

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

Sheenan Harpaz Agricultural Research Organization
Beit Dagan, Israel

Zvi Yaron Dept. of Zoology
Tel Aviv University
Tel Aviv, Israel

Angelo Colorni National Center for Mariculture, IOLR
Eilat, Israel

Rina Chakrabarti Aqua Research Lab
Dept. of Zoology
University of Delhi

Ingrid Lupatsch Swansea University
Singleton Park, Swansea, UK

Jaap van Rijn The Hebrew University
Faculty of Agriculture
Israel

Spencer Malecha Dept. of Human Nutrition, Food
and Animal Sciences
University of Hawaii

Daniel Golani The Hebrew University of Jerusalem
Jerusalem, Israel

Emilio Tibaldi Udine University
Udine, Italy

Copy Editor

Ellen Rosenberg

Published under auspices of
**The Society of Israeli Aquaculture and
Marine Biotechnology (SIAMB),
University of Hawaii at Manoa Library**

and
**University of Hawaii Aquaculture
Program** in association with
AquacultureHub

<http://www.aquaculturehub.org>



UNIVERSITY
of HAWAII[®]
MĀNOA
LIBRARY



AquacultureHub
educate • learn • share • engage

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>

FIELD REPORT

MICROFLORA ON THE SKIN OF EUROPEAN EEL (*ANGUILLA ANGUILLA* L., 1758) SAMPLED FROM CREEK YUVARLAKÇAY, TURKEY

Aysel Ugur*, Fevzi Yılmaz and Nurettin Sahin

Muğla University, Faculty of Arts and Sciences, Department of Biology,
TR-48187 Kötekli, Muğla, Turkey

(Received 30.12.01, Accepted 11.2.02)

Key words: *Anguilla anguilla*, European eel, microflora, skin, Turkey

Abstract

Bacterial skin microflora of eel obtained from Muğla (Turkey) province were studied. The aerobic bacteria associated with skin and slime were estimated using the dilution plate technique. The dominant bacterial species were *Pseudomonas* spp. (17.23%), *Acinetobacter baumannii* (15.51%) and *Stenotrophomonas maltophilia* (12.05%). A lower frequency of Gram-positive bacteria (18.93%) was found in samples.

Introduction

Fish, as is true of any other food, can cause health problems. It can be contaminated at any time from the moment of capture until it is eaten. Contamination can occur because pathogenic microorganisms form part of the normal flora of the fish. In other cases, toxic substances are introduced through cross-contamination, recontamination or faulty handling and processing.

The flesh of healthy live or newly caught fish is sterile as the immune system of the

fish prevents bacteria from growing on the flesh. When the fish dies, the immune system collapses and bacteria proliferate freely. On the skin surface, the bacteria to a large extent colonize the scale pockets. During storage, they invade the flesh by moving between the muscle fibers.

The natural distribution of *Anguilla anguilla* is in rivers near the North Atlantic, Baltic and Mediterranean Seas. It has been introduced to Asia, South and Central America

* Corresponding author. Tel: 90-252-223-8002, fax: 90-252-223-8656, e-mail: augur@mu.edu.tr

but does not reproduce there. Its spawning area is in the Western Atlantic (Sargasso Sea; Wellcomme, 1988). They are also found along the coast of Europe from the Black Sea to the White Sea. Males remain in fresh water for 6-12 years, females for 9-20 years. They live on the bottom, beneath stones, in the mud, or in crevices. At the end of their growth period, they become sexually mature and migrate to the Sargasso Sea (McClave et al., 1988). The leptocephali are brought by the Gulf Stream to the coast of Europe. This drifting migration lasts up to three years (Tesch, 1977). They evolve into elvers and lastly small eels before moving to freshwater basins (Keith et al., 1992). They can reach a total length of 150 cm and a weight of 6000 g. Their foods include virtually the whole aquatic fauna (Deelder and Anders, 1986). They are an important food for human beings and are utilized fresh, dried/salted, smoked or frozen and can be fried, boiled or baked (Frimodt, 1995).

Bacteria associated with the skin and slime of various fish have been reported in several studies (Shewan, 1977; Beri et al., 1989; Cahill, 1990). However, no information exists about the skin microflora of the eel, *A. anguilla*. Fish quality, in terms of safety and keeping time, is highly influenced by non-visible factors such as autolysis and contamination and growth of microorganisms. The aims of microbiological examination of fish products are to detect the presence of bacteria or other organisms with public health significance and to determine their hygienic quality in relation to factors such as temperature abuse during handling and processing and their impact on shelf life and marketing potential. Consequently, the aim of this study was to identify the bacterial flora associated with the skin and slime of the eel *A. anguilla*.

Materials and Methods

Fresh wild fish samples were taken from Creek Yuvarlakçay, Muğla Region, Turkey, from June 2000 to May 2001. Approximately 1-g samples of the skin and slime, taken on the same day, were thoroughly rinsed three times in sterile 0.85% saline to remove non-

floral bacteria and then homogenized in aseptic conditions with a homogenizer. Different fish samples were diluted in sterile saline and 0.1 ml of the dilution was spread on the surface of TSAg plates (Ringo and Strom, 1994) which contained TSA (tryptic soy agar, Difco) at 40 g/l and glucose at 5 g/l. The plates were incubated at 25°C and inspected daily for one week to allow growth of slow-growing strains. The dominant colony on each TSAg plate was determined. Representative colonies of each dominant type of bacteria were selected for characterization and subcultured three or four times to obtain pure cultures. After confirmation of culture purity, the bacteria were cultivated in tryptic soy broth (Difco) and stored in glycerol (20%) at -20°C for further identification.

The bacteria were identified using standard biochemical tests. Each isolate was tested for Gram reaction, oxidase and catalase, pigmentation, growth on MacConkey agar plates, and API 10S (bioMérieux) microtube systems. Extracellular enzymatic activities of dominant bacterial species were also determined with API ZYM systems (bioMérieux). Finally, Gram-negative and Gram-positive bacteria were identified as described in Holt et al. (1994) and the diagnostic scheme (Ringo, 1993), respectively.

Results and Discussion

The 116 bacterial isolates obtained from fish skin were classified. The dominant bacterial species were *Pseudomonas* spp. (17.23%), *Acinetobacter baumannii* (15.51%) and *Stenotrophomonas maltophilia* (12.05%; Table 1). Most of the isolates were Gram-negative rods and able to ferment some organic acids and sugars (Fig. 1). Most of the isolated *Acinetobacter* strains resembled the saprophytic *Pseudomonas* in being able to use any of a large number of organic carbons as an energy source. (Holt et al., 1994). We compared results with previously reported data but, due to changes in the taxonomical positions of bacteria, some of these identifications are now out of date. The genus *Pseudomonas*, for example, is divided into several genera such as *Comamonas*, *Stenotrophomonas*, etc., and

Table 1. Distribution of skin microflora on the European eel *Anguilla anguilla*.

	Σ	February	March	April	May-January
<i>Gram-negative</i>					
<i>Pseudomonas</i> spp.	17.23	++	+	-	++
<i>Acinetobacter baumannii</i>	15.51	+	++	-	++
<i>Stenotrophomonas maltophilia</i>	12.05	++	+	+	-
<i>Enterobacter aerogenes</i>	8.62	-	-	-	++
<i>Chryseobacterium-Sphingobacterium</i> spp.	8.61	++	+	+	-
<i>Citrobacter freundii</i>	6.89	-	-	++	+
<i>Proteus penneri</i>	5.14	-	+	-	+
<i>Methylobacterium extorquens</i>	3.44	-	+	+	-
<i>Serratia</i> sp.	1.72	-	-	-	+
<i>Aeromonas</i> sp.	1.72	-	-	-	+
<i>Gram-positive</i>					
Coryneforms	6.88	+	+	+	-
<i>Micrococcus</i> sp.	5.17	-	-	-	++
<i>Staphylococcus</i> sp.	3.44	-	++	-	-
Unidentified bacil	3.44	-	+	-	+
Σ		25.84	27.54	12.05	34.43

+ = 1.71-3.44%

++ = 5.17-8.62%

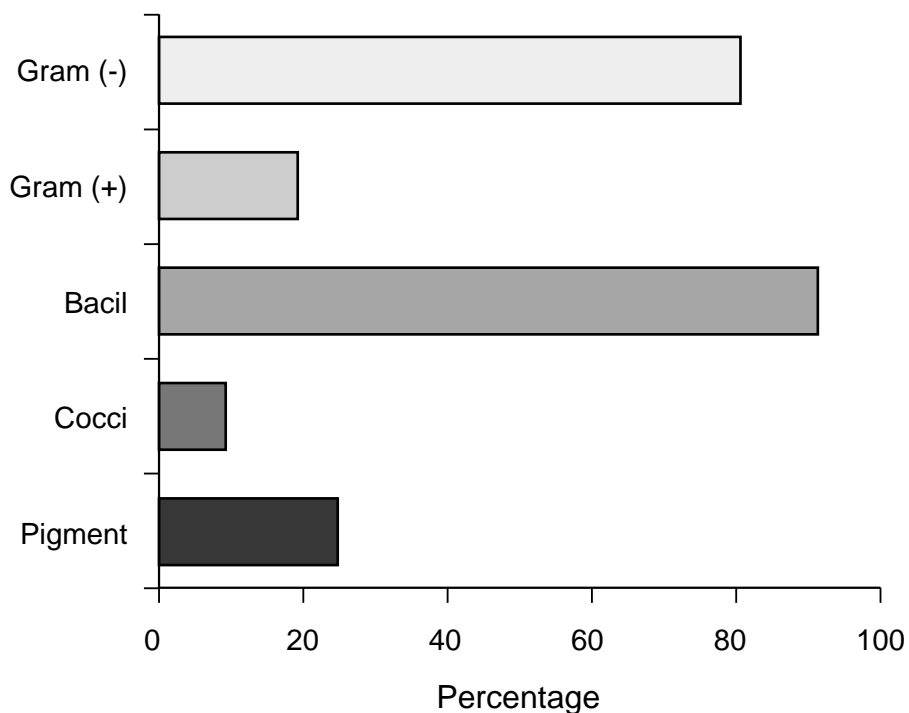


Fig. 1. Percent of skin microflora with morphological characteristic.

some *Pseudomonas* species have been revised under new genera such as *Stenotrophomonas* (formerly *Pseudomonas*) *maltophilia*. Extracellular enzymatic activity tests showed that the dominant microflora have high alkaline and acid phosphatases and lipase activity (Fig. 2). During February-March, the distribution and variety of bacteria were greater than during the rest of the sampling periods (Table 1). In this study, Gram-negative bacteria belonging to genera *Pseudomonas* and *Acinetobacter* constituted the major flora in skin/slime during all sampling periods. Similar findings were reported for several other species of fishes (Beri et al., 1989; Cahill, 1990). Gram-positive bacteria formed the lowest proportion of skin flora; the majority of Gram-positive bacteria were coryneforms and cocci. *Pseudomonas*, *Stenotrophomonas maltophilia* (formerly *Pseudomonas maltophilia*)

and *Acinetobacter* spp. are probably true resident microflora of eel skin. *Citrobacter*, *Proteus*, *Chryseobacterium*, *Sphingobacterium*, *Aeromonas*, *Methylobacterium* and *Serratia* constituted a lesser proportion of the bacterial skin flora of the eel.

The bacterial flora on newly caught fish depends on the environment in which the fish are caught rather than on the fish species (Shewan, 1977). Fish caught in very cold, clean waters have the lowest number of bacterial flora, whereas fish caught in warm waters have slightly higher counts. Very high numbers, i.e., 10^7 cfu/cm², are found on fish from polluted warm waters. In polluted waters, high numbers of Enterobacteriaceae may be found. In clean temperate waters, these organisms disappear rapidly, but it has been shown that *Escherichia coli* and *Salmonella* can survive

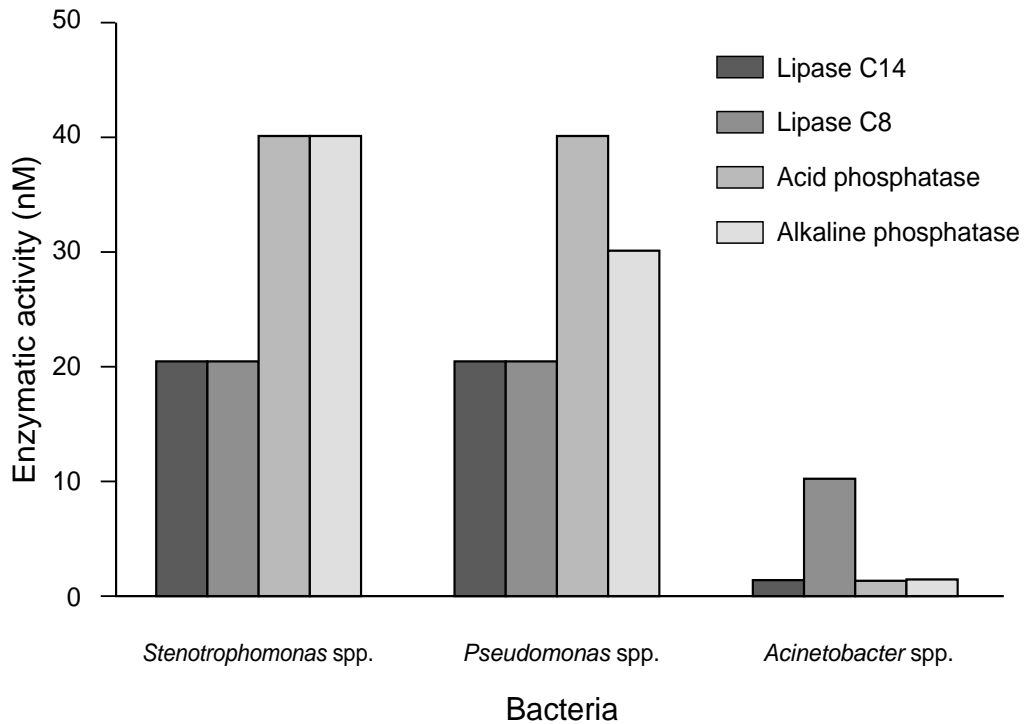


Fig. 2. Strength of extracellular enzymatic activities of dominant bacterial groups.

for very long periods in tropical waters and, once introduced, may become almost indigenous to the environment (Fujioka et al., 1988). Previous studies reported that Gram-negative bacteria dominate fish skins (Cahill, 1990). Bacteria belonging to Enterobacteriaceae are mostly related to water quality (Nieto et al., 1984).

Bacteria on fish caught in temperate waters enter an exponential growth phase almost immediately after the fish have died. This is also true when fish are iced, probably because the microflