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PILOT STUDY

Characterization of the Cultivable Gut Microflora in Wild-Caught Mediterranean Fish Species

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Abstract: ***Background*:** Microflora of the gastrointestinal tract plays important roles in food digestion, nutrient absorption and in host defense against ingested pathogens. Several studies have focused on the microflora of farmed fishes, but the gut flora of wild fishes remains poorly characterized. The aim of this work was to provide an overview of the bacteria colonizing the gut of wild-caught fishes and to determine whether some bacterial species can be pathogenic.

***Results*:** We isolated cultivable bacteria from fifteen wild-caught Mediterranean fish species corresponding to different habitat, diet and origin. Bacterial species identity was determined by 16s rRNA gene sequencing for the 61 isolates. The potential pathogenicity of isolated bacteria was investigated using fruit fly (*Drosophila melanogaster*) and zebrafish (*Danio rerio*) as model organisms. Two bacterial strains (*Serratia* sp. and *Aeromonas salmonicida*) were lethal when microinjected to *Drosophila,* while zebrafish did not develop any disease when exposed to any of 34 isolated bacterial strains. However, it was interesting to note that two bacterial strains (*Shewanella* and *Arthrobacter*) isolated from marine fishes were able to colonize the guts of freshwater zebrafish.

***Conclusion*:** The results of this study give an overview of the bacterial species found in the guts of wild fishes living off Beirut seashore. It shows that some parameters believed to be limiting factors to host-gut colonization by bacteria can be overcome by some species. This pilot study could be extended by sampling a larger number of fish species with several specimens per fish species, and by identifying uncultivable bacteria that reside in the fish guts. Our results may have implications for the utilization of certain bacterial species in fish farming or their use as bio-indicators for water and/or food quality.

Keywords: Bacteria, *Drosophila*, gut flora, infection, mediterranean fish, zebrafish.

INTRODUCTION

Microflora refers to the living microscopic organisms that grow inside and on the surface of living creatures, including fishes. These organisms are usually found on the skin, tissues and inside guts [1, 2]. The composition of microbial communities within fish guts is believed to differ significantly from those living in the surrounding environment in both diversity and specificity [1, 3, 4]. Some of these bacteria occur permanently within the microflora while others appear to be transient [1]. 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. 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 [5]. The composition of the gut microflora is believed to change in response to a variety of factors affecting the host physiology, such as feeding strategies, developmental stages, and changing environmental conditions [1, 6-10].

Different studies used culture-based techniques to identify bacteria providing valuable insights into the composition of microbial communities. Gamma-Proteobacteria such as *Aeromonas* spp., *Escherichia coli*, *Photobacterium* spp., *Pseudomonas* spp. and *Vibrio* spp., dominated the gut microbiome of most fishes [7-9, 11-21]. It is 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 [2].

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 the leading causes of human and marine mammals' casualties, although several members of this genus are nonpatho-

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genic and are the dominant bacteria in and on marine fishes [22]. They have been commonly reported as members of the gut microflora in both farmed and wild fishes [1, 7, 18, 21-24]. Most infections involving these two bacteria occur through the consumption of raw or undercooked seafood leading to gastroenteritis and septicemia [25]. 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 [22, 26]. *Photobacterium damselae,* which is a virulent strain, can also cause septicemia and internal hemorrhage in fishes and septicemia and wound infections in humans [27, 28]. *Streptococcus inae*, *Aeromonas hydrophilia*, *Edwardsiella tarda*, *E. rhusopathiae*, *Mycobacterium marimum* and other *Vibrio* spp. are additional pathogens leading to human diseases [29-31].

The naturally occurring bacteria in the guts of wild fishes remain poorly characterized. This is particularly true for species living in the Mediterranean Sea, some of which are introduced [32]. In fact, not much is known about the original gut bacteria associated with introduced species and whether they retain their original flora or acquire a new one, similar to that of native species.

Also, fishes inhabiting water polluted by human sewage can be the vectors of human diseases representing a great public health threat [33] and it is interesting to determine if the presence of certain bacteria can be used as an indicator of contaminated water. The aim of this work was to provide an overview of the bacteria colonizing the gut of wild-caught native and exotic marine fishes collected from the eastern Mediterranean, off the coast of Lebanon (Beirut) and to compare them according to their habitat, diet and origin. We also attempted to determine whether bacterial species' distribution over the host fish displayed nestedness and investigated the potential pathogenicity of some bacteria using the model organisms *Drosophila melanogaster* (Fruit fly) and *Danio rerio* (Zebrafish).

