The Open Access Israeli Journal of Aquaculture – Bamidgeh

As from **January 2010** The Israeli Journal of Aquaculture - Bamidgeh (IJA) will be published exclusively as **an on-line Open Access (OA)** quarterly accessible by all AquacultureHub (http://www.aquaculturehub.org) members and registered individuals and institutions. Please visit our website (http://siamb.org.il) for free registration form, further information and instructions.

This transformation from a subscription printed version to an on-line OA journal, aims at supporting the concept that scientific peer-reviewed publications should be made available to all, including those with limited resources. The OA IJA does not enforce author or subscription fees and will endeavor to obtain alternative sources of income to support this policy for as long as possible.

Editor-in-Chief

Dan Mires

Editorial Board

Rina Chakrabarti	**Aqua Research Lab, Dept. of Zoology, University of Delhi, India**
Angelo Colorni	**National Center for Mariculture, IOLR Eilat, Israel**
Daniel Golani	**The Hebrew University of Jerusalem Jerusalem, Israel**
Hillel Gordin	**Kibbutz Yotveta, Arava, Israel**
Sheenan Harpaz	**Agricultural Research Organization Beit Dagan,**
Gideon Hulata	**Agricultural Research Organization Beit Dagan,**
George Wm. Kissil	**National Center for Mariculture, IOLR, Eilat, Israel**
Ingrid Lupatsch	**Swansea University, Singleton Park, Swansea, UK**
Spencer Malecha	**Dept. of Human Nutrition, Food & Animal Sciences, CTAHR, University of Hawaii**
Constantinos Mylonas	**Hellenic Center for Marine Research, Crete, Greece**
Amos Tandler	**National Center for Mariculture, IOLR Eilat, Israel**
Emilio Tibaldi	**Udine University Udine, Italy**
Jaap van Rijn	**Faculty of Agriculture, The Hebrew University of Jerusalem, Israel**
Zvi Yaron	**Dept. of Zoology, Tel Aviv University, Tel Aviv, Israel**

Copy Editor **Ellen Rosenberg**

Published under auspices of
The Society of Israeli Aquaculture and Marine Biotechnology (SIAMB),
University of Hawai'i at Mānoa Library
&
University of Hawai'i at Mānoa Aquaculture Program
in association with
AquacultureHub
http://www.aquaculturehub.org

ISSN 0792 - 156X

© Israeli Journal of Aquaculture - BAMIGDEH.

PUBLISHER:

Israeli Journal of Aquaculture - BAMIGDEH -
Kibbutz Ein Hamifratz, Mobile Post 25210,
ISRAEL
Phone: + 972 52 3965809
http://siamb.org.il

The *IJA* appears exclusively as a peer-reviewed on-line open-access journal at http://www.siamb.org.il. To read papers free of charge, please register online at registration form.
Sale of *IJA* papers is strictly forbidden.

Isolation of *Shewanella putrefaciens* from Goldfish *(Carassius auratus auratus)*

Soner Altun[1]*, Ayşe Gül Büyükekiz[1], Muhammed Duman[1], Musa Özgür Özyiğit[2], Süheyla Karataş[3], Emre Turgay[3]

[1] *Department of Aquatic Animal Disease, Faculty of Veterinary Medicine, Uludag University, 16059 Bursa, Turkey*

[2] *Department of Pathology, Faculty of Veterinary Medicine, Uludag University, 16059 Bursa, Turkey*

[3] *Department of Fish Disease, Faculty of Fisheries, Istanbul University, 34470 Istanbul, Turkey*

(Received 7.2.13, Accepted 3.4.13)

Key words: *Shewanella putrefaciens*, PCR, sequencing, *Carassius auratus auratus*

Abstract

In the present study, the etiological agent that caused mortality in goldfish at an aquarium company located in Bursa in northwestern Turkey was identified. Diseased fish were lethargic, displaying a loss of appetite, ascites, and exophthalmia. Internal examination revealed that the kidney was surrounded by exudate and that the spleen and liver were pale. Microbiological examination showed that the kidney, spleen, and liver were infected by gram-negative, cytochrome oxidase, catalase, and H_2S positive rod-shaped bacteria identified as *Shewanella putrefaciens*. Identification of the bacteria was confirmed by 16S rRNA gene sequencing. Histopathological examination of the kidney, spleen, and liver revealed necrosis in kidney tubules and the presence of hepatocytes and hemosiderin in melanomacrophages. According to the disk diffusion method, isolates were sensitive to enrofloxacin (5 µg), florfenicol (30 µg), gentamycin (120 µg), and sulfamehoxazole+ trimethoprim (25 µg).

