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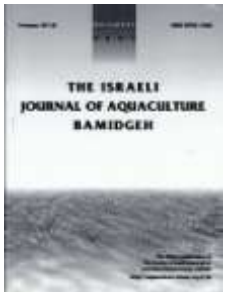

Infections by Lactic Acid Bacteria in Marine Fish from Southern Israel (Red Sea): New Records

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Abstract

Gram-positive chain-forming bacteria were isolated from the spleen, kidney, and blood of dead wild Red Sea fish (broomtail wrasse *Cheilinus lunulatus*, grouper *Epinephelus fasciatus*, goatfish *Parupeneus* sp.) that washed ashore on the Eilat Nature Reserve beach (Israel, Red Sea). Gram-positive bacteria were similarly isolated from rabbitfish *Siganus rivulatus*, white grouper *Epinephelus aeneus*, and grey mullet *Mugil cephalus* cultured in the Israel Oceanographic and Limnological Research, National Center for Mariculture, Eilat. On the basis of their phenotypical characteristics and 16S rRNA gene partial sequence analyses, the bacteria were identified as *Streptococcus parauberis* (from *C. lunulatus*), *Lactococcus lactis* (from *E. aeneus* and *M. cephalus*), *Streptococcus iniae* (from *E. fasciatus*, *Parupeneus* sp. and *S. rivulatus*), *Streptococcus agalactiae* (from *M. cephalus*), and *Carnobacterium* sp. (from *E. aeneus*). These lactic acid bacteria, isolated between 2009 and 2011, are added to the growing list of bacterial pathogens detected in wild or farmed marine fish in the Gulf of Eilat in recent years, suggesting a rising trend in areas subjected to intense anthropogenic impact (including aquaculture), where newly emerging pathogens and previously unexposed host populations are brought together.

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Introduction

Infections by gram-positive chain-forming bacteria have been reported since the late 1980s in wild and cultured fish in marine and freshwater environments in Israel and were recognized in the late 1990s as an emerging problem in worldwide aquaculture (Austin, 1999). Until a few years ago, the etiological agents of these diseases in Israel were identified as *Streptococcus iniae*, *Streptococcus difficile* (syn. *difficilis*, *agalactiae*), and *Lactococcus garvieae* (Eldar et al., 1994; Zlotkin et al., 1998a,b; Colorni et al., 2002, 2003; Kvitt and Colorni, 2004). These diseases had both a sporadic and an epizootic character. *Streptococcus iniae* is perhaps the most virulent and cosmopolitan member of this group of cocci (Agnew and Barnes, 2007), but several other species are known to be highly contagious and develop into lethal septicemia in fish (Austin and Austin, 2007). Clinical signs vary with the species of coccus and the species and size of the affected host but, in general, fish become lethargic and swim erratically or in spirals as a result of an evident meningoencephalitis. Exophthalmos and hyphema (bloody eyes), petechial hemorrhages, edema with accumulation of serosanguinous fluid in the peritoneal cavity and intestine, a pale liver, and a dark red spleen are the most common clinical signs of these coccoses.

While fish farms in northern Israel have suffered severe losses since 1986 when *S. iniae* was first detected in trout *Oncorhynchus mykiss* (Walbaum) and tilapia *Oreochromis* spp. Günther (Eldar et al., 1994,1995; Bachrach et al., 2001), until the early 1990s the Eilat (Red Sea) region was considered "streptococcus-free" (Diamant and Colorni, 1992). In 1996, however, *S. iniae* was isolated for the first time in red drum *Sciaenops ocellatus* (L.) reared in sea cages off the shore of Eilat's northern coast and has recurred repeatedly ever since in various species of cultured and wild Red Sea fish (Colorni et al., 2002; Kvitt and Colorni, 2004). In 2002, *L. garvieae* was isolated from a moribund clown wrasse *Coris aygula* Lacépède collected by divers who observed it lying motionless on its side in shallow water near the Eilat Nature Reserve (Colorni et al., 2003). The present report adds four new members, *Streptococcus parauberis*, *Lactococcus lactis*, *Streptococcus agalactiae*, and *Carnobacterium* sp., all isolated for the first time in Israel from marine fish, to the above lactic acid bacteria (LAB) infectious agents.