MATERIALS AND METHODS

Sampling Design

Sampling was conducted in the coastal water of Lebanon, in the vicinity of Beirut. A total of fifteen marine fish species (9 families) were sampled using a beach seine. The net was hauled over soft bottom at a 0-5 m depth. Fishes were identified and categorized according to their diet, origin and swimming mode [34]. Fishes were immediately transported on crushed ice to the laboratory for examination. They were dissected under aseptic conditions and their guts were removed and processed.

Bacterial Isolation

Three small slices from different places of each dissected gut (upper, middle and lower portion) were ground with their content in 200 µl of LB, diluted to reach a volume of 1 ml, and different volumes (between 10 to 100 µl) were plated on LB agar. Dishes were kept incubated overnight at room temperature for colonies to grow. Bacterial colonies were checked for their colors and patterns and individual colonies were isolated. The obtained bacterial colonies were inspected and divided into different categories based on their appearance, relative abundance and color. For each morphological category, one representative bacterial colony was isolated and further analyzed per fish.

Polymerase Chain Reaction PCR

Bacterial genomic DNA was prepared from liquid culture according to the standard protocols and used as template for PCR. Each PCR mix consisting of: 28µl distilled water, 4 µl 10X buffer, 3.5 µl $MgCl_2$ (25 mM), 0.75µl dNTPs (2 mM), 0.75 µl 16sF, 0.75 µl 16sR and 0.25 µl Taq polymerase was added to 2 µl 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, steps 2-4 were repeated 30 times, step 5: 72 °C for 5 min and step 6: 4 °C forever.

DNA Purification, Sequencing and Bacterial Species Identification

PCR product was Phenol/Chloroform (Sigma-Aldrich, CA, USA) extracted and resuspended in nuclease free water according to the standard manufacturer's protocol. DNA concentration was measured using a Nanodrop apparatus (Thermoscientific). The samples were diluted to 80 ng/µl and sequenced (dideoxy nucleotides method) using 16s-RP2 (CCCGGGATCCAAGCTTACGGCTACCTTGTTACGAC TT) or 16s-FD1 (CCGAATTCGTCGACAACAGTTT GAT CCTGGCTCAG) primers. NCBI nucleotide blast (www. ncbi.nlm.nih.gov) was used to compare the 16s sequences, obtained as database sequences, and each 16s sequence was then assigned to the closest match in the database from an identified species.

Drosophila Injection

32nl bacterial suspension of OD= 0.15 were injected into the thorax of wild type *Drosophila melanogaster* using a Nanoject II apparatus (Drummond Scientific, CA). Each experiment was performed using 15 flies in fresh vials and survival was monitored by counting the flies at regular intervals after injection. The graph shown in Fig. (**1**) is representative of 3 independent experiments. For this experiment, all the bacteria that were successfully grown in liquid culture were assayed. Using both Log-rank (Mantel-Cox) Test and Gehan-Breslow-Wilcoxon Test, the results were statistically highly significant ($p<0.05$).

Bacterial Exposure Experiment

50 µl of each bacterial mix (OD = 40) was added to small cups containing 100 ml of water. Three specimens of zebra fish were added and left for 45 min. The fish were transferred to 3 liters containers filled with 2 liters of tap water and 1 liter of aquarium water and aerated with bubblers. The behavior of the fish was monitored at different time points. Two weeks later, fish were sacrificed and gut content plated on LB. In this experiment a subset of 34 bacterial isolate was assayed.

Table 1. Summary information about the analyzed fish specimens.

