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SHORT COMMUNICATION

CLINICAL AND PATHOLOGICAL INVESTIGATION OF *PSYCHROBACTER IMMOBILIS* INFECTION IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*, WALBAUM)

Olca Hisar, Telat Yanik* and Sükriye Aras Hisar

Fisheries Department, Agricultural Faculty, Atatürk University, Erzurum 25240, Turkey

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Key words: bacteria, pathology, *Psychrobacter immobilis* infection, rainbow trout

Abstract

The pathogenicity of *Psychrobacter immobilis*, isolated from naturally infected rainbow trout *Oncorhynchus mykiss*, was investigated. In experimentally-infected trout, no mortality was recorded during 35 days but the following external and internal symptoms were observed. Externally, skin was darkened, gills were pale and swimming was abnormal. Internally, there were dilatations of the vascular structures on cross-sections of the liver; vascular congestion, inflammatory mononuclear cell infiltration and degeneration in the gills; interstitial inflammatory mononuclear cell infiltration and free bleeding in patches of the posterior kidney; vascular congestion and free bleeding in the spleen; degeneration, atrophy, polymorphonuclear leukocyte infiltration and liquefaction necrosis in muscle fibers; edema, inflammatory mononuclear cell infiltration and basophilic degeneration among muscle fibers of the heart; and congestion in vascular structures on cross-sections of the brain tissue. It is suggested that *P. immobilis* is an opportunist pathogen that causes secondary infections.

Introduction

In the occurrence of a disease, many factors such as pollution, pathogens, genetic disorders, physical injuries, malnutrition and the synergy between them play an important role (Kinne, 1980). A wide variety of diseases

caused by pathogenic bacteria at different growing stages in fish production have been reported, e.g., *Escherichia vulneris* in rainbow trout (Aydın *et al.*, 1997), *Psychrobacter glancincola* in Antarctic sea ice (Bowman *et*

* Corresponding author. E-mail: talatyanik@yahoo.com

al., 1997), *P. immobilis* in wild *Salmo trutta* and *Esox lucius* in farmed *Oncorhynchus mykiss* (Gonzalez et al., 2000).

P. immobilis can be isolated from fresh water and freshwater fish, mainly after post mortem storage (Gonzales et al., 2000). Most strains of *P. immobilis* associated with fish and processed meat and poultry products are characterized as gram negative, non-motile, aerobic and lipolytic (Holt et al., 1994; Gennari et al., 1999). *P. immobilis* infects humans, causing physical damage such as traumatic wounds or by opportunistic colonization and infection of compromised hosts. It can cause meningitis and ocular infections in infants (Gini, 1990; Lloyd et al., 1991; Lozano et al., 1994).

Many bacterial genera have been described as pathogens of freshwater and/or marine fish. These include *Aeromonas*, *Flavobacterium*, *Acinetobacter* and *Pseudomonas* (Austin and Austin, 1999; Daly, 1999). Psychrotrophic bacteria in foods (Pacova and Urbanova, 1998; Pacova et al., 2001), in Antarctic krill (*Euphausia superba* Dana; Denner et al., 2001), in mussels (Vásquez et al., 2002) and in marine environments (Romanenko et al., 2002) were also studied. However, the pathogenicity of *P. immobilis* in salmonids has not been reported. The present study reports on the pathogenicity of *P. immobilis* isolated from naturally infected rainbow trout and pathological changes observed in experimentally infected trout.

Material and Methods

Year-old rainbow trout (mean avg wt 150±15 g) which were reared in 8.5°C well water on a farm at the Research and Extension Center, Atatürk University, were transferred to the Central Laboratory in the Aquarium Fish Rearing Facility in June 1999. Fish were kept in three 785-l circular fiberglass tanks (100 x 100 cm) in natural light conditions with a constant water flow of 1.5 l/min of aerated dechlorinated tap water and a temperature of 9-11°C. Dissolved oxygen was 8-9 ppm, pH was 7.8 and total hardness was 102 mg as CaCO₃. The fish were acclimated in these conditions for two weeks.

P. immobilis inoculum. Naturally infected fish were slaughtered in sterile conditions. Inoculums were taken from the kidney, liver and abdominal cavity of the fish and used to isolate the bacteria. The inoculums were streaked on a blood agar base (Tryptone soya agar-Oxoid + 5% sterile sheep blood) at 25°C for 24-72 h. After that, individual colonies were enriched in Tryptone soya (TS) broth (Oxoid) at 25°C for 24-72 h, followed by re-streaking on TS agar.

Experimentally infected fish. Eight fish were stocked in each tank (a total of 24 fish). The fish in two tanks were injected intramuscularly with 0.5 ml of a suspension containing *P. immobilis* bacteria (approximately 10⁶ cell/ml) at the base of the dorsal fin according to the McFarland-1 standard (Plumb, 1981). Fish in the third tank served as the control and were injected with 0.5 ml physiological saline.

Clinical and histopathological examination. During the incubation period, the symptoms of sick fish and the gross external and internal clinical symptoms of sacrificed fish were studied. Tissues from experimentally infected fish were excised and fixed in a 10% formalin solution. After these tissues were embedded in paraffin wax, the paraffin blocks were cut into 5 µm-thick cross-sections which were then stained with hematoxylin-eosin (H&E; Collins, 1993). The hepatosomatic (HSI) and splenosomatic (SSI) indices were calculated for the sacrificed fish (Shimma et al., 1982).

Identification of bacteria. At the end of the bacteriological examinations, *P. immobilis* was re-isolated from lesion areas and from the kidney. Gram painting, motility, catalase and oxidase tests were carried out to determine the characteristics of the pathogenic *P. immobilis* strains (Ekingen, 1980). Finally, the MIDI-Sherlock® Microbial Identification system was used to identify the *P. immobilis* (Brown and Leff, 1996).

Results

No mortality was recorded during the 35-day experimental period. Externally, abnormal swimming, darkened skin, anorexia and hemorrhages on the skin were observed in infected

fish. Slightly hyperemic gills, hemorrhages on the ends of gill lamellas and enlargement of the spleen in the liver with extremely pale and hemorrhaged points were observed in autopsies. There were hemorrhages and lesions (2 x 3 cm) in muscle tissues where the fish were injected and edema on the body surface between the dorsal fin and the back of the head.

Average HSI and SSI were calculated as 2.97 ± 0.03 and 0.45 ± 0.03 , respectively, in infected fish. These values were higher than the normal values (HSI = 1.22, SSI = 0.13) reported by Shimma *et al.* (1982). The reason for this may be the occurrences of hepatomegaly and splenomegaly in the present study.

Vascular congestion, inflammatory mononuclear cell infiltration and degeneration in gill cartilage were seen in infected fish (Fig. 1) and may indicate *P. immobilis* infection. No such pathologic findings in cross-sections of healthy fish were observed.

No histopathological differences in the cross-sections of anterior kidney tissue of infected fish were observed. However, interstitial inflammatory mononuclear cell infiltration, atrophied glomeruli and free bleeding in patches of the posterior kidney were present. The appearance of inflammatory cell infiltration indicated the presence of infection. The free bleeding in patches of the kidney, however, seemed to account for the atrophied glomeruli (Fig. 2).

Vascular congestion and free bleeding were observed in the spleen. The absence of congestion in histopathological cross-sections of the spleen accounts for splenomegaly in autopsy findings (Fig. 3).

Some dilatations were observed in the vascular structures in cross-sections of the liver of infected fish. The structure of the bile ducts was normal. The histopathological findings were hemorrhage in the cross-sections and enlarged pale livers in autopsies (Fig. 4).

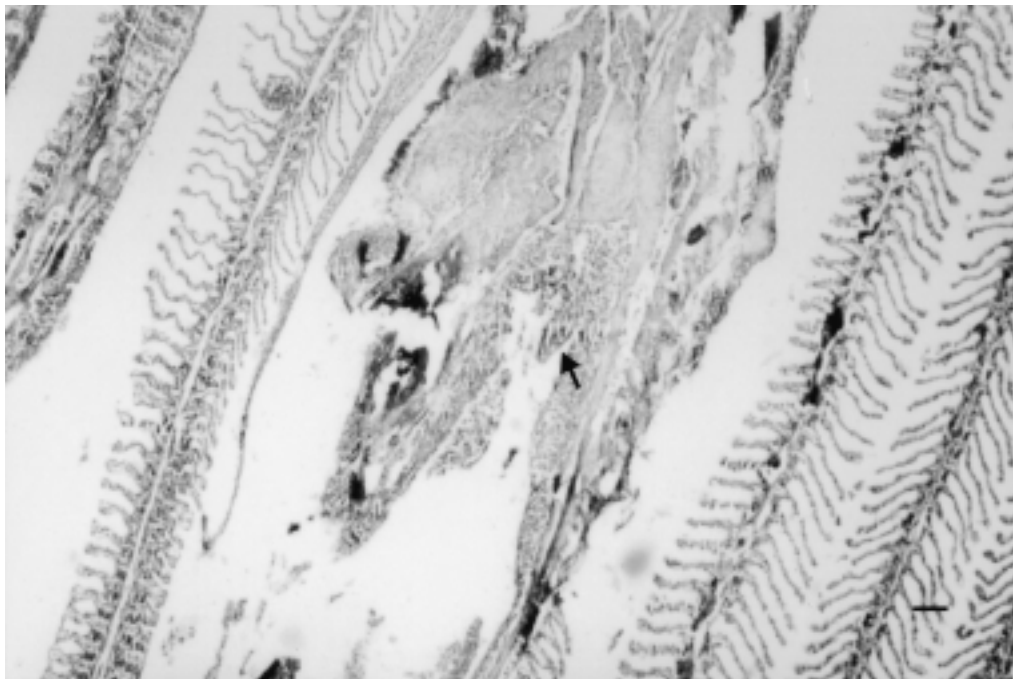


Fig. 1. Pathological changes in the gill of rainbow trout caused by *P. immobilis* infection 35 days after inoculation. Arrow shows inflammatory mononuclear cell infiltration (H&E x 40). Bar = 80 μ m.

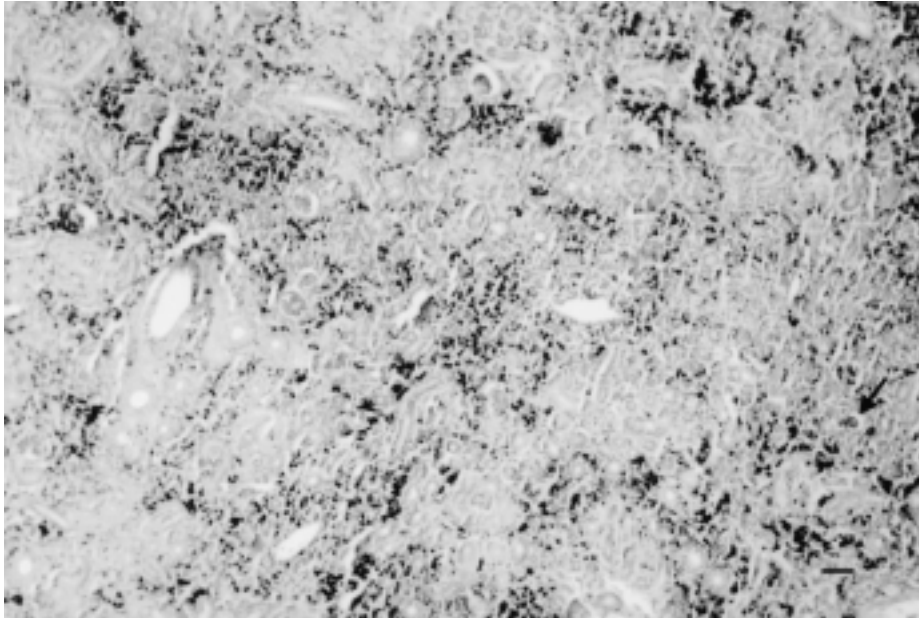


Fig 2. Pathological changes in the posterior kidney of rainbow trout caused by *P. immobilis* infection 35 days after inoculation. Arrow shows an atrophied glomerulus (H&E x 40). Bar = 80 μ m.

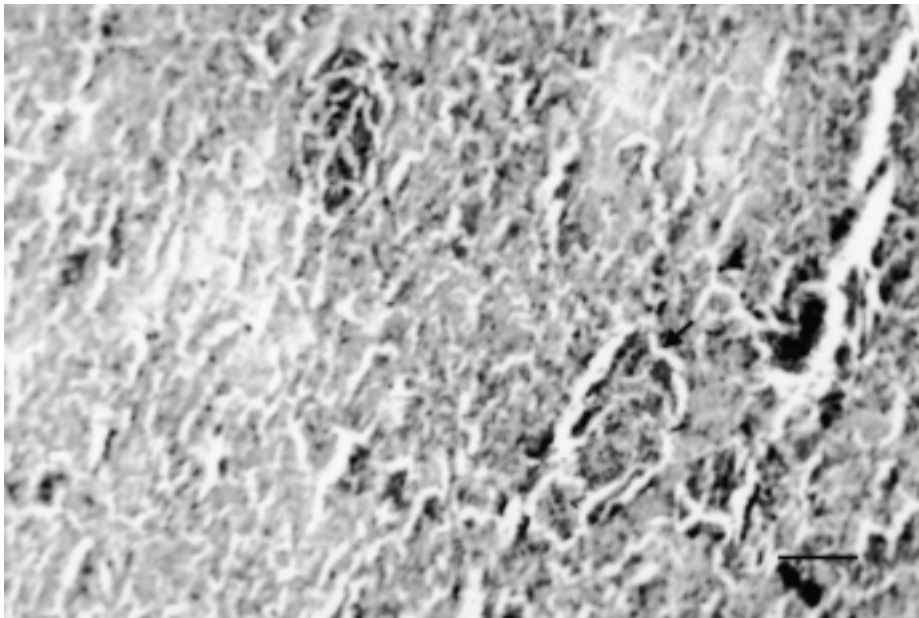


Fig. 3. Pathological changes in the spleen of rainbow trout caused by *P. immobilis* infection 35 days after inoculation. Arrow shows vascular congestion (H&E x 100). Bar = 80 μ m.

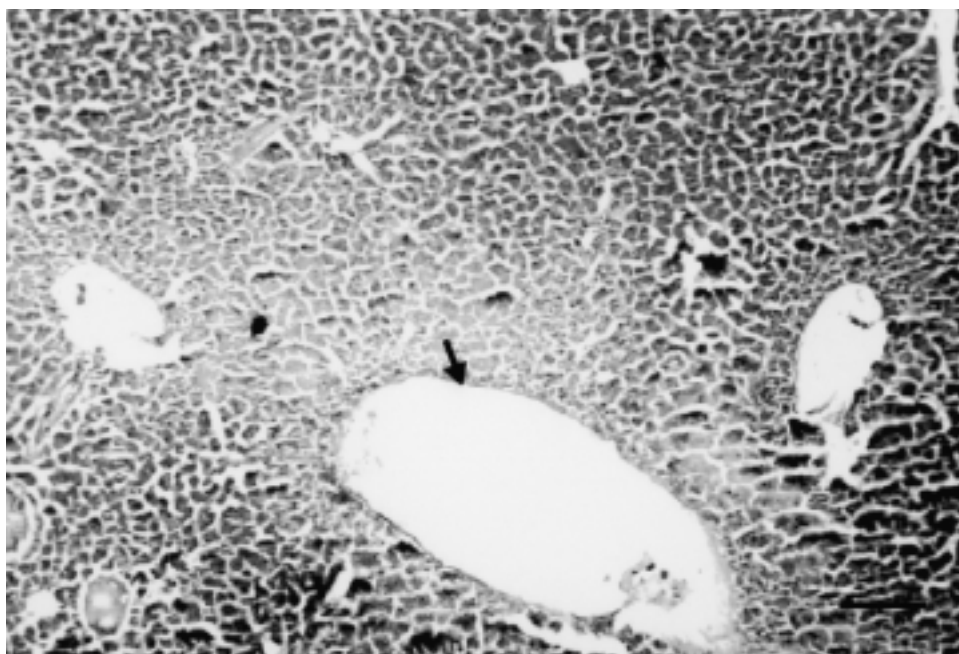


Fig. 4. Pathological changes in the liver of rainbow trout caused by *P. immobilis* infection 35 days after inoculation. Arrow shows vascular dilatation (H&E x 100). Bar = 80 μ m.

Degeneration, atrophy, PNL infiltration and liquefaction necrosis were present in muscle fibers. There were edema at the injection point and lesions and hemorrhage in the muscle (Fig. 5).

Edema, inflammatory mononuclear cell infiltration and basophilic degeneration were observed in muscle fibers of the heart (Fig. 6).

Congestion in vascular structures in cross-sections of brain tissue was observed. On the other hand, no histopathological findings were seen in the eyes. Infection from many diseases can be distinguished by the presence of congestion in the vascular structures of brain tissue (Fig. 7).

Discussion

Although *P. immobilis* has been isolated and identified in many experiments, little is known about its pathogenicity in fish. A total of 1500 strains, consisting mainly of *Pseudomonas*, *Flavobacterium*, *Psychrobacter*, *Acinetobacter* and *Shewanella*, were isolated from the skin

and gills of Adriatic sardines (fresh ice-stored for four days and spoiled for eight), but no information was recorded about the pathogenicity of the bacteria (Gennari *et al.*, 1999). Six *P. immobilis* strains were isolated and characterized from the experimentally infected fish in the present study. The strains were able to grow at 5°C, gram negative, non-motile, aerobic and non-pigmented. They reacted positively to catalase and oxidase tests and could not hydrolyze urea. In agreement with our study, 64 strains of *Psychrobacter*, characterized as gram negative, non-motile and aerobic, were isolated from *O. mykiss*, *S. trutta* and *E. lucius* (Gonzales *et al.*, 2000).

Since no mortality was observed, it is difficult to conclude whether *P. immobilis* is a primary pathogen or a secondary invader of already damaged tissues. Based on the findings of the present study, it is our hypothesis that the organism is a secondary invader. Further investigations are needed to determine its precise pathogenicity in different fish species.

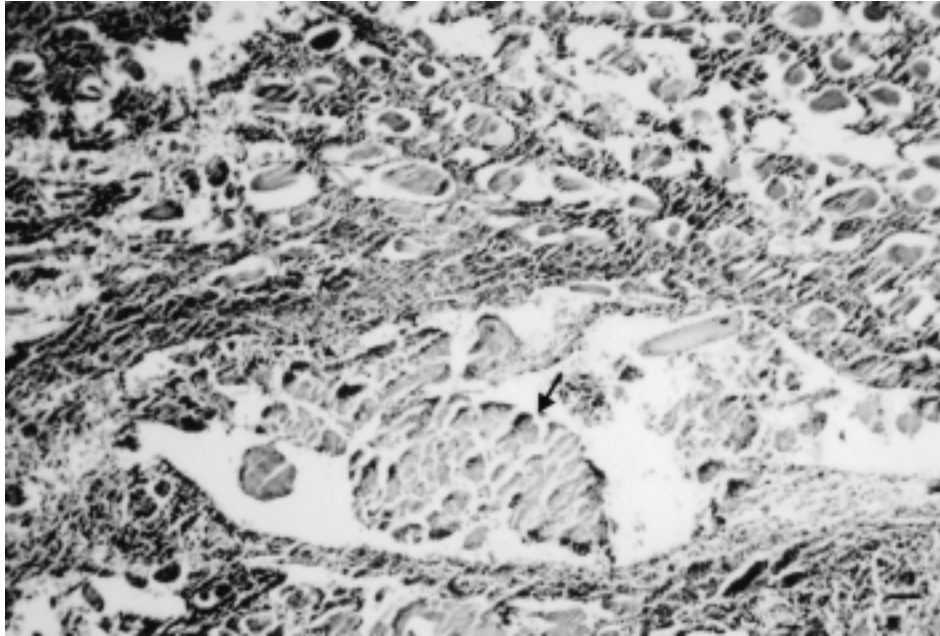


Fig. 5. Pathological changes in the muscle of rainbow trout caused by *P. immobilis* infection 35 days after inoculation. Arrow shows liquefaction necrosis in muscle fibers (H&E x 200). Bar =20 μ m.

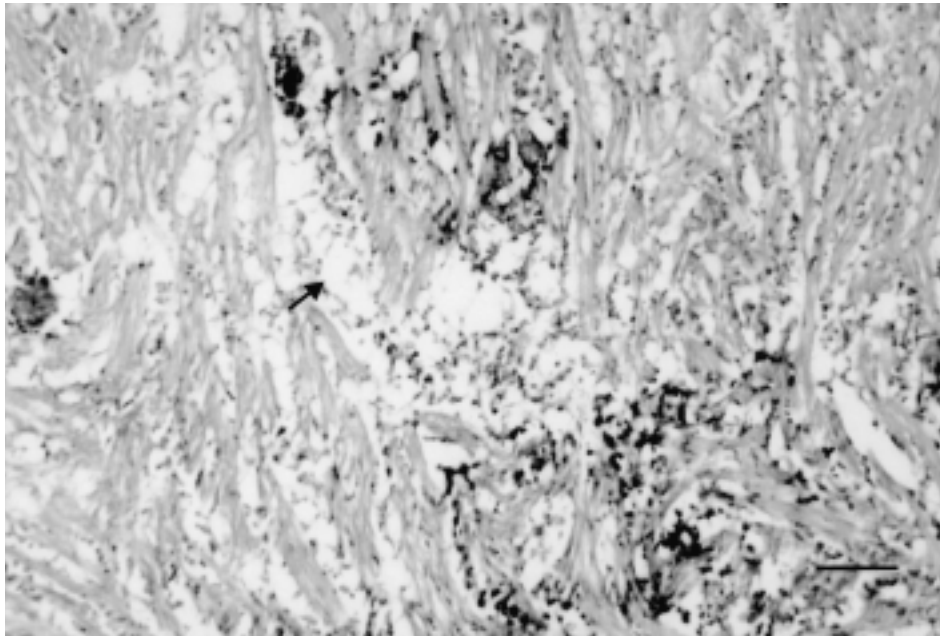


Fig. 6. Pathological changes in the heart of rainbow trout caused by *P. immobilis* infection 35 days after inoculation. Arrow shows edema among the muscle fibers (H&E x 100). Bar = 80 μ m.

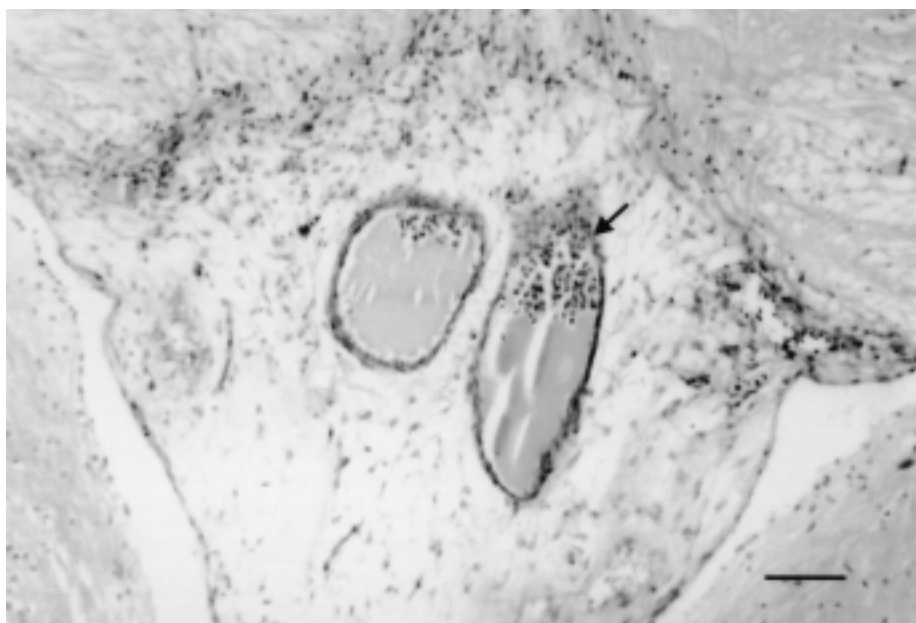


Fig. 7. Pathological changes in the brain of rainbow trout caused by *P. immobilis* infection 35 days after inoculation. Arrow shows vascular congestion (H&E x 100). Bar = 80 µm

References

- Austin B. and D.A. Austin**, 1999. *Bacterial Fish Pathogens: Disease of Farmed and Wild Fish*. 3rd rev. ed. Springer-Praxis, Godalming.
- Aydın S., Çelebi S. and I. Akyurt**, 1997. Clinical, hematological and pathological investigation of *Escherichia vulneris* in rainbow trout (*Oncorhynchus mykiss*). *Fish Pathol.*, 32:29-34.
- Bowman J.P., Nichols D.S. and T.A. McMeekin**, 1997. *Psychrobacter glacincola* sp. nov., a halotolerant, psychrophilic bacterium isolated from Antarctic Sea ice. *Syst. Appl. Microbiol.*, 20:209-215.
- Brown B.J. and L.G. Leff**, 1996. Comparison of fatty acid methyl ester analysis with the use of API 20 E and NFT strips for identification of aquatic bacteria. *Appl. Environ. Microbiol.*, 62:2183-2185.
- Collins R.**, 1993. In: L. Brown (ed.). *Principles of Disease Diagnosis. Aquaculture for Veterinarians, Fish Husbandry and Medicine*. Pergamon Press, Oxford, New York, Seoul, Tokyo. 72 pp.
- Daly J.G.**, 1999. Other bacterial pathogens. Chap. 16. In: P.T.K. Woo and D.W. Bruno (eds.). *Fish Diseases and Disorders, Vol. 3. Viral, Bacterial and Fungal Infections*. CABI Publ.
- Denner E.B., Mark B., Busse H.J., Turkiewicz M. and W. Lubitz**, 2001. *Psychrobacter proteolyticus* sp. nov., a psychrotrophic, halotolerant bacterium isolated from the Antarctic krill *Euphausia superba* Dana, excreting a cold-adapted metalloprotease. *Syst. Appl. Microbiol.*, 24:44-53.
- Ekingen G.**, 1980. Balıklarda Bakteriyel Hastalıkların Laboratuvar Diagnozu. Fırat Üniv. *Vet Fak. Derg.*, 5:69-82.
- Gennari M., Tomaselli S. and V. Cotrona**, 1999. The microflora of fresh and spoiled sardines, *Sardina pilchardus*, caught in Adriatic (Mediterranean) Sea and stored in ice. *Food Microbiol.*, 16:15-28.
- Gini G.A.**, 1990. Ocular infection caused by *Psychrobacter immobilis* acquired in a hospital. *J. Clin. Microbiol.*, 28:400-401.

- Gonzalez C.J., Santos J.A., Garcia-Lopez M.L. and A. Otero**, 2000. Psychrobacters and related bacteria in freshwater fish. *J. Food Protect.*, 63:315-321.
- Holt J.G., Krieg N.R., Sneath P.H.A., Staley J.T. and S.T. Williams**, 1994. *Bergey's Manual of Determinative Bacteriology*. Williams and Wilkins, Baltimore, MD. 94 pp.
- Kinne O.**, 1980. pp. 12-13. In: *Disease of Marine Animals, Vol. 1. General Aspects, Protozoa to Gastropoda*. Wiley, Chichester.
- Lloyd-Puryear M., Wallace D., Baldwin T. and D.G. Hollis**, 1991. Meningitis caused by *Psychrobacter immobilis* in an infant. *J. Clin. Microbiol.*, 29:2041-2042.
- Lozano F., Florez C., Recio F.J., Gamboa F., Gómez-Mateas J.M. and E. Martín**, 1994. Fatal *Psychrobacter immobilis* infection in a patient with AIDS. *AIDS (London, England)*, 8:1189-1190.
- Pacova Z. and E. Urbanova**, 1998. The first isolation of *Psychrobacter immobilis* from foods in the Czech Republic. *Veterinarni Medicina-Praha*, 43(4):111-114.
- Pacova Z., Urbanova E. and E. Durnova**, 2001. *Psychrobacter immobilis* isolated from foods: characteristics and identification. *Veterinarni Medicina-Praha*, 46(4):95-100.
- Plumb J.A.**, 1981. Relationship of water quality and infectious diseases in cultured channel catfish. pp. 290-303. In: J. Oláh, K. Molnár and Z. Jeney (eds.). *Fish Pathogens and Environment in European Polyculture*. Haltenyesztesi Kutató Intézet, Szarvas, Hungary.
- Romanenko L.A., Schumann P., Rohde M., Lysenko A.M., Mikhailov V.V. and E. Stackebrandt**, 2002. *Psychrobacter submarinus* sp. nov. and *Psychrobacter marincola* sp. nov., psychrophilic halophiles from marine environments. *Int. J. Syst. Evol. Microbiol.*, 52(4):1291-1297.
- Shimma Y., Shimnia H. and K. Ikeda**, 1982. Plasma constituents of 2-year-old rainbow trout raised with fish meal and SCP combined feeds. *Bull. Natl. Res. Inst. Aquacult.*, 3:61-73.
- Vásquez M., Grüttner C., Möeller B. and E.R.B. Moore**, 2002. Limited selection of sodium channel blocking toxin-producing bacteria from paralytic shellfish toxin-contaminated mussels (*Aulacomya ater*). *Res. Microbiol.*, 153(6):333-338.