* Corresponding author. Tel.: +90-224-294-1264, fax: +90-224-294-1202, e-mail: saltun@uludag.edu.tr

Introduction

The genus *Shewanella* is affiliated with the subclass Protobacteria within the family Vibrionaceae (Ziemke et al., 1998). The first description of a species eventually assigned to this genus was provided in 1931 by Derby and Hammer who isolated an unknown bacterial taxon from putrefied butter and water supplies of dairies and named it *Achromobacter putrefaciens*; in 1941, Long and Hammer proposed that this species be transferred to the genus *Pseudomonas* under the name *P. putrefaciens* (Holt et al., 2005). In 1985, phylogenetic studies resulted in the reclassification of this organism to the family Vibrionaceae, and the description of a new genus, *Shewanella* (MacDonell and Colwell, 1985).

The genus *Shewanella* comprises gram-negative, oxidative, and non-oxidative bacilli widely distributed in marine and freshwater environments. *Shewanella putrefaciens* is generally isolated from sea water samples and putrefied fish (Shewan, 1977; Jergensen and Huss, 1989; Stenstram and Molin, 1990). Beside aquatic reservoirs (sea water, fresh water, sewage), other isolation sources include natural energy reservoirs (oil and gas), poultry, meat, and milk products (Levin, 1972; Semple et al., 1989; Stenstram and Molin, 1990; Vogel et al., 1997). Characteristic phenotypic attributes are the production of hydrogen sulfide gas on triple sugar iron (TSI) slants (Weyant et al., 1995; Ziemke et al., 1998) and good growth on conventional solid media, including MacConkey agar where the bacteria produce 1-2 mm yellowish-brown colonies after incubation for 18-24 h (Holt et al., 2005). In addition, *S. putrefaciens* produces acid from maltose and glucose and sometimes from sucrose and arabinose (Holt et al., 2005). Bacteria of the genus *Shewanella* are important in the turnover of organic material and capable of dissimilatory reduction of various metals and other substances such as nitrate, nitrite, thiosulphate, and trimethylamine-N-oxide (Holt et al., 2005).

The first isolation of *S. putrefaciens* from fish, more specifically from cultured rabbit fish (*Siganus rivulatus*), was achieved in 1987 by Saeed et al. (1990). Since then, the bacterium was isolated from common carp (*Cyprinus carpio* L.) and rainbow trout (*Oncorhynchus mykiss*) in Poland (Kozinska and Pekala, 2004) and from European sea bass (*Dicentrarchus labrax*) in Turkey (Korun et al., 2009).

The aim of the present study was to identify the causative agent of infection in five adult goldfish *(Carassius auratus auratus)* that were referred to the Aquatic Animal Diseases Department in Bursa, Turkey, after displaying a swollen abdomen, bilateral exophthalmia, abnormal swimming, loss of appetite, and stagnancy. Conventional tests, API 20 E rapid diagnostic kits, and VITEK II test kits were used to identify the cause of infection. The isolation of *S. putrefaciens* from goldfish shows the zoonotic ability of the bacterium.

Materials and Methods

Sampling and identification. In March 2012, five diseased and moribund adult goldfish (10-15 g) were transferred alive to our laboratory from an aquarium located in Bursa in northwestern Turkey and euthanized by an overdose of Quinaldine. Liver, spleen, and kidney tissue samples were streaked on tryptic soy agar (TSA), blood agar, and MacConkey agar and incubated 22°C for 48 h for bacteriologic isolation. The predominant colonies that grew on the plates were purified and identified by classic bacterial tests (Gram staining, motility, oxidation/fermentation, oxidase, catalase test, etc.). In addition, a commercial miniaturized API 20 E rapid test kit (bioMérieux, France) was used according to the manufacturer's instructions but with a modified incubation temperature (26°C for 24 h) and checked in APIweb, and VITEK II (bioMérieux, France) was used with a Gr(-) id card for 24 h incubation at 37°C (Whitman, 2004; Austin and Austin, 2007).

