSGAGTAATACTTAKGAATCTACCTAGYARTGGGGGATAGCTCGGGRAAACTCGAATTAATACCGCATACRAC CTACGGKAGAAAGGGGGCAACTTGTTGCTCTCGCTATTAGATGAGCCTAAGYCGGATTAGCTAGAYGGTGGGGTAAA GGCCTACCATGGCGACGATCTGTAGCTGGTCTGAGAGAGGATGATCAGCCACMCCGGGACTGAGACACRGCCCGGACTC CTACGGGAGGCAGSAGYGGGGAATATTGGACAATGGGGGCAACCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGG CCTTTTGSTTGTAAAGCACTTTAAGCASCGAAKAAKACTCCATGGTTAATACCCATGGACGATGACATTAGCTGCMGA ATAAGCACCGGCTAACTTGGYGCCAGCRGCCGCGGGTAATACASAGGGTGCAAGCGTTAATCGGAATTACTGGGGSGY AAAGCGAGCGTASGTGGCTTGATAAGWCAGATGWGAAAACCCCGGGCTTWAACCTGGGAACTGCATCTGATACTGT TAGGYTAGAATAGKYGAGAGGGAAGGTAGAAATCCCACGGCGTAG

5a, Psychrobacter maritimus:

ACMACCATGCAAGTCGAGCGGAAACGATGATAGCTTGCTAYCAGGCSYCGAGCGGCGGACGGGTGAGTAATACTTA GGAATCTACCTAGTAGKGGGGGATAGCTCGGGGAAACTCGAATTAATACCGCATACGACCTACGGGGGAAAAGGGGG CAACTTGTTGCTCTCGCTATTARATGAGCCTAAGTCGGGATTAGCTAGATGGTGGGGTAAAGGCCTACCATGGCGACG ATCTGTAGCTGGTCTGAGAGGGATGATCAGCCACACCGGGACTGAGACACGGCCCGGACTCCTACGGGAGGCAGCAGT GGGGAATATTGGACAATGGGGGCAACCCTGATCCAGCCATGCGCGCGKGKGTGAAGAAGGCCTTTTGGTTGTAAAGCA CTTTAAGCAGTGAAGAAGACTCCRTGGTTAATACCCAYGGACGATGACATTAGCTGCAGAAATAAGCACCGGCTAACT CTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGSGAGCGTAGGTGGC TTGATAAGTCAGATGTGAAATCCCCGGGCTTAACCTGGGAACTGCATCTGATACTGTTAGGCCAGAATAAGGTGAGAG GAAGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGATGGCGAAAAGCAGCCTTCTGG YATCATATTGACACTGAGGTTCGAAAGCGTGGTAGCARACAGGATTAGATACCCGGGGAACCCCGCGAAAGCAGCCTTCTGG TCTACTAGTCGTTGGGTCCTTGAGACTAATGACGCMGCTAMMSMATAAGTAGACCSCCTGGGRTACGCGCAGAGC TCAATGATGWCGGGGCCGCCAGCGTGRCATKGTTAATTCATGCACSSGAGAAACATAAYG

5b, Planococcus sp.:

5c, Shewanella baltica:

CAAAGTGGTGAGCGCCCCCCGAAGGTTAAGCTACCCACTTCTTTTGCAGGAAACCCGTGGTGTGACGGGCGGTGTG TACAAGGCCCGGGAAGAATTCACCGKGGCATTCTGATCCACGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTG CAGACTCCAATCCGGACTACGACGAGCTTTGTGAGATTAGCTCCACCTCGCGGCTTTGCAACCCTCTGTACTCGCCAT TGTAGCACGTGTGTAGCCCTACTCGTAAGGGCCATGATGACTTGACGTCGTCCCCACCTTCCTCCGGGTTTATCACCGG CAGTCTCCCTAGAGTTCCCACCATTACGTGCTGGCAAATAAGGATAGGGGTTGCGCTCGTTGCGGGACTTAACCCAAC ATTTCACAACACGAGCTGACGACAGCCATGCAGCACCTGTCTCACRGTTCCCGAAGGCACTAAGYTATCTCTAGCGA ATTCYSTGGATGTCAAGAGTAGGTAAGGTTCTTCGCGTTGCACTGGAATTAAACCACATGCTCCACCGCTTGTGCGGGC CCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTCTACTTAATGCGTTAGCTTGAGAGCCC AGTGTTCAAGACACCAAACTCCGAGTAGACATCGTTTACGGCGTGGACTACCAGGGTATTCTAATCCTGTTTGCTCCCC ACGCTTTCGTGCCTGAGCGTCAGTCTTTGTCCAGGGGGGGCGCCTTCGCCACCGGGTATTCCTCCAGATCTCTACGCAT TTCACCGCTACACCTGGAATTCTACCCYCCCTCTACAAGACTCTAGTTCGCCMRGTTCGAAATGGMATTYCCCAGGTT GAAGCCGGGGGGATTTTCMCATCTTCGCTTAAACCAAAACCGGCCTKGCGCACCRGCTTTTTAMRCSCCCACRCAWA AATTYTC