Fish ID	Fish Species	Common Name	Diet	Swimming Mode	Origin	Weight (gram)	Length (cm)	Gut Weight (gram)
1	*Sargocentrom rubrum*	Redcoat	C	P	E	67.05	15.5	0.86
2	*Pagellus acarne*	Axillary seabream	C	P	N	47.64	14.9	1.56
3	*Pomadasys incisus*	Bastard grunt	C	B	N	30.96	12.5	0.60
4	*Siganus rivulatus*	Marbled spinefoot	H	P	E	65.47	17.1	3.50
5	*Dentex macrophthalmus*	Large-eye dentex	C	B	N	30.61	13.0	0.74
6	*Diplodus vulgaris*	Common two-banded seabream	C	B	N	37.97	13.1	1.02
7	*Dicologlossa cuneata*	Wedged sole	C	B	N	49.06	16.3	1.99
8	*Oblada melanura*	Saddled seabream	C	P	N	91.71	18.9	0.92
9	*Pagellus erythrinus*	Common pandora	C	B	N	19.71	10.1	0.50
10	*Pomadasys stridens*	Striped piggy	C	P	E	71.71	16.4	1.12
11	*Plotosus lineatus*	Striped eel catfish	C	B	E	21.43	14.8	1.34
12	*Pempheris rhomboidea*	Vanikoro sweeper	C	P	E	49.93	15.5	0.80
13	*Sardinella maderensis*	Maderian sardinella	C	P	N	12.57	11.7	0.34
14	*Lithognathus mormyrus*	Sand steenbras	C	P	N	25.50	11.9	0.45
15	*Liza aurata*	Golden grey mullet	C	P	N	171.51	26.8	6.87

Fish ID, scientific name, common name, predominant diet, mode of swimming and origin are listed for each fish. C = carnivorous, H = herbivorous, B = benthic, P = pelagic, N = native and E = exotic. The last three columns give respectively the size of the specimen studied, its weight and the weight of its dissected gut.

RESULTS

Fish Sampling and Bacteria Isolation

Lists of studied fish and bacteria are provided in Tables **1** and **2**, respectively. In total, 61 distinct bacterial colonies were isolated on LB agar plates and genomic DNA was extracted from each. Then, 16s rRNA gene was amplified and sequenced for identification purposes and obtained sequences were deposited at Genbank under the accession numbers KX650092-KX650146. The results were as follows: Three bacterial species were cultured from *Sargocentrom rubrum* (*Staphylococcus hominis, Shewanella baltica* and *Psychrobacter faecalis*). Four bacterial isolates were cultured from *Pagellus acarne* (two *Psychrobacter* spp*., Shewanella* sp. and *Aeromonas* sp.). Seven bacterial isolates were cultured from *Pomadasys incisus* (two *Psychrobacter faecalis*, two *Planococcus* sp*., Shewanella putrefaciens, Psychrobacter* sp. and *Arthrobacter* sp.). Three bacterial species were cultured from *Siganus rivulatus* (*Shewanella* sp*., Kocuria rhizophila* and *Psychrobacter* sp.). Four bacterial isolates were cultured from *Dentex macrophthalmus* (*Psychrobacter maritimus, Planococcus* sp*., Shewanella baltica* and *Psychrobacter* sp.). Five bacterial isolates were cultured from *Diplodus vulgaris* (*Psychrobacter cibarius, Psychrobacter faecalis,* two *Psychrobacter* sp. and *Arthrobacter* sp.). Three bacterial isolates were cultured from *Dicologlossa cuneata* (*Shewanella* sp*., Shewanella baltica* and *Psychrobacter* sp.). Four bacterial species were cultured from *Oblada melanura* (*Psychrobacter maritimus, Shewanella baltica,* and two *Psychrobacter faecalis*). Three bacterial species were cultured from *Pagellus erythrinus* (*Shewanella putrefacien*, *Vibrio metschnikovii* and *Arthrobacter* sp.). Two bacterial species were cultured from *Pomadasys stridens* (*Psychrobacter psychrophilus* and *Aeromonas* sp.). Two bacterial isolates were cultured from *Plotosus lineatus* (*Serratia* sp. and *Aeromonas salmonicida*). Seven bacterial isolates were cultured from *Pempheris rhomboidea* (*Shewanella baltica,* two *Psychrobacter* spp*., Arthrobacter* sp*., Planococcus* sp*., Planococcus* sp. and *Bacillus* sp.). Three bacterial isolates were cultured from *Sardinella maderensis* (two *Shewanella baltica* and *Psychrobacter* sp.). Four bacterial species were cultured from *Lithognathus mormyrus* (*Shewanella baltica, Psychrobacter* sp*., Psychrobacter cryohalolentis* and *Arthrobacter arilaitensis*). Seven bacterial isolates were cultured from *Liza aurata* (*Kocuria* sp*., Kocuria palustris, Exiguobacterium* sp*., Chryseobacterium* sp*., Psychrobacter faecalis, Psychrobacter* sp. and *Rothia* sp.).