Materials and Methods

Fish. The analyzed fish are listed in Table 1. In April 2009, a gram-positive chain-forming bacterium was isolated from a broomtail wrasse *Cheilinus lunulatus* (Forsskål) washed ashore on the Eilat Nature Reserve beach. In February 2010, a gram-positive chain-forming bacterium was isolated from the white grouper *Epinephelus aeneus* Saint-Hilaire and grey mullet *Mugil cephalus* L. cultured in the facilities of the Israel Oceanographic and Limnological Research, National Center for Mariculture (IOLR-NCM) in Eilat. In June 2010 and again in May and June 2011, a gram-positive chain-forming bacterium was

Table 1. Wild and cultured fish from the Eilat region infected by gram-positive bacteria.

Date of isolation	*Fish species*		*Eilat location*	*Identified bacteria*
April 2009	Broomtail wrasse	*Cheilinus lunulatus*	Nature Reserve beach	*Streptococcus parauberis* strain *maris rubri*
February 2010	White grouper	*Epinephelus aeneus*	IOLR-NCM	*Lactococcus lactis* strain *eilaticus*
February 2010	Grey mullet	*Mugil cephalus*	IOLR-NCM	*Lactococcus lactis* strain *eilaticus*
June 2010	Grey mullet	*Mugil cephalus*	Private farm	*Streptococcus agalactiae*
November 2010	Goatfish	*Parupeneus* sp. (*rubescens*?)	Southern coast	*Streptococcus iniae*
November 2010	Grouper	*Epinephelus fasciatus*	Southern coast	*Streptococcus iniae*
December 2010	Rabbitfish	*Siganus rivulatus*	Tidal lagoon (north beach)/IOLR-NCM	*Streptococcus iniae*
May-June 2011	Grey mullet	*Mugil cephalus*	IOLR-NCM	*Streptococcus agalactiae*
December 2011	White grouper	*Epinephelus aeneus*	IOLR-NCM	*Carnobacterium eilaticum*

isolated from a number of grey mullet *M. cephalus* cultured in the facilities of an Eilat private farm and of IOLR-NCM, respectively, with fish mortalities nearing 100% on both occasions and at both locations. Between November 2010 and January 2011, a gram-positive chain-forming bacterium was isolated from a goatfish, *Parupeneus* sp. Bleeker (prob. *rubescens*), washed ashore along the Eilat southern coast, a grouper *Epinephelus fasciatus* (Forsskål), and several rabbitfish *Siganus rivulatus* (Forsskål) that had been caught some weeks before in a tidal lagoon at the end of an artificial channel that collects the effluents from industrial salt ponds and IOLR-NCM facilities (Eilat north beach). In December 2011 a gram-positive asporogenous rod was isolated from the kidney of a white grouper *E. aeneus* broodstock cultured at IOLR-NCM.

Bacteriology. Bacteria were isolated from the spleen, kidney, and blood of freshly dead or dying fish, or from fish that displayed obvious signs of disease. Tryptic Soy Agar (TSA) and Brain Heart Infusion (BHI) agar (Acumedia, Baltimore, MD), prepared with 25% aged seawater, alone or supplemented with 5% outdated human blood-bank blood, were used for culture. The inoculated media were incubated at least one week at 24±1°C. Several subcultures and Gram staining were made to ensure the purity of the colonies. For comparison, all eight coccus isolates were grown in both atmospheric and CO_2 ambient (Anaeropack, Mitsubishi Gas Chemical, Japan). The isolates were cryopreserved in 8-15% glycerol at -80°C until further use. Hemolysis was verified on TSA blood agar and BHI blood agar, and a catalase test was performed on colonies from plain TSA agar. Two miniaturized commercial kits, API 20 Strep and API 50 CH (bioMérieux, Marcy-l'Etoile, France), were used to obtain a biochemical profile of each coccus isolate. The manufacturer's instructions were followed except for the incubation temperature, which was maintained at 24±1°C instead of the recommended 36±1°C, and the intermediate readings, which were taken after 18 h of incubation. Final results were read 72 h after inoculation. Since a limited number of aquatic bacteria are included in the kit database, the characteristics of the isolates were compared with those reported in Austin and Austin (2007). The gram-positive rods isolated in December 2011 from *E. aeneus* were subjected to analysis using the bioMérieux Vitek 2 system.