The identity of the isolated bacterium was confirmed by 16S rRNA gene sequencing (Suau et al., 1999; Zhang et al., 2000; Hall, 2004; CLSI, 2010). Proliferation in trypticase soy broth (TSB) with 0, 3%, 5%, 7%, or 9% NaCl and on thiosulfate citrate bile sucrose (TCBS) agar as well as hemolysis on sheep blood agar and sensitivity to 10 mg vibriostatic agent results were checked. FeS production on TSI agar medium (Difco)

was determined after incubation at 22°C for 48 h. The isolates were also incubated on TSA medium for 5 days to check their ability to proliferate at 5°C, 22°C, 37°C, and 42°C.

Genomic DNA extraction. The isolated strain was inoculated into nutrient broth and incubated overnight at 22°C in a shaking incubator. Genomic DNA was extracted with a High Pure PCR Template Preparation Kit (Roche Applied Science) according to the manufacturer's directions and used as the template for PCR.

PCR and 16S rRNA gene sequencing. Genomic target DNA was amplified using the 16S rRNA gene universal bacteria-specific primer set: primer S-20 - 5′ AGA GTT TGA TCC TGG CTC AG 3′ and primer A-18 - 5′ GWA TTA CCG CGG CKG CTG 3′ (Suau et al., 1999). The PCR mixture (50 µl) included approximately 100 ng template DNA (2 µl), 10 pmol of each primer (2 x 2 µl), 2X PCR Master Mix (Fermentas; 25 µl), and nuclease-free water (Fermentas; 19 µl). Amplification was performed using a thermal cycler (Biometra) with the following parameters: initial denaturation at 95°C for 3 min, followed by 30 cycles of amplification (denaturation at 95°C for 30 s, primer annealing at 56°C for 1 min, extension at 72°C for 1 min), and a final extension step of 72°C for 4 min. After amplification, 10 µl of the PCR sample was loaded on a 1.5% (wt/vol) agarose gel in Tris-acetate-EDTA containing ethidium bromide (0.5 ug/ml) and electrophoresis was performed for 40 min at 90 V. PCR products were visualized on a UV transilluminator and size was estimated against the GeneRuler 100 bp DNA Ladder (Fermentas SM0241). PCR products were cleaned and sequenced by BM Labosis (Ankara, Turkey) using the given primers. Sequence editing and analysis was performed using Bioedit v7.0.0 (Hall, 2004) and BLASTN 2.2.20 algorithm (Zhang et al., 2000).

Histopathological examination. Spleen, kidney, and liver tissue samples were taken during necropsy. The samples were fixed in a 10% buffered formalin solution for 24 h and further processed following standard techniques. The tissue samples were embedded in paraffin wax, cut into 5-µm thick sections, mounted on slides, stained with hematoxylin-eosin (H&E) and Brown & Brenn stains for bacteria, and examined under a light microscope.

Determination of antimicrobial sensitivity. The bacteria isolates were tested for antimicrobial susceptibility by the disc diffusion method on Mueller-Hinton agar and incubated at 22ºC for 24 h. The tested antibiotics (Oxoid) included amoxicillin (25 µg), doxycycline (30 µg), enrofloxacin (5 µg), erythromycin (15 µg), florfenicol (30 µg), gentamycin (120 µg), lincomycin (2 µg), neomycin (10 µg), oxytetracycline (30 µg), and sulfamehoxazole+trimethoprim (25 µg). Incubation zone diameters were measured after 24 and 48 h of incubation and classified as sensitive (S) or resistant (R) according to the size of the zone of bacteria growth inhibition according to the Clinical and Laboratory Standards Institute (CLSI, 2010) In addition, ATB VET (bioMérieux) test was carried out to determine sensitivity of the bacteria to different antibiotic agents.

Results

Clinical and autopsy findings. The diseased fish displayed stagnancy, abnormal swimming, lack of appetite, swelling of the abdomen, and bilateral exophthalmia (Fig. 1). Internal examination revealed a pale liver as well as ascites and enlargement of the spleen. The kidney was difficult to identify due to exudate. Pure culture colonies were opaque, 1-2 mm in diameter, smooth, slightly convex, and had a pinkish color with a bright surface on TSA plates after 48 h at 22°C. Bacteria appeared as gram-negative rods after Gram staining, were cytochrome oxidase and catalase positive, and were motile according to the hanging drop motility test (Table 1). The oxidation-fermentation test revealed that the bacteria were nonoxidative. No hemolysis was seen on sheep blood agar. The isolate was resistant to 10 mg/ml vibriostatic agent and grew in TSB with 0%, 3%, 5%, or 7% NaCl but not 9% NaCl or on TCBS agar.