Effects of Isolated Bacteria on Lab Model Organisms

The virulence of 50 bacterial isolates was assayed using the laboratory model organism *Drosophila melanogaster*. For this, a bacterial suspension with an OD of 0.15 was microinjected into the thorax of wild-type flies and survival was monitored. A virulent strain of laboratory *Staphylococcus aureus* was used as a positive control in these experiments. In agreement with previous reports [35], *Serratia* sp. was highly pathogenic when injected into *Drosophila melanogaster*. Another bacterial species, *Aeromonas salmonicida*, triggered rapid death rates in the flies (Fig. **1**). However, the majority of the tested bacteria led to low or no pathogenicity to *Drosophila* (Supplementary Fig. **1**).

In parallel, the effect of a selection of 34 bacterial isolates (listed in Table **3**) was assessed using *D. rerio* as a

Table 2. List of all bacteria isolated from the different fish guts.

Bacterial ID	Bacterial Species	Blast ID Match (%)	Appearance	Abundance
1a	*Staphylococcus hominis*	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* sp.	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.	97	Cream-pink, jelly	++
4c	*Kocuria rhizophila*	88	Yellow, bright	+
4e	*Psychrobacter* sp.	92	Cream	++
5a	*Psychrobacter maritimus*	97	Cream	++
5b	*Planococcus* sp.	98	Orange	+
5c	*Shewanella baltica*	96	Cream-pink, jelly	++
5e	*Psychrobacter* sp.	97	Cream	++
6a	*Psychrobacter cibarius*	90	Cream	++
6b	*Psychrobacter faecalis*	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 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*	99	Cream	++
8d	*Psychrobacter faecalis*	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* sp.	83	Cream	++
11b	*Aeromonas salmonicida*	99	Cream	++
12a	*Shewanella baltica*	95	Cream, jelly	++

(Table 2). contd…

Bacterial ID	Bacterial Species	Blast ID Match (%)	Appearance	Abundance
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	*Exiguobacterium* sp.	96	Orange	+
15d	*Chryseobacterium* sp.	97	Mustard orange	+
15e	*Psychrobacter faecalis*	96	Cream	++
15g	*Psychrobacter* sp.	92	Cream	++
15h	*Rothia* sp.	99	Mustard, light	+

Bacteria ID number refers to the fish it was isolated from, and the letter to independent isolates. The bacterial names given are based on the best match obtained after 16s sequence BLAST and the percent identity with database sequences is given in the third column. The last two columns describe the general appearance of the colony at the time of isolation and the abundance of each isolate among other bacterial colonies obtained from the same fish (+++ = very abundant/predominant, ++ = common and + = only few colonies obtained).

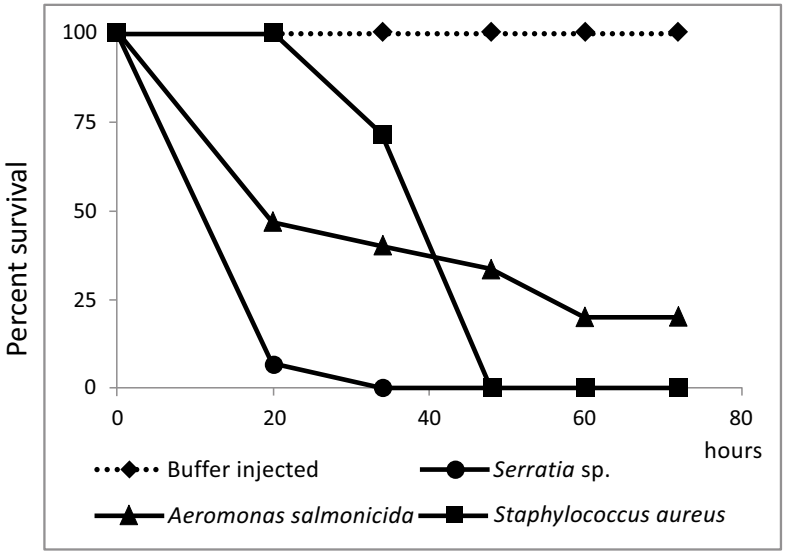

Fig. (1). Survival of *Drosophila* after bacterial microinjections.
This figure shows the survival of fruit flies after bacterial microinjection with some of the isolated bacteria including the only two isolates (*Serratia* sp. and *Aeromonas salmonicida*) that caused high death rates as compared to the buffer injected controls ($p<0.05$). *Staphylococcus aureus* is a virulent gram+ bacteria used in laboratory *Drosophila* infection experiments and is included for comparison purposes. The X-axis indicates the time post injection in hours. The Y-axis indicates the percentage of surviving flies. The complete survival graph including infections with the 50 isolates tested is shown in Supplementary Fig. (**1**).

Table 3. List of the bacterial species used in the zebrafish exposure experiment.

Bacterial ID	Bacterial Species	Ability to Colonize Zebrafish Gut
1a	*Staphylococcus hominis*	-
1b	*Shewanella baltica*	+
1c	*Psychrobacter faecalis*	-
2a	*Psychrobacter* sp.	-
3a	*Psychrobacter faecalis*	-
3d	*Shewanella putrefaciens*	+
3e	*Planococcus* sp.	-
3f	*Psychrobacter* sp.	-
3g	*Arthrobacter* sp.	+
4e	*Psychrobacter* sp.	-
5a	*Psychrobacter maritimus*	-
6b	*Psychrobacter faecalis*	-
6c	*Psychrobacter* sp.	-
6d	*Arthrobacter* sp.	+
7c	*Shewanella baltica*	+
7d	*Psychrobacter* sp.	-
8a	*Psychrobacter maritimus*	-
8c	*Psychrobacter faecalis*	-
8d	*Psychrobacter faecalis*	-
9b	*Vibrio metschnikovii*	-
9c	*Arthrobacter* sp.	+
11a	*Serratia* sp.	-
11b	*Aeromonas salmonicida*	-
12a	*Shewanella baltica*	+
12c	*Psychrobacter* sp.	-
12d	*Arthrobacter* sp.	-
12e	*Planococcus* sp.	-
12f	*Planococcus* sp.	-
14d	*Arthrobacter arilaitensis*	+
15a	*Kocuria* sp.	-
15b	*Kocuria palustris*	-
15e	*Psychrobacter faecalis*	-
15g	*Psychrobacter* sp.	-
15h	*Rothia* sp.	-

Zebrafish were exposed to a subset of the bacterial isolate. The + sign indicates that the bacteria was abundantly recovered from the fish guts 2 weeks after the initial exposure.

model organism. This selection has been made in order to reduce unnecessary multiple sequencing of isolates belonging to the same species and to privilege the isolates that grew to high OD in liquid culture. No lethality was observed after exposure to any of these bacteria (see methods). However, *D. rerio* treated with *Kocuria palustris*, *Psychrobacter faecalis* and *Kocuria* sp. showed transient distress symptoms (abnormal swimming and rapid respiration) in the first nine hours following the exposure, but the zebrafish completely recovered afterwards. To test the ability of bacteria to colonize the guts of zebrafish, two weeks after the exposure experiment, we checked for the presence of the bacterial isolate in the guts. In this aim, one of the fish exposed to each bacterial species was dissected, and bacteria from its gut isolated and identified as in the procedure used for the initial identification of bacteria from marine fish species. No cultivable bacteria were obtained from controls *Danio* that were not exposed to any bacterial isolate. After the exposure experiments, eight out of 34 bacterial strains were able to colonize the intestinal lining of *D. rerio*: four corresponded to distinct *Shewanella* sp. isolates (obtained from different fish species: *S. rubrum, P. incisus, D. cuneata* and *P. rhomboidea*) and four to distinct *Arthrobacter* sp. isolates (obtained from *P. incisus, D. vulgaris, P. erythrinus* and, *L. mormyrus*).

DISCUSSION AND CONCLUSION

In this pilot study, cultivable bacteria were isolated from the guts of 15 wild-caught marine fish species. The most represented bacterial genera among the 61 isolate were *Psychrobacter* and *Shewanella*. All the bacterial isolates belong to aquatic species except *Staphylococcus hominis* which is a human skin commensal and could result of a contamination. One of the identified bacterial species, *Aeromonas salmonicida*, is a known fish pathogen [36] and its presence in the fish guts possibly indicates environmental degradation.