16S rRNA gene sequence. Identifications obtained on the basis of phenotypic characteristics were confirmed by partially sequencing the 16S ribosomal RNA gene. PCR products from template DNA of needle-touched bacteria colonies (three replicates) were amplified using the universal primers fD1 (27F), and either rP2 (Weisburg et al., 1991) or 1100R (Turner et al., 1999). GoTaq Green Master Mix (Promega, Madison, WI) was used in a total volume of 50 µl, and PCR assays were performed with the Eppendorf Mastercycler gradient (Eppendorf, Hamburg, Germany). Typical cycling parameters were: 1 min denaturation at 94°C, 1 min annealing at 50°C, and 1.5 min extension at 72°C for 30 cycles. The initial denaturation step was extended to 4 min, and the final extension step was extended to 10 min. PCR products were analyzed in 1.2% agarose gels containing ethidium bromide and visualized under UV light. PCR products were purified using a PCR purification kit (Qiagen, Hilden, Germany) and the quantity and purity (260/280 ratio) of double-stranded PCR products were estimated in a microplate spectrophotometer (Power-Wave™ XS, BioTek, Winooski, VT). Internal universal primers 537F (5' AGCAGCCGCGGTAATACG 3') and 550R (5' CGCTCGAGACCTACGTATTACC 3') designed by us were used for the sequencing analysis. DNA was sequenced at Hy Laboratories Ltd. (Hylabs, Rehovot, Israel). The sequences of the isolates were compared with isolates in the GenBank database (NCBI/BLAST) and deposited in the Genbank (NCBI), with accession numbers JQ780604 to JQ780608.

Antibiogram. The sensitivity of the isolates to antibiotics commonly used in the Israeli food and ornamental fish industries was tested using the disk-diffusion method of antibiotic-impregnated disks (Oxoid, UK) on BHI agar. Zone diameters were measured and sensitivity was determined by comparing the zones of inhibition with the zone diameter breakpoints recommended by the manufacturer and the BSAC (1998).

Results

All the isolates were gram-positive. With the exception of the December 2011 isolate from white grouper *E. aeneus*, which was rod-shaped, all isolates were chain-forming, catalase-negative cocci or coccoid bacteria. They grew on all the media but the most robust growth was obtained on BHI blood agar in CO_2 atmosphere, where they formed punctiform, translucent colonies that become round, convex, and grey to whitish after 48 h. After 72-96 h, those isolated from the broomtail wrasse *C. lunulatus* were non-hemolytic and 1.75±0.25 mm in diameter; those isolated from the white grouper *E. aeneus* and grey mullet *M. cephalus* from Feb. 2010 were non-hemolytic and 1.25±0.25 mm in diameter. Those isolated from *E. fasciatus*, *Parupeneus* sp., and *S. rivulatus* in Nov-Dec 2010 and Jan 2011 were α-hemolytic, beige, and 1.75±0.25 mm in diameter. Those isolated from the mullets *M. cephalus* in June 2010 and 2011 were non-hemolytic, 1.50±0.25 mm in diameter, and formed sticky filaments when picked up with the inoculation loop.

Bacteriology. Phenotypical characteristics of the coccus isolates are given in Table 2; analysis of the gram-positive rods identified as *Carnobacterium* sp. is given in Table 3.

Table 2. Biochemical profiles of the cocci isolates (± = weak positive; d = delayed reaction).

	Fish species							
	Cheilinus lunulatus	*Siganus rivulatus*	*Parupeneus* (*rubescens*?)	*Epinephelus fasciatus*	*Mugil cephalus*	*Epinephelus aeneus*	*Mugil cephalus*	*Mugil cephalus*
Sample no.	250409	291210	251110	041110	020210	010210	080610	050611
Year of isolation	2009	2010	2010	2010	2010	2010	2010	2011
Infected by...	*Streptococcus parauberis* strain *maris rubri*	*Streptococcus iniae* strain *maris rubri*			*Lactococcus lactis* strain *eilaticus*		*Streptococcus agalactiae* strain *eilaticus*	
Gram stain	+	+	+	+	+	+	+	+
Catalase	-	-	-	-	-	-	-	-
Hemolysis	-	α	α	α	-	-	-	-
Voges-Proskauer (acetoin production)	+	-	-	-	+	+	+	+
Hippurate hydrolase	-	-	-	-	+	+	+	+
Esculin (β-glucosidase)	+	+	+	+	+	+	-	-
Pyrrolidonyl arylamidase	-	+	+	+	+	+	-	-
α-galactosidase	-	-	-	-	-	-	-	-
β-glucuronidase	-	+	+	+	-	-	-	-
β-galactosidase	-	-	-	-	-	-	-	-
Alkaline phosphatase	+	+	+	+	-	-	-	-
L-leucine arylamidase	+	+	+	+	+	+	-	-
Arginine dihydrolase	+d	-	-	-	+	+	-	-
Glycerol	-	±d	±d	±d	-	-	-	-
Erythritol	-	-	-	-	-	-	-	-
D-Arabinose	-	-	-	-	-	-	-	-
L-Arabinose	-	-	-	-	-	+	-	-
D-Ribose	+	+	+	+	+	+	-	-
D-Xylose	-	+d	+d	±d	+	+d	-	-
L-Xylose	-	-	-	-	-	-	-	-
D-Adonitol	-	-	-	-	-	-	-	-
Methyl-βD-xylopyranoside	-	-	-	-	-	-	-	-
D-Galactose	+	+	+	+	+	+	-	-
D-Glucose	+	+	+	+	+	+	+	+
D-Fructose	+	+	+	+	+	+	+	+
D-Mannose	+	+	+	+	+	+	+	+
L-Sorbose	-	-	-	-	-	-	-	-
L-Rhamnose	-	-	-	-	-	-	-	-
Dulcitol	-	-	-	-	-	-	-	-
Inositol	-	-	-	-	-	-	-	-
D-Mannitol	+	+	+	+	-	+	-	-
D-Sorbitol	+	-	-	-	-	-	-	-
Methyl-αD-mannopyranoside	-	-	-	-	-	-	-	-

Table 2 (cont.).

Methyl-αD-glucopyranoside	-	-	±d	-	-	-	-	-
N-Acetyl glucosamine	+	+	+	+	+	+	+	+
Amygdalin	+	+d	+d	+d	+	+	-	-
Arbutin	+	+	+	+	+	+	-	-
Esculin ferric citrate	+	+	+	+	+	+	-	-
Salicin	+	+	+	+	+	+	-	-
D-Cellobiose	+	+	+	+	+	+	-	-
D-Maltose	+	+	+	+	+	+	+	+
D-Lactose	+	-	-	-	+	+	-	-
D-Melibiose	+	-	-	-	-	-	-	-
D-Saccharose	+	+	+	+	+	+	+	+
D-Trehalose	+	+	+	+	+	+	-	-
Inulin	+	-	-	-	+	+	-	-
D-Mellezitose	-	+	+	+	+	+	-	-
D-Raffinose	+	-	-	-	+	+	-	-
Amidon (starch)	-	+	+	+	+	+	-	-
Glycogen	-	+	+	+	-	-	-	-
Xylitol	-	-	-	-	-	-	-	-
Gentiobiose	+	+d	+d	+d	+	+	-	-
D-Turanose	-	-	-	-	-	-	-	-
D-Lyxose	-	-	-	-	-	-	-	-
D-Tagatose	-	-	-	-	-	-	-	-
D-Fucose	-	-	-	-	-	-	-	-
L-Fucose	-	-	-	-	-	-	-	-
D-Arabitol	-	-	-	-	-	-	-	-
L-Arabitol	-	-	-	-	-	-	-	-
Potassium gluconate	-	-	-	-	+	±d	-	-
Potassium 2-ketogluconate	-	-	-	-	-	-	-	-
Potassium 5-ketogluconate	±d	±d	±d	±d	±d	±d	+d	+d

Table 3. Biochemical profiles of gram-positive rods isolated in December 2011 from *Epinephelus aeneus* broodstock and molecularly identified as *Carnobacterium* sp.