API 20E rapid test and VITEK II GR(-) id card. In addition to conventional tests, API 20 E and VITEK II tests identified the isolate as *S. putrefaciens* with 98.3% and 94% probability, respectively (Table 1).

Fig. 1. Asymmetry and ascites in goldfish caused by *Shewanella putrefaciens.*

Gene sequencing. Our partial 16S rRNA gene sequence was identical to *S. putrefaciens* strain HAC411 (DQ307731) and showed 99% similarity to *S. putrefaciens* strain N22 (AB182185), isolated from the intestinal tract of carp, *Cyprinus carpio* (Namba et al., 2007). The isolated strain was named UAAD7 and the corresponding partial 16S rRNA gene sequence was deposited in the GenBank database under accession number JX518487.

Histopathological findings. Histological examination of the goldfish naturally infected with *S. putrefaciens* indicated depletion of white and red pulpas and deposition of hemosiderin in melanomacrophage centers in the spleen (Fig. 2). The kidney was severely affected, displaying necrosis in renal tubules (Fig. 3). In addition, the kidney was swollen and

Table 1. Phenotypic characterization and API 20E test results of *Shewanella putrefaciens* isolated from goldfish and compared with earlier studies.

Phenotypic character	*Our Shewanella putrefaciens*	*Korun et al. (2009) isolate*	*Saeed et al. (1987) isolate*	*Kozinska and Pekala (2004) isolate*
Gram staining	-	-	-	-
Cytochrome oxidase	+	+	+	99**
Catalase	+	+	nd	nd
Oxidation/fermentation	-/-	-/-	-/-	-/-
Mobility	+	+	+	+
Growth on MacConkey agar	+	nd	nd	+
Hemolysis on blood agar	-	+	nd	nd
Growth on TCBS agar	-	+	nd	nd
O/129 (10 µg/disc)	-	+	nd	nd
Growth on TSB agar in...				
0% NaCl	+	+	-	+
3% NaCl	+	+	nd	nd
5% NaCl	+	+	+	+
7% NaCl	+	+	+	60**
9% NaCl	-	nd	+	60(w)**
5°C	+	+	nd	+
Growth on TSA agar at...				
22°C	+	+	nd	nd
37°C	+	+	+	+
42°C	-	nd	+	-
FeS production on TSI agar	+	nd	nd	nd
API 20E test results				
Beta galactosidase	-	-	-	-
Arginin dihidrolaz	-	-	-	-
Lysine decarboksilase	-	-	-	-
Ornithine decarboxylase	-	+	+	80**
Citrate utilization	+	+	+	75**
H_2S utilization	+	+	+	75**
Urease utilization	-	-	-	1**
Tryptophan deaminase	-	nd	-	-
Indole utilization	-	-	-	-
Voges Proskauer	-	-	-	-
Gelatin hydrolysis	-	+	+	75**
Glucose*	+	-	-	1**
Mannitol*	-	-	-	-
Inositol*	-	-	-	-
Sorbitol*	-	nd	-	-
Ramnose*	-	nd	-	-
Sucrose*	-	-	-	1**
Melibiose*	-	nd	-	-
Amygdalin*	-	nd	-	-
Arabinose*	-	-	-	2**
NO_2	+	+	+	96**
N_2	-	nd	nd	nd

* acid utilization from carbohydrates
** percent of positive isolates/strains
nd = no data
w = weak growth

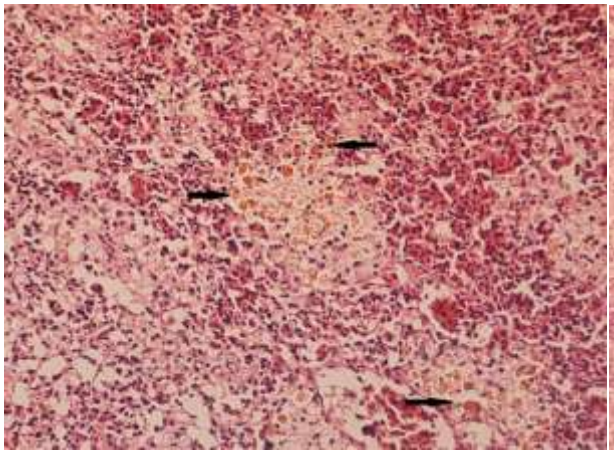

Fig. 2. Hemosiderin in melanomacrophages in the center of the spleen (arrows) of goldfish infected by *Shewanella putrefaciens*, H&E stain.