5e, Psychrobacter sp.:

6a, Psychrobacter cibarius:

GGYTTTTCCTTCCGGCTCGCCCGAKGCGGCGGGACGGGKTGGAGTAAWACTTTAGGAAATCCTACCCTAGKAAGTGG GGGGATAGCACGGGAAACTCGTATTTAATACCGCATACGACTACGGGAGAAAGGGGGGCAGTTACTGCTCTCGCTAT TTAATGAGCCTAAKCGGATTASTAGATGGKGGGGGAAAGGGCCTACCTGGGSAACAATCTGWASTGGGYCTGAAAG GATGATCASCCCCCGGGACTGAAACCCGGCCCGGACTCTACGGGGAGGMRGMRKGGGGGAAATATTGGAACAAT GGGGGGAAAACCCTGGATCCRSCCATGGCCGCGCGGGGKGKGKGAAGAAAGGCCTTTTTGGGTTGTAAAAGCACTTTTAAG CAGTGAAAGAAAGACTCCCRTGGGTTAATACCCCATGGGACGATGACTTTAGCTGCAGAATAAGCACCGGCTAACTC TGTGCCAGCAGCCGCGGGGTAATACAGAGGGTGCAAAGCGTTAATCGGAATTACTGGGCGTAAAAGGAGCGTAGGTG GCTCTATAAGTCAGATGTGAAATCCCCGGKCTTAAYCTGGGAACTGCATCTGAAACTGTAGAGCTAGAGAAGGCAGCGTAGG AGGAAGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGACTGGAGGAATACCGATGGCGAAGGCAGCCTTCT GGCATAATACTGACACTGAGGCTCGAAAGCGTGGGTAGCAACAGGATTAGATACCCTGGTAGTCCACGCCGTAACGA TGTCTACTAGTCGTTGGGTCCCTTGAGGACTTAGTGACGCAGCAATAAGTAGACCGCCTGGGGGAGTACGG GCCGCAAGGTTAAACTCAAATGAATTKGACGGGGGGCC

6b, *Psychrobacter faecalis*:

6c, Psychrobacter sp.:

6d, Arthrobacter sp.:

GGGCGGCGTGCTTACACATGCAAGTCGAACGATGACCCCGTGCTTGCACGGGTGATTAGTGGCGAACGGGTGAGTAA CACGTGAGTAACCTGCCCCTGACTCTGGGATAAGCCCGGGAAACTGGGTCTAATACTGGATATGACCTTTAACCGCA TGGTTTTTGGTGGAAAGATTTATCGGTTGGGGATGGACTGGCGCCAATCAGCTTGTTGGTGAGGTAATGGCTCACCA AGGCGACGACGGGTAGCCGGCCTGAGAGGGTGACCGGCCACACTGGGACTGAGACACGGCCCARACTCCTACGGGA GGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACSCCGCGTGRGGGATGACGGYCYTCCGG GTTKTAAACCTYTTTCAGTAGGGAAGAAGCGAAAGCGAAAGTGRCGGGTACCCTGCAGAARAAAGCGCCGGSCTAACTACKTG CCAGSMRGCCGSSGGTAAATACGTAAGGSGRARSGYTTTYYCCGKATTTTATTGGGSMRAAAARASCTCMWARGSSK TTTTSCCSSYCKYGCCRWAAAWYCMAGRGYMCCMCCCCCMRAYTYGGGGGGGGSMSGGSAAAMWWRGWGGWTGGG GGGAAWGTGATTTYYSKGKGTRASSGRAAWGCCMAAWTWYMGGGGRAMMCCCTGTGMARGSGGSTCTKGGGCYY TYWYGASCKAGAGAGAGAGAGAATKGGAAAAAAAAAAAAAAAACACCYGYGAGACCCKSGSCAAACGTTGGMCTMTGT GGGGAAATMYCRTGTTCGCGCGTCTACTATMGTATTACGCCCCCTGTGAT

6e, Psychrobacter sp.:

TGGGCGGGSAGGCTTAACACATGCAAGTCGAGCGRAAACSMYGAKMKCKTGCTATCAGGATYRKCGAGCGGCGGAC GGGTGAGTAATACTTARGAATCTACCTAGTAKGGGGGGGATAGCTCGGGRAAACTCSWATTAATACCGCAKACSACCT ACSGGAGAAAGGGGGCWACTTGKTGCTCTCGCTWTTAGATGAGCCTAAGTCGGATTAGCTAGATGGKGGGGTAAAG GCCTACCATGGCGACGATCTGTAGCTGGTCTGARAGGATGATCAGCCMCMCCGGGACTGARACMCGGCCCGGACTC CTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGGGCAACCCTGATCCAGCCATGCCGCGTGTGTGAAAGAAGG CCTTTTGGTTGTAAAGCACTTTAMGCAGTGAARAAGACTCCATGGTTAATACCCRTGGACGATGACATTAGCTGCASA ATAAGCACCGGCTAACTCTGTGCCAGCAGCGCGGGGAAAACGGGGTGCRAGCGTTAATCGGAATTACTGGGCGTA AAGCGAGCGYAGGKGGMTTGATAAGTCARATGTGAAATCCCCGGKSTTAACCTGGRAACTGCATCTGATWCTGTTAG GCTRGAATAGGTGAGAGRAAGGTAGAATTKCCAGGTGTAGCGGWGAAATGCSTAKAGATCTGGAGGAATACCGATG GYSAASGSAGCCTTCTGGCATCATATTGACACTGAAGGWTCGAAAGCGTGASTMKGRACAGGATTASATACCCTGGTA GTCCACGSCGTRACGATGTCTACTAGTYTYGSGTCCCTTGWRGASYTATTGACGCMCCYYACYSGATAAGWMGACCS CTGGGKARTACYGCCGCSMAKGTAGAAGCTCAMYGMAATGACGGGACCGCCAGCRTTGAGCMWGSGTTAATCWATG CAACGCGGRRAAAGA

7b, Shewanella sp.:

AAACGCCCTYYCGAAGGTTAAGCTATSTACTTCTGGTGCAGGAACCCATGGKGTGACGGGCGGTGTGTACAAGGCCC GGGAAGATTCACCGKGGCATTCTGATCCACGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATC CGGACTACGACAAGCTTTGTGAGATTAGCTCCACCTCGCGGGCTTTGCAACCCTCTGTACTTGCCATTGTAGCACGTGT GTAGCCCTACTCGTAAGGGCCATGATGACTTGACGTCGTCCCCACCTTCCTCCGGTTTATCACCGGCAGTCTCCCTAA AGTTCCCACCATTACGTGCTGGCAAATAAGGATAAGGGTTGCGCTCGTTGCGGGGACTTAACCCAACATTTCACAACA CGAGCTGACGACAGCCATGCAGCACCTGTCTCAGAGYTCCCGAAGGCACTAARCYATCTCTRGCRAATTCTCTGGAT GTCAAGAGTAGGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCCACCGCTTGTGCGGGGCCCCCGTCAATT CATTTGAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTCTACTTAATGCGTTAGCTTGRGAACCCAGTGTTCAAGA CACCAAATTCCGAGTAGACATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGTA CCTGAGCGTCAGTCTTTGTCCAGGGGGCCGCCTTCGCCACCGGTATTCCTTCAGATTCTACGCATTTCACCGCTACAC CTGAAATTCTACCCCCCTCTACAAGACTCTAGTYTGCCAGTTCGAAATGCARTTCCCAGGGTTGAGCCCGGGGGCCTC ACATCTCGCTAACAAACCGTCCTGGCGTACCGCCTTTTTACGSCCCCCRAGTTAAT

7c, Shewanella baltica:

7d, *Psychrobacter* sp.:

GAGGAAAGGGGAGGKGTGACGGGGGGGGGGACAGGCGGGAARAAAARGRGRRCGGCATTCTGATCCGCGATTACTAGC GATTCCTACTTCATGGAGTCGAGTTGCAGACTCCAATCTGGACTACGATAGGCTTTTTGAGATTCGCATCACATCGCT GTGTAGCTGYYCTCTGTACCTACCATTGTAGCACGTGTGTAGCCCTGGTCGTAAGGGCCATGATGACTTGACGTCGTC CCCGCCTTCCTCCAGTTTGTCACTGGCAGTATCCTTAKAGTTCCCGGCTTAACCCGCTGGTAACTAAGGACAAGGGTT GCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTATTCTAATTCCC GAAGGCACTCCCGCWTCTCTGCAGGATTCTAGATATGTCAAGACCAGGTAAGGTTCTTCGCGTTGCATCGAATTAAA CCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAACCCTTGCGGCCGTACTCCCCAGGCGGT CTACTTATTGMGTTAGCTGCGGTCACTAAGTCCTCAAGGGACCCAACGACTAGTAGACATCGTTTACGGCGTGGACTA CCAGGGTATCTAATCYTGTTTGCTACCACGYTTTCRAACCTCAGTGYCAATATGATGCCAGAAGGCTGCCTTCGCCAT CCGGGATTCCCTCCAGATCTCCTACGCATTTCACCSGCTWMCACCKGAAATTCTWCCTTYCCTCCTCACCMTATTCW AGCCTAMACAGATATSCAKAATGGCRKCTCCCARGATTAAGGCCCCGGGGCAWTT

8a, Psychrobacter maritimus:

8b, *Shewanella baltica*:

GGTTAAGCTACCCACTTCTTTTGCASCCCACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCA CCGTGGCATTCTGATCCACGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGACTACGAC GAGCTTTGTGAGATTAGCTCCACCTCGCGGCTTTGCAACCCTCTGTACTCGCCATTGTAGCACGTGTGTAGCCCTACTC GTAAGGGCCATGATGACTTGACGTCGTCCCCACCTTCCTCCGGTTTATCACCGGCAGTCTCCCTAGAGTTCCCACCAT TACGTGCTGGCAAATAAGGATAGGGGTTGCGCTCGTTGCGGGGACTTAACCCAACATTTCACAACACGAGCTGACGAC AGCCATGCAGCACCTGTCTCACGGTTCCCGAAGGCACTAAGCTATCTCTAGCGAATTCCGTGGATGTCAAGAGTAGG TAAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCCACCGCTTGTGCGGGGCCCCCGTCAATTCATTTGAGTTTTA ACCTTGCGGCCGTACTCCCCAGGCGGTCTACTTAATGCGTTAGCTTGAGAGCCCAGGTTTCAAGACACAAACTCCGA GTAGACATCGTTTACGGCGTGTACTACCAGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGTGCCGAGCGTCAGCTCAGTCT TTGTCCAGGGGGCSGCCTTC

8c, Psychrobacter faecalis:

8d, Psychrobacter pµlmonis:

9a, Shewanella putrefaciens:

9b, Vibrio metschnikovii:

AAGGAAGCTTGCTTTCTTTGCTGACGAGCGGCGGACGGGTGAGTAATGCCTGGGAAATTGCCCTGATGTGGGGGGATA ACCATTGGAAACGATGGCTAATACCGCATGATGCCTACGGKYCTAAKAAGGGGACCTTCGGGCCTCTCACCGCARGG TCTGTCCMGGYGGGATTAGCTAGKTGGKGAGGWAATGGYTCRCCARGGMRAGGATCCCTARSTGGACTGASAGGAT GAGCASTCACACTGKAACTGASCCCCCTGCCACACTCCTACGGKAGGSASTCCTGCGGAATATTGCACWGTGGAAGM TWGCCTGATGCRSSCATGCCTGGTGYATGAATAASGCCTGCRTGATGAAAAGCTCTTTCWKGCRAGAGGAGGGGG ATCGWTAATAGSGGTATTCTTTGACGTTRGMTTCTTAGAARKYACCGACTAACTACGYGCCRGCTRMCTCCGTGATA CSGAGGGYGCKAGCGTTRATCGTAATTACTGKRATCRRAGTTACTGCASGTGRWTTGYWAAGTCAKATGTGGAAGCC CGGGACTCAACCTCSCGAGTTSTCTTTGAMACTGKYRSGTTAGAGTACTGYAGASTRGAGTAGAATTTARGGTGTWAG AATTTCATGYGTAGAGATCTAATGSGAAWACCRKCTGGARAAAATACCGCTG

9c, Arthrobacter sp.:

AGGTGGTTAGGCCATCGGCTTCGGGTGTMYYCAACTTTCGTGACTTGACGGGCGGTGTGTACAAGGCCCGGGAACGT ATTCACCGCAGCGTTGCTGATCTGCGATTACTAGCGACTCCGACTTCATGGGGTCGAGTTGCAGACCCCAATCCGAAC TGAGACCGGCTTTTAGGGATTAGCTCCACCTCACAGTATCGCAACCCATTGTACCGGCCATTGTAGCATGCGTGAAGC CCAAGACATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGAGTTGACCCCGGCAGTCTCCCATGAGTCC CCACCTTTACGTGCTGGCAACATGGAACGAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGC TGACGACAACCATGCACCACCTGTGAACCAGCCCCGAAGGGAAACCCCATCTCTGAGGCGGTCTGGAACAAGGAC CCTTGGTAAGGTTCTTCGCGTTGCATCGAATTAATCCGCATGCTCCGCCGCTTGTGCGGGGCCCCCGTCAATTCCTTTGA GTTTTAGCCTTGCGGCCGTACTCCCCAGGCGGGGCACTTAATGCGTTAGCTACGCGGGGGAAACGTGGAATGTTCCCAC ACCTAGTGCCACGTTACGCATGACTACAGGTATCTATCCTGGTCGCTCCCATGCTTCGCTCCTCAGCGTCAGTAGATG CCAGAGACTGCCTTCCGCCATGGGGTTCCTCCTGGAWWTCTTG

10a, *Psychrobacter psychrophilus*:

GGCTTAACACATGCAAGTCGAGCGGAAACGATGATAGCTTGCTATCAGGCGWCGAGCGGCGGACGGGTGAGTAATR CTTRGGAAWCTRCCTAGTRGTGGGGGGATAGCTCSGGGAAACTCGAATTAATACCGCATACGACCTACGGGAGAAAGG GGCAACTTGTTGCTCTCGCTATTAGATGAGCCTAAGTCGGATTAGCTAGATGGKGGGGTAAAGGCCTACCATGGCG ACRATCTGTAGCTGGTCTGAGAGGATGATCAGCCACACCGGGACTGAGACACGGCCCGGACTCCTACGGGAGGCAGC AGTGGGGAATATTGGACAATGGGGGCAACCCTGATCCAGCCMTGCCSCGTGKGTGAAGAAGGSCTTTTGGTTGTAAA GCACTTTAAGCAGTGAAGAAGACTCCATGGTTAATACCCATGGACGATGACATTAGCTGCAGAATAAGCACCGGCTA ACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGACGGTGGG GGCTTGATAAGTCAGATGTGAAATCCCCGGGCTTAACCTGGGAACTGCATCTGATACTGTTAGGSTAGAATAGGTGA GAGGAAGGTAGAATTCCAGGTGTAAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGATGGCGAAGGCAGCCTTC CTGGCATCATATTGACACTGAGTTCGAAGCGKGGGTAGCAAMAGGATTA

10b, Aeromonas sp.:

GGGCGGGCRGGCCTAACACATGCAAGTCGAGCGGCAGCGGGAAAGTAKCTTGCTAYTTTGCCGGCGAGCGGCGGA CGGGTGAGTAATGCCTGGGGATCTGCCCAGTCGAGGGGGGATAACAGTTGGAAACGACTGCTAATACCGCATACGCCC TACGGGGGAAAGGAGGGGACCTTCGGGCCTTTCGCGATTGGATGAACCCAGGTGGGATTAGCTAGTTGGTGGGGTAA TGGCTCACCAAGGCGACGATCCCTAGCTGGTCTGAGAGGGATGATCAGCCACACTGGAACTGAGACACGGYCCAGACT CCTACGGGAGGCAGCAGKGGGGAATATTGCACAATGGGGGGAAACCCTGATGCAGCCATGCCGCGTGTGTGAARAAG GCCTTCGGGTTGTAAAGCACTTTCAGCGAGGAGGAGGAAAGGTTGGCGCCTAATACGTGTCAACTGTGACGTTACTCGCA RAARAAGCACCGGCTAACTCCGTGCCAGCAGCGCGCGGGAAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCG TAAAGCGCCAGCAGGCGGTTGGATAAGTTAKATGTGAAAGCCCCGGGCTCAACCTGGGAATTGCATTTAAAACTGTC CAGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGT GGCGAAGCGGCCCYCTGGACAAAGACTGACGCTCAKGTGCGAAAGCGTGGGG

11a, Enterobacteriaceae:

AACTGCCTGATGGAGGGGGATAACTACTGGAAACGGKAGCTAATACCGCATAACGTCTTCRGACCAAAGKGGGGGA CCTTCGGGCCTCRCSCCMTCAKATGTGCCCAGATGGGATTATCTARTAGGKGGGGTAATGTCTCACCTASGCGACRAT CCCTATSTGGTCTGAGARGATGACCASCCACWCTGRAACTGACACACSCCCCACACTCCTACGGGGGGSARCWGTGG GGAATATTGCACAGTGSGCGCAMGCCTGATGCACCCATGCCGCGTGTGRGAARAAGGCCTTCGGGKTGTAAAGCACT TTCWKCGARGAGGAAGGGKASRGTGKTAATASCYCTCTGYWGTGACRWTACTCRCAGAARAAACACCSTCTMTCTC CGTGCCAKCASSCGCGGTAATACAGAGGGTGCKWGCGTTWWGCASAATTACTGSGCGTARAGCGCACGCRCGSGGT GTGWTAAGTCAKATGTGATATCCKCKCKCCCACGTGGGMWCTGYWTTTGA

11b, Aeromonas salmonicida:

TACGGCTGGGCGGCAGGCCTAACACATGCAAGTCGAGCGGCAGCGGGAAAGTAGCTTGCTACTTTTGCCSGCGAGCG GCGGACGGGTGAGTAATGCCTGGGGATCTGCCCAGTCGAGGGGGATAACAGTTGGAAACGACTGCTAATACCGCAT ACGCCCTACGGGGGAAAGGAGGGGGCCCTTCGGGGCCTTTCGCGATTGGATGAACCCAGGTGGGATTAGCTAGTTGGTG GGGTAATGGCTCACCAAGGCGACGATCCCTAGCTGGTCTGAGAGGGATGATCAGCCACACTGGAACTGAGACACGGTC CAGACTCCTACGGGAGGCAGCAGTGGGGGAATATTGCACAATGGGGGAAACCCTGATGCAGCCATGCCGCGTGTGTG AAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGAGGAGGAGGAAAGGTTGGCGCCTAATACGTGTCAACTGTGACGTTA CTCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGGTAATACGGAGGGGGCAAGCGTTAATCGGAATTAC TGGGCGTAAAGCGCACGCAGGCGGTTGGATAAGTTAGATGTGAAAGCCCCGGGCTCAACCTGGGAATTGCATTTAAA ACTGTCCAGCTAGAGTCTTGTAGAGGGGGGGTAGAATTCCAGGTGTAGCGGTGGAAATGCGTAGAGATCTGGAGGATAC CGGTGGCGAAAGCGGCCCCCTGGTACAAGACTGACGCTCAGKGCGAAAGCGTGGGGAGCAAAC

12a, Shewanella baltica:

12c, Psychrobacter sp.:

CGGCAGGCTTAACACATGCAAGTCGAGCGGAAACGATGGTAGCTTGCTACCAGGCGTCSMYCGGCGGACGGGTGAG TAATACTTAGGAATCTACCTAGTAGTGGGGGGATAGCTCGGGGAAACTCGAATTAATACCGCATACGACCTACGGGGG AAAGGGGGCAGTTTACTGCTCTCGCTATTAGATGAGCCTAAGTCGGGATTAGCTAGATGGTGGGGGTAAAGGCCTACCA TGGCGACGATCTGTAGCTGGTCTGAGAGGATGATCAGCCACACCGGGACTGAGACACGGCCCGGACTCCTACGGGAG GCAGCAGTGGGGGAATATTGGACAATGGGGGGAAACCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGCCTTTTGGTT GTAAAGCACTTTAAGCAGTGAAGAAGACTCCGTGGTTAATACCCACGGACGATGACATTAGCTGCMGAATAAGCAC CGGCTAACTCTGTGCCAGCAGCCGCGGGTAATACAGAGGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGGGAGC GTAGGTGGCTCGATAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCTGATACTGTTGAGCTAGAG ATGTGAGAGGAAGGTAGAATTCCAGGTGTAGCGGYGAAATGCGGTAGAGATCCTGGAGGAATACCGATTGGTGAA

12d, Arthrobacter sp.:

GGATGACCCCGTGCTTGCACGGGTGATTAGTGGCGAACGGGTGAGTAACACGTGCGTACCTGCCGTGATTTKGTAGGTTGTTGGTTTTTGATTGCGTGTTWATGCTATTMGTAKTGTMTGCCTGGGTG

12e, Planococcus sp.:

12f, Planococcus sp.:

12g, Psychrobacter sp.:

GATGATAGCTTGCTATCAGGCGTCSMSCGGCGGACGGGTGAGTAATACTTAGGAATCTACCTAGTAGTGGGGGGATAG CACGGGGAAACTCGTATTAATACCGCATACGACCTACGGGAGAAAGGGGGCAGTTTACTGCTCTCGCTATTAGATGA GCCTAAGTCGGATTAGCTAGATGGTGGGGGTAAAGGCCTACCATGGCGACGATCTGTAGCTGGTCTGAGAGGAGGATGATC AGCCACACCGGGACTGAGACACGGCCCGGACTCCTACGGGGAGGCAGCAGTGGGGAATATTGGACAATGGGGGAAAC CCTGATCCAGCCATGCCGCGTGTGTGAAGAGAGGCCTTTTGGTTGTAAAGCACTTTAAGCAGTGAAGAAGACTCCGTG GTTAATACCCACGGACGATGACATTAGCTGCAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCGGCGGAATACCGG GGCTTTAATCGGAACTGCGAATTACTGGGCGTAAAGGGAGCGTAGGTGGCTCTATAAGTCAGATGTGAAAATCCCG GGCTTTAACCTGGGAACTGCATCTGAAACTGTAGAGCTAGAGTAGTGGAGGAAGGTAGAATTCCAGGTGTAGCGG TGAAATGCGTAGAGAWYCTGGAGGAATACCGRATGGCGRAARGCMGSCCTTTYTGGG

12h, Bacillus sp.:

AGAGCTTGCTCTTATGAAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTC CGGGAAACCGGGGCTAATACCGGATAACATTTTGAACCGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTT ATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGA GGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATG GACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAAC AAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCAGCCGCG GTAATACGTAGGTGGCAACGCTTATCCGGAATTATTGGGCGTAAAAGCCACGGCGCAGGAGGAAAGTCGAATGTG AAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCAT GTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACMCCAGTGGCGAAAGC

13a, Shewanella baltica:

AGGTTAAGCTACCCACTTCTTTTGCAGCCACACCSGKGGKGTGACGGGCGGTGTGTACAAGGCCCGGGAACYTWTTC ACCGTGGCATTCTGATCCACGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGACTACGA CGAGCTTTGTGAGATTAGCTCCACCTCGCGGGCTTTGCAACCCTCGTACTCGCCATTGTAGCACGTGTGTAGCCCTACT CGTAAGGGCCATGATGACTTGACGTCGTCCCCACCTTCCTCCGGTTATCACCGGCAGTCTCCCTAGAGTTCCCACCA TTACGTGCTGGCAAATAAGGATAGGGGTTGCGCTCGTTGCGGGGACTTAACCCAACATTTCACAACACGAGCTGACGA CAGCCATGCAKYWYCTGTCTCACGGTTCCCGAAGGCACTAAGCTATCTCTAGCGAATTCCGTGGATGTCAAGAGTAG GTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCCACCGCTTGTGCGGGGCCCCCGTCAATTCATTTGAGTTTT AACCTTGCGGCGTACTCCCCAGGCGGTCTACTTAATGCGTTAGCTTGAGAGCCAGTGTTCAGACACCAAACTCCGAGG GGGCTCGCACGGTGACTACAGGTATCTATCCTGGGTGCTTCCACGCTTCSTGCTGACGTCAGTCTTGTCAGGGG CGGCTCGCACGGTATTCCTCC

13b, Shewanella baltica:

13c, Psychrobacter sp.:

GACGCCTGGCGGCAGGCTTAACACATGCAAGTCGAGCGGTAACATTTCTAGCTTGCTAGAAGATGACGAGCGGCGGA CGGGTGAGTAATACTTAGGAATCTACCTAGTAGTGGGGGGATAGCACGGGGGAAACTCGTATTAATACCGCATACGACC TACGGGAGAAAGGGGGCAGTTTACTGCTCTCGCTATTAGATGAGCCCTAAGTCGGATTAGCTAGATGGTGGGGTAAAG GCCTACCATGGCGACGATCTGTAGCTGGTCTGAGAGGATGATCAGCCACACCGGGACTGAGACACGGCCCGGACTCC TACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGGGAAACCCTGATCCAGCCATGCCGCGKGTGTGAAGAAGAC CTTTTGGTTGTAAAGCACTTTAAGCAGTGAAGAAGACTCCATGGTTAATACCCATGGACGATGACATTAGCTGCAGA ATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTA AAGGGAGCGTAGGTGGCTCTATAAGTCAGATGTGAAATCCCCGGGCTTAACCTGGGAACTGCATCTGAAACTGTAGA GCTAGAGTATGTGAGAGGAAGGTAGAATTCCAAGGTGTAGCGGTGGAAATGCGTAGAGATTAGCTGCAGG CGAAGGCAGCCTTCTGGCATAATACTGACACTGAGGCTCGAAAGCGTGGGTAGCAACAGGATTAGATACCCGGTGG CGAAGGCAGCCTTCTGGCATAATACTGACACTGAGGCTCGAAAGCGTGGGTAGCAACAGGATTAGATACCCGGTGG GGGAGTACCGCCCGCAAGGTAWACTCAATGGATTGAYCGGGGTCGCAGCGGGGGCGGCAGGTGGAAATYCATGCACGCG AGAAACCATACCTG

14a, Shewanella baltica:

ATCCCGGGGGCAGCGGGRAGATASTTTGCTATCTTTGCCGGCGAGCGGSGGACGGGTGAGTAATGCCTAGGGATCTG CCCAGTCGAGGGGGATAACAGTTGGAAACGACTGCTAATACCGCATACSCCCTACGGGGGAAAGGAGGGGACCTTC GGGCCTTCCGCGATTGGATGAACCTAKGTGGKATTAKCTAKKTGGWGAGGTAATGGCTCACCRRGGMSACKATCCCT AKSTGYTCTGAGAGGATGATCASCCACWCTGRSACTGASACACGSCCCASACTCCTACGGGAGGCAGCWGKGGRRAA TATTGCACARTGGGRRAAACCCTGATGCMSCCRTGCCGCGTGTGWGAARAASGCCTTCGGKTTGTAAAGCACTTTCA RTAGGGAGGAAAGGTAGCAKCTTAATACKCTTKTGCTGTGACKTTMCCTACARAARAAGGACCGGCTAACTCCGTGC CMGCMSCCGCGGTAATACRGAGGGTCCGAGCGTTAATCRGAWTTACTGGGYGTAAAGCGTGCRCASGCGKTKTGTT AAGMGAGATGTGAAMGCCCCGSKCTCAACCTGAAWAKTGCATTTCRAACTGGMGAWCTAGASTCTTGTAGAGGGG GATAGWAYTCCATGTGTAGCGGWGAWATGCGTAGAGATCTGGAGTATACCTGGTGGMGAAGSCGCCC

14b, Psychrobacter sp.:

TGGCGGCAGGCTTAACACATGSAAGTCSAGCGGAAACGATGATAGCTTGCTATCAGGCGTCGAGCGGCGGACGGGTG AGTAATACTTAGGAATCTACCTAGTAGTGGGGGGATAGCACGGGGGAAACTCGTATTAATACCGCATACGACCTACGGG AGAAAGGGGGCAGTTTACTGCTCTCGCTATTAGATGAGCCTAAGTCGGATTAGCTAGATGGTGGGGTAAAGGCCTAC CATGGCGACGATCTGTAGCTGGTCTGAGAGGAGGATGATCAGCCACACCGGGACTGAGAACGGCCCGGGACTCCTACGGG AGGCAGCAGTGGGGAATATTGGACAATGGGGGAAACCCTGATCCAGCCATGCCGCGTGTGGAAGAAGGCCTTTTGG TTGTAAAGCACTTTAAGCAGTGAAGAAGACTCCATGGTTAATACCCATGGACGATGACATTAGCTGCAGAATAAGCA CCGGCTAACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAAATTACTGGGCGTAAAGGAG CGTAGGTGGCTCTATAAGTCAGATGTGAAATCCCCGGGCTTAACCTGGGAACTGCATCGAAACTGTAGAGCTAGAG TATGTGAGAGGAAGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGATGGCGAAAGGC AGCYTTCTGGCATAATAC

14c, Psychrobacter cryohalolentis:

GGAAACGATGATAGCTTGCTATCAGGCGTCGAGAGGCGGACGGGTGAGTAATACTTAGGAATCTACCTAGTAGYYSS SGWTAGCTCGGGGAAACTCGAATTAATACCGCATACGACCTACGGGGGAAAAGGGGGCAGTTTACTGCTCTCGCTATT AGATGAGCCTAAGTCGGATTAGCTAGATGGTGGGGGAAAAGGCCTACCATGGCGACGATCTGTAGCTGGTCTGAGAGG ATGATCAGCCACACCGGGACTGAGACACGGCCCGGACTCCTACGGGAGGCAGCAGTGGGGGAATATTGGACAATGGG GGAAACCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGACGCCTTTTGGTTGTAAAGCACTTTAAGCAGTGAAAAAGAA TCTTCGGTTAATACCCGGWKWCKWTKWCATTAGCTGCAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCGCGGT AATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGGAGCGTAGGTGGCTCGATAAGTCAGATGTGA AATCCCCCGGGCTCAACCCTGGGAACTGCATCTGATACTGTTGAGCTAGAGTATGTGAGAGGAAGGTAGAAATTYCC AGGYGTAGCGSTGAAATGCGTAGAGAT

14d, Arthrobacter arilaitensis:

TCGAACGATGAAGCCCAGCTTGCTGGGTGGATTAGTGGCGAACGGGTGAGTAACACGTGAGTAACCTGCCCCCGACT CTGGGATAAGCCCGGGAAACTGGGTCTAATACCGGATATTACCTCTTGCCGCATGGCAGGTGGTGGAAAGATTTATC GGTGGGGGATGGACTCGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGGGAAAGATTTATC GAGAGGGTGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCAC AATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGTAGGGAA GAAGCGAAAGTCGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCGGCGGGAAAACTCCGAGGCCC AAGCGATATCCGGATTTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCGTGACAGCGCGGGAAAGTCCGAGGCTCAA CCTCGGATCTGCGGTGGGTACGGGCAGACTAGAGTGATGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAAAGC GCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTCTCTGGGCACTTGCGCGTGAGGAGCGAAAGCATGGG GAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAACGTTGGGCACTAGGTGGGGGACATTCCACGTTTTC CGCGCCGTAGCTAACGCATTAAGTGCCCCGCCTGGGGGAGTACGGCGCAAGGCTAAACTCAAAGGAATTGRMSGGG GGGCCSCACAGCGGCGGARCATGSGGATWATTTCGATGCACGCGGAGACCTTACCAGCTTGACATGGTGGCGCAGACCGC CTYCTCTAGA

15a, Kocuria sp.:

GGCGYGCTTAACACATGCAAGYCGAACGCTGAAGCTTGGTGCTTGCACTGGGTGGMTGAGTGGCGAACGGGTGAGT AATACGTGAGTAACCTGCCCTTGACTCTGGGATAAGCCTGGGAAACTGGGTCTAATACTGGATACGACATGTCACCG CRTGGTGGTGTGTGGAAAGGGTTTTACTGGTTTTGGATGGKCTCACGGCCTWTCASCTTGTTGGTGGGGTAATGGCTC ACCARGGCGACGACGGGGAGGCGGCCTGAGAGGGTGACCGGCCACACTGGGACTGAGACACGGCCCAGYCTCCTAC GGGAGGCAGCAGCGGGGAATATTGCACMATGGGCGAAAGCCGACACTGGGACGACGCGCGGTGAGGGGATGACGGCCT CGGGTTGTAAACCTCTTTCAGCACGGAAGAGCGAAAGTGACGGYACGTGCAGAAGAAGCGCCGGCTAACTACGTG CCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTC GCGTCTGCTGTGAAAGCCCGGGGCTTAACCCCGGGTGTGCAGTGSKTACGGGCAGACTTGAGTGCAGTAGGGGGAGAC TGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATMTCAGGAGGAACACCGATGGCGAAGGCAGGTCTCTGGGCTGTT ACTGACKCTGRGAGCGAAAGCATGGGGAGCGAACAGGATTAGATACCCTGGTAGWCCATGCCGTAAACGTTGGGCA CTAGGYGTGGKGAACATTCCAYGTTTTTCCGCGCGTAGCTAACGCATTAMGTGACCCSGCTGGAGAGTACCGGCGCA GGCTAAACTCRAGTATGACGGGGTCCG

15b, Kocuria palustris:

15c, Exiquobacterium sp.:

15d, Flavobacteriaceae:

15e, Psychrobacter faecalis:

15g, Psychrobacter sp.:

15h, Rothia sp.:

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