Test		*Test*	
D-amygdalin	+	Polymixin B resistance	+
Phosphatidylinositol-phospholipase C	-	D-galactose	+
D-xylose	-	D-ribose	+
Arginine dihydrolase 1	+	L-lactate alkalinization	-
β-galactosidase	-	Lactose	-
α-glucosidase	-	N-acetyl-D glucosamine	+
Ala-Phe-Pro arylamidase	-	D-maltose	+
Cyclodextrin	-	Bacitracin resistance	+
L-aspartate arylamidase	+	Novobiocin resistance	+
β-galactopyranosidase	-	Growth in 6.5% NaCl	+
α-amannosidase	-	D-mannitol	-
Phosphatase	-	D-mannose	+
Leucine arylamidase	+	Methyl-B-D-glucopyranoside	+
L-Proline arylamidase	-	Pullulan	-
r-β-glucuronidase	-	D-raffinose	-
α- galactosidase	-	0/129 resistance (comp. Vibrio)	+
L-pyrrolidonyl-arylamidase	+	Salicin	+
β-glucuronidase	-	Saccharose/sucrose	+
Alanine arylamidase	+	D-trehalose	+
Tyrosine arylamidase	+	Arginine dihydrolase 2	+
D-sorbitol	-	Optochin resistance	+
Urease	-		

16S rRNA gene sequence. Comparison of the partial sequencing of the 16S ribosomal RNA gene with isolates in the GenBank database confirmed the biochemical identifications. The 2009 isolate from broomtail wrasse *C. lunulatus* was compatible with the cultural and biochemical profile of *S. parauberis* and the sequencing of its 16S ribosomal RNA gene was identical to *S. parauberis* isolates deposited in the GenBank (accession no. JQ780604). The 2010 isolates from white grouper and mullet were compatible with the biochemical profile of *L. lactis*, although they differed in their ability (the former) or inability (the latter) to ferment L-arabinose

and D-mannitol. Both had a 16S sequence identical to *L. lactis* isolates deposited in the GenBank (accession no. JQ780605). The winter 2010-2011 isolates from the three wild Red Sea fish, *E. fasciatus*, *Parupeneus* sp. (prob. *rubescens*), and *S. rivulatus*, were compatible with the biochemical profile of *S. iniae* and had 16S sequences identical to *S. iniae* isolates deposited in the GenBank (accession no. JQ780607). Both 2010 and 2011 summer isolates from mullets were compatible with the biochemical profile of *S. agalactiae* and had 16S sequences identical to *S. agalactiae* isolates deposited in the GenBank (accession no. JQ780606). The gram-positive rods isolated in December 2011 from *E. aeneus* broodstock produced a biochemical profile somewhat similar to that of *L. garvieae*. However, partial sequencing of the 16S ribosomal RNA gene showed 100% similarity to a *Carnobacterium* sp. and our sequence was deposited in the GenBank with the accession no. JQ780608.

Antibiogram. The sensitivity of the isolates to antibiotics is given in Table 4.

Table 4. Resistance of fish bacterium to commonly used antibiotics in aquaculture (R = resistant; S = sensitive, MS = moderately sensitive).

		Fish species								
		Epinephelus aeneus	*Cheilinus lunulatus*	*Siganus rivulatus*	*Parupeneus* sp.	*Epinephelus fasciatus*	*Mugil cephalus*	*Epinephelus aeneus*	*Mugil cephalus*	*Mugil cephalus*
Sample no.		021211	250409	291210	251110	041110	020210	010210	080610	050611
Year of isolation		2011	2009	2010	2010	2010	2010	2010	2010	2011
Infected by...	*Carnobacterium* sp., strain *eilaticum*		*Streptococcus parauberis* strain *maris rubri*		*Streptococcus iniae* strain *maris rubri*		*Lactococcus lactis* strain *eilaticus*		*Streptococcus agalactiae* strain *eilaticus*	
Antibiotic	*Dose (µg)*									
Oxytetracycline	30	R	R	S	S	S	R	S	S	S
Chloramphenicol	30	S	S	S	S	S	S	S	S	S
Florfenicol	30	S	S	S	S	S	S	S	S	S
Baytril (enrofloxacin)	5	R	R	S	S	S	S	S	MS	MS
Doxycycline	30	R	R	S	S	S	R	S	S	S
Sulphamethoxazole/ trimethoprim	25	MS	MS	S	S	S	R	R	MS	R
Clindamycin	2	R	S	S	S	S	S	S	S	S

Discussion

Although *S. parauberis* is better known for causing bovine mastitis, it has been isolated from fish, including cultured turbot (*Scophthalmus maximus*) in Spain, where it seems to be endemic (Toranzo et al., 1994, 2005; Doménech et al., 1996), sea bass (*Dicentrarchus labrax*) in France (Michel et al., 2007), and olive flounder (*Paralichthys olivaceus*) in South Korea (Kim et al., 2006; Nho et al., 2009) and Japan (Kanai et al., 2009).

Lactococcus lactis (formerly *Streptococcus lactis*) is better known for its importance in the dairy industry where it is extensively used to produce cheese and fermented foodstuffs (Madigan and Martinko, 2005). Nevertheless, it is an opportunistic pathogen (Aguirre and Collins, 1993) and clinical infections are being reported with increasing frequency in both humans and animals (Facklam et al., 1990; Mannion and Rothburn, 1990; Facklam and Elliot, 1995; Goyache et al., 2001; Akhaddar et al., 2002; Antolín et al., 2004; Güz et al., 2006). Differing in their fermentative activity of at least two carbohydrates (L-arabinose, D-mannitol) and sensitivity to antibiotics (oxytetracycline, doxycycline), our two isolates are most likely not the same strain. Because of their high phenetic similarities to *L. garvieae*, aquatic isolates of *L. lactis* were generally misidentified as *L. garvieae*, with sensitivity to clindamycin being the only test that could differentiate between the sensitive *L. lactis* and the resistant *L. garvieae* (Elliott and

Facklam, 1996; Zlotkin et al., 1998a). This test, however, may not be universally discriminative, at least with regard to marine isolates (Colorni et al., 2003).

The host range of *L. garvieae* is not limited to fish. Clinical infections by *L. garvieae* have been reported in terrestrial animals as well as humans, suggesting a zoonotic potential of this species (Vendrell et al., 2006 and references therein; Foschino et al., 2008 and references therein), while there is potential risk for consumers from milk and cheese isolates (Fortina et al., 2007). However, comparison at the molecular level of *L. garvieae* isolates from dairy products and diseased fish exhibited a low genetic relatedness (Foschino et al., 2008).

Our isolates from grey mullet were fairly unreactive, non-hemolytic, and mannitol negative, matching the biochemical profile described by Eldar et al. (1994) for *Streptococcus difficile* (*agalactiae*). *Streptococcus agalactiae* is known to cause mastitis in cattle, camels, goats, and sheep, and neonatal septicemia and urogenital infections in dogs and cats (Karamy, 1990; Younan, 2002; Carter and Wise, 2004), although various degrees of genomic diversity appear to exist among isolates from these hosts (Evans et al., 2007). In Israel, it was first described in freshwater fish by Eldar et al. (1994) who considered it a new species and named it *S. difficile* (later corrected to *difficilis* by Euzéby 1998 and found to be indistinguishable from *S. agalactiae* by Vandamme et al., 1997). In marine animals, *S. agalactiae* was isolated in Kuwait from cultured gilt-head sea bream *Sparus aurata*, wild mullet *Liza klunzingeri* (Evans et al., 2002) and other feral species (Qasem et al., 2008), cultured silver pomfret *Pampus argenteus* (Duremdez et al., 2004), and a bottlenose dolphin *Tursiops truncatus* (Evans et al., 2006). A captive dolphin of the same species (*T. truncatus*) died in Italy of *S. agalactiae*-sustained necrotizing fasciitis and myositis (Zappulli et al., 2005). More recently, *S. agalactiae* was held responsible for an outbreak of septicemic infections in giant grouper *Epinephelus lanceolatus* and other marine fish species in Queensland, Australia (Bowater et al., 2012).

Identification of gram-positive chain-forming bacteria by traditional methods is complicated as phenotypic characteristics among species are often too similar to allow validation with a satisfactory degree of confidence. A multiplex PCR has been developed, and successfully recognized - from cultures and fish tissues of the four species of gram-positive chain-forming cocci most commonly associated with infections in marine fish: *S. iniae*, *S. parauberis*, *S. agalactiae*, *L. garvieae* (Mata et al., 2004; Toranzo et al., 2005). In addition to these common bacteria, we detected *L. lactis* displaying pathogenic properties, and a *Carnobacterium* sp. for the first time in marine fish in Israel. In our study, the *Carnobacterium* sp. produced a biochemical profile similar to that of *L. garvieae* (with only APPA and dMAN contraindicating the typical biopattern) when using the bioMérieux Vitek 2 system for gram-positive cocci and non-spore-forming bacilli, but its correct identification was obtained when the isolate was analyzed at the molecular level. Partial sequencing of the 16S ribosomal RNA gene turned out to be highly similar to that of *Carnobacterium divergens* isolated from salmon *Salmo salar* (Rudi et al., 2004). *Carnobacterium* is allegedly common in fish gastrointestinal tracts (Ringø et al., 2001), but its isolation from the kidney is unusual. In fact, to the best of our knowledge, this is the first report of a white grouper kidney infection by this bacterium.

The presence of *S. parauberis*, *S. agalactiae, L. lactis,* and *Carnobacterium* sp. (*eilaticum* = possibly *divergens*) in diseased wild and cultured fish in the northern Red Sea region within a relatively short time span (3-4 years) is ominous. These bacteria, isolated for the first time in marine fish in Israel, are to be added to other bacterial pathogens such as *Mycobacterium marinum*, *Photobacterium damselae* ssp. *piscicida*, *S. iniae*, and *L. garvieae,* detected in the last two decades in wild or cultured fish in the Gulf of Eilat (Colorni, 1992; Colorni et al., 2002, 2003; Colorni, unpubl. data). The ability of streptococci and mycobacteria in particular to survive in macrophages is a further indication of pathogenicity (Thulin et al., 2006; Jayachandran et al., 2007; Phelps and Neely, 2007). Additional cases of moribund or dead wild Red Sea fish were associated with various *Vibrio* spp. One case in 2003 in a parrotfish *Scarus ferrugineus*, due to a simultaneous infection by the enteric LAB *Enterococcus faecalis* and *Proteus mirabilis*,

was suspected to be the result of human or sewage contamination (Diamant et al., 2004). At the time, however, 16S rRNA analysis was not conducted and the possibility that *E. faecalis* was a misidentification of *S. parauberis* cannot be ruled out; Verner-Jeffreys et al. (2011) encountered the same problem with the latter species isolated from turbot and misidentified by a commercial miniaturized phenotypic testing system.

Survey of dead wild fish in the Gulf of Eilat has been limited to shallow waters along the Israeli coast to a depth of 7-8 m. Whether these are sporadic occurrences or a sign of severely altered microbial dynamics in the northern Red Sea fish populations remains to be established. However, the perception that prevalence of infections and diseases in feral fish is on the increase in the northern Gulf of Eilat has been strongly felt for a number of years by Israeli scientists working in various disciplines (Diamant et al., 2004). Our results further add to the suspicion that only the "tip of the iceberg" has been revealed, while the actual extent of bacterial infections and related mortalities among wild fish may be considerably more devastating. Disease outbreaks in the wild constitute a rising trend affecting marine organisms at a global level in areas subjected to intense anthropogenic impact (including aquaculture), where newly emerging pathogens and previously unexposed host populations are brought together (Diamant and Colorni, 1992; Diamant et al., 2004).

For convenience, the LAB strains isolated in recent years in our region were labeled *eilaticus*/*eilaticum* (from Eilat) or *maris rubri* (of the Red Sea). In fact, however, they may not be endemic to the Red Sea. While the biogeographical origin, reservoirs, and paths of infection remain elusive, the fact that bacteria generally associated with udder inflammation in livestock or production of cheese are able to infect fish underscores the urgent need for thorough epidemiological and molecular phylogenetic studies. In particular, the latter should aim at determining whether an extraordinary adaptability of this group of bacteria allows the same strains to occupy a wide spectrum of ecological niches or, as some investigators have demonstrated (Kvitt and Colorni, 2004; Evans et al., 2007; Foschino et al., 2008), intraspecific variations, often phenotypically undetectable, can differentiate between freshwater and marine strains, aquatic and terrestrial strains, or harmless environmental and pathogenic strains.

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