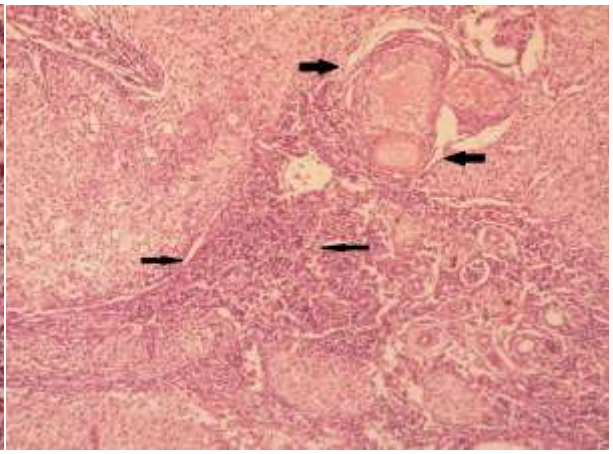

Fig. 3. Necrotic areas (thick arrows) and mononuclear cell infiltrations (thin arrows) in the kidney of goldfish infected by *Shewanella putrefaciens*, H&E stain.

there was exudate on the cut surface. There were mononuclear cells and connective tissue around the necrotic areas on the kidney (Fig. 4). Similar areas were observed on liver samples.

Antimicrobial sensitivity. According to the disk diffusion method, the isolated strain was resistant to amoxicillin, doxycycline, lincomycin, and neomycin, but sensitive to enrofloxacin, florfenicol, gentamycin, and sulfamehoxazole+ trimethoprim. Results of the ATB VET test are given in Table 2.

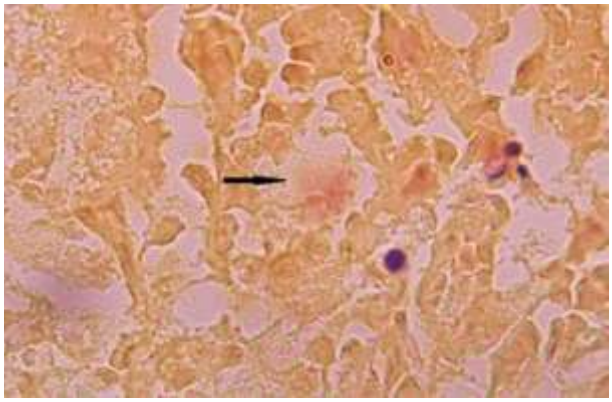

Fig. 4. *Shewanella putrefaciens* in the kidney tissue of goldfish, B&B stain.

Table 2. Antibiotic sensitivity results of *Shewanella putrefaciens* using ATB VET (bioMérieux, France).

Antimicrobial drug	*mg/l*	*Result*	*Antimicrobial drug*	*(mg/l*	*Result*
Amoxicillin	4	R	Kanamycine	8	R
Amoxicillin/ clavulanic acid	4/2	R	Lincomycine	2	R
Apramycine	16	S	Metronidazole	4	R
Cefoperazone	4	S	Nitrofurantoine	25	S
Cefalotine	8	R	Oxacilline	2	R
Chloramphenicol	8	S	Oxolinic acid	2	S
Colistine	4	S	Penicillin	0.25	R
Cotrimoxazole	2/38*	S	Pristinamycine	2	R
Doxycycline	4	R	Rifampicine	4	S
Enrofloxacin	0.5	S	Spectinomycine	64	R
Erythromycine	1	R	Streptomycine	8	R
Flumequine	4	S	Sulfamethizole	100	R
Fucidic acid	2	R	Tetracycline	4	R
Gentamycin	4	S	Tylosine	2	R

R = resistant, S = sensitive
* 2 mg trimethoprim and 38 mg sulfamethoxazole/l

Discussion

Shewanella putrefaciens is an opportunistic bacterial pathogen that can cause disease in fish under stressful conditions (Kozinska and Pekala, 2004). Human infections by members of the genus *Shewanella* are rare, and have been reported mainly from warm climatic areas in the USA, Australia, Asia, South Africa, and southern Europe or during especially warm summers in temperate climates such as that of Denmark (Holt et al., 2005). Immunosuppressive people with pre-existing cutaneous ulcers should be especially careful of Shewanellosis after exposure to sea water or a marine environment (Tsai et al., 2008). As *S. putrefaciens* has zoonotic potential, any direct contact between humans and infected aquatic animals such as the infected goldfish in the present study is

risky (Chen et al., 1997).

Shewanella algae has been misidentified as *S. putrefaciens* as a result of the failure of conventional phenotypic characteristic testing and commercial bacteria identification systems such as ID 32 GN and VITEK to differentiate *S. putrefaciens* from *S. algae* (Tsai et al., 2008). Therefore, we used sequencing to confirm the identity of our conventionally-isolated isolate. Our isolate was correctly identified by test systems and our partial 16S rRNA gene sequence was identical to an *S. putrefaciens* entry in the EMBL-bank but had only 91.3% sequence similarity (excluding the missing bp's) to the *S. algae* type strain LMG 18393 (accession no. AF005249).

While our *S. putrefaciens* isolate showed homogeneity with earlier studies (Saeed et al., 1990; Kozinska and Pekala, 2004; Korun et al., 2009), API 20E and conventional tests found differences in hemolysis on blood agar, growth on TCBS agar, O/129 (10 µg/disc), 9% NaCl, 42°C, ornithine decarboxylase, gelatin hydrolysis, and acid production from glucose, indicating that there may be variation between *S. putrefaciens* strains regarding decarboxylase, gelatin, and glucose metabolism. The *S. putrefaciens* isolate isolated from freshwater fish by Kozinska and Pekala (2004) showed negative hemolysis, good growth in TSB without NaCl, and weak growth in TSB with 9% NaCl, similar to our results. However, *S. putrefaciens* isolated from diseased rabbit fish did not grow in TSB with 1% NaCl but did grow in TSB with 9% NaCl (Saeed et al., 1990). Taken together, these differences suggest that some strains may have adapted to fresh water. Our isolated bacterium grew at 5°C, 22°C, and 37°C but not at 42°C, consistent with the findings of Kozinska and Pekala (2004) but different from those of Saeed et al. (1990), indicating that *S. putrefaciens* isolated from freshwater fish and those isolated from marine fish may have different phenotypes.

Inappetence, skin ulcers, darkening of the skin, exophthalmia, and ascites were reported in outbreaks of *S. putrefaciens* (Saeed et al., 1990; Kozinska and Pekala, 2004; Korun et al., 2009). In our study, hemosiderin was deposited in melanomacrophages on red and white pulpas of the spleen and there were mononuclear cells around necrosis on liver and renal tubules of diseased goldfish. Our findings agree with other histopathological examinations related to infection with this bacterium in different fish (Saeed et al., 1990; Kozinska and Pekala, 2004; Korun et al., 2009). Our *S. putrefaciens* isolate was sensitive to enrofloxacin, florfenicol, oxolinic acid, and oxytetracycline, frequently used antibiotics in aquaculture. These results are very similar to antibiogram findings of Kozinska and Pekala (2004) - sensitivity to enrofloxacin, flumequine, gentamycin, and oxytetracycline. However, while our isolate is resistant to erythromycin and kanamycin, the isolate of Korun et al. (2009) is sensitive to these antibiotics.

In conclusion, the histopathological changes and antibiotic susceptibility of our *S. putrefaciens* isolate are in accordance with previous reports but results of some biochemical tests indicate a variation between *S. putrefaciens* strains. The identification of *S. putrefaciens* in ornamental fish is important due to the bacterium's zoonotic potential. Thus, the pathogenesis of *S. putrefaciens* and factors affecting the transmission of Shewanellosis infection should be of interest and further studied.

References

Austin B. and D.A. Austin, 2007. *Bacterial Fish Pathogens, Diseases of Farmed and Wild Fish*, 4th ed. Springer-Praxis, Godalming, England. 552 pp.

Chen Y.S., Liu Y.C., Yen M.Y., Wang J.H., Wang J.H. and S.R. Wann, 1997. Skin and soft-tissue manifestations of *Shewanella putrefaciens* infection. *Clin. Infect. Dis.*, 25:225-229**.**

CLSI, 2010. *Performance Standards for Antimicrobial Susceptibility Testing, 20th Informational Supplement*. Clinical and Laboratory Standards Institute, M100-S20 & M100-S-20-U.

Hall T., 2004. *BioEdit version 7.0.0*. Distributed by the author, website: www.mbio.ncsu.edu/BioEdit/bioedit.html.

Holt H.M., Gahrn-Hansen B. and B. Bruun, 2005. *Shewanella algae* and *Shewanella putrefaciens*: clinical and microbiological characteristics. *Clin. Microbiol. Infect.*, 11:347-352.
Jergensen B.R. and H.H. Huss, 1989. Growth and activity of *Shewanella putrefaciens* isolated from spoiling fish. *Int. J. Food Microbiol.,* 5:1-62.
Korun J., Akgün-Dar K. and M. Yazıcı, 2009. Isolation of *Shewanella putrefaciens* from cultured European sea bass, (*Dicentrarchus labrax*) in Turkey. *Revue Méd. Vét.,* 160, 11:532-536.
Kozinska A. and A. Pekala, 2004. First isolation of *Shewanella putrefaciens* from freshwater fish - a potential new pathogen of fish. *Bull. Eur. Ass. Fish Pathol.,* 24(4):189.
Levin R.E., 1972. Correlation of DNA base composition and metabolism of *Pseudomonas putrefaciens* isolates from food, human clinical specimens, and other sources. *Antonie Van Leeuwenhoek,* 38(2):121-127.
MacDonell M.T. and R.R. Colwell, 1985. Phylogeny of the Vibrionaceae, and recommendation for two new genera, *Listonella* and *Shewanella*. *Syst. Appl. Microbiol.*, 6:171-182.
Namba A., Mano N. and H. Hirose, 2007. Phylogenetic analysis of intestinal bacteria and their adhesive capability in relation to the intestinal mucus of carp. *J. Appl. Microbiol.,* 102(5):1307-1317.
Saeed M.O., Alamoudi M.M. and A.H. Al-Harbi, 1990. Histopathology of *Pseudomonas putrefaciens* associated with disease in cultured rabbitfish, *Siganus rivulatus. J. Fish Dis.*, 13:417-422.
Semple K.M., Doran J.L. and D.S. Westlake, 1989. DNA relatedness of oil-field isolates of *Shewanella putrefaciens*. *Can. J. Microbiol*., 35:925-931.
Shewan J.M., 1977. The bacteriology of fresh and spoiling fish and the biochemical changes induced by bacterial action. pp. 51-66. In: *Proc. Conf. Handling, Processing and Marketing of Tropical Fish*. Tropical Products Institute, London.
Stenstram J.M. and G. Molin, 1990. Classification of the spoilage flora of fish, with special reference to *Shewanella putrefaciens. J. Appl. Bacteriol.*, 68:601-618.
Suau A., Bonnet R., Sutren M., Godon J.J., Gibson G., Collins M.D. and J. Dore, 1999. Direct analysis of genes encoding 16S rRNA from complex communities reveals many novel molecular species within the human gut. *Appl. Environ. Microbiol*., 65:4799-4807.
Tsai M.S., You H.L., Tang Y.F. and J.W. Liu, 2008. *Shewanella* soft tissue infection: case report and literature review. *Int. J. Infect. Dis.*, 12(6):119-124.
Vogel B.F., Jørgensen K., Christensen H., Olsen J.E. and L. Gram, 1997. Differentiation of *Shewanella putrefaciens* and *Shewanella alga* on the basis of whole-cell protein profiles, ribotyping, phenotypic characterization, and 16S rRNA gene sequence analysis. *Appl. Environ. Microbiol*., 63:2189-2199.
Weyant R.S., Moss C.W., Weaver R.E., Hollis D.G., Jordan J.G., Cook E.C. and M.I. Daneshvr, 1995. *Identification of Unusual Pathogenic Gram-Negative Aerobic and Facultative Anaerobic Bacteria*, 2nd ed. Williams & Wilkins, Baltimore, Md.
Whitman K.A., 2004. *Finfish and Shellfish Bacteriology Manual: Techniques and Procedures.* Iowa State Press. Ames, Iowa. pp. 107-155.
Zhang Z., Schwartz S., Wagner L. and W. Miller, 2000. A greedy algorithm for aligning DNA sequences. *J. Comp. Biol*., 7:203-214.
Ziemke F., Hofle M.G., Lalucat J. and R. Rossello-Mora, 1998. Reclassification of *Shewanella putrefaciens* Owen's genomic group II as *Shewanella baltica* sp. nov. *Int. J. Syst. Bacteriol.*, 48:179-186.