It is 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. However, a similar analysis of a second batch of fishes including duplicate specimens of some of those reported in Table **1** confirmed that most of the isolated bacterial species were found again in the same hosts (supplementary Table **1**). Another limitation was that this study focused only on the cultivable bacteria present in the fish guts. Most of the bacteria that thrive in the digestive system of fishes don't grow on artificial media. Therefore, to have a 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 high throughput sequencing.

When isolated bacteria were assayed for their virulence by microinjection into *D. melanogaster*, from the 50 isolates tested, only two (*Serratia* sp. and *Aeromonas salmonicida*) were highly pathogenic to the flies. *Drosophila* has been used in several previous studies as a model to assess the pathogenicity of bacteria, fungi and other microbes because of its ease of manipulation and infection [37-40]. *Aeromonas salmonicida* is a fish pathogen [36] that tolerates salinity changes [41]. However, this isolate (and 33 others tested) were not harmful to zebrafish. Indeed, *A. salmonicida* was not able to persist in the guts of zebrafish after the exposure

experiment. It should be noted that the virulence of bacteria in *Drosophila* and in zebrafish cannot be really compared for two main reasons: 1- the immune systems of insects rely on innate responses unlike that of vertebrates that rely on an adaptive component in addition to the innate responses [42]; 2- in this study infection of *Drosophila* was achieved by microinjection into the body cavity, while infection of zebrafish was attempted *via* the oral route.

Other than providing an overview of the bacterial species that compose the flora of wild Mediterranean fishes, the most interesting finding was that some isolated bacteria were able to colonize the guts of a freshwater fish. Indeed, the exposure experiment proved that the isolated bacteria weren't accidentally present in the wild-caught fishes' guts since eight of these isolates successfully colonized the gut of aquarium kept zebrafish. This experiment proved that *Shewanella* sp. and *Arthrobacter* sp. were adapted to live in the gut independently of whether the host is a freshwater or a marine fish species.

This result somehow challenges the current ideas that variations in salinity and temperature play a major role in the composition microflora communities in fishes. Indeed, [6, 7] documented shifts in the composition of fish gut microflora coinciding with salinity variations encountered in estuarine environments. Other studies showed that many freshwater fishes harbor *Aeromonas* sp. within their guts while *Vibrio* sp. was documented in estuarine and marine species [1, 4, 43]. The composition of gut microflora has been shown to be altered by varying environmental conditions [5, 44]. An example is the potentially pathogenic *Vibrio vulnificus* that was detected in the sheepshead (*Archosargus probactocephalus*, Sparidae) in the Gulf of Mexico [45, 46], whose presence and abundance increased with increasing water temperature [47-49]. However, these changes of environmental factors are more likely to affect the transient microflora while the stable resident flora is expected to be less affected.

The results of this preliminary study give an overview of the bacterial species found in the guts of wild fishes living off Beirut seashore. It shows that some parameters believed to be limiting factors to host-gut colonization by bacteria (such as differences in water salinity) can be overcome by some species. A further step could be to test *Shewanella* for a potential utilization in fish farming as probiotics. Our study has shown that *Shewanella* is widely distributed among the saltwater species and can occur in freshwater zebrafish. It may be therefore be used to inoculate farmed fish and prevent harmful bacteria that develop under crowded conditions from colonizing the guts of farm reared fishes. Another possible application to similar studies would be the identification of certain bacterial species, such as *A. salmonicida*, that can be used as indicators of poor water quality or of contaminated fish destined for human consumption.

LIST OF ABBREVIATIONS

LB	=	Luria-Bertani Broth
nl	=	Nanoliter
OD	=	Optical Density
sp.	=	Species
µl	=	Microliter
^{o}C	=	Degree Celsius

AVAILABILITY OF DATA AND MATERIAL

16s sequences for isolated bacteria were deposited at Genbank under the accession numbers KX650092-KX650146.

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AUTHORS' CONTRIBUTIONS

AJ, HD, MB and ZK designed and performed experiments and analyzed the data. AJ and ZK wrote the manuscript. All authors read and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publishers Web site along with the published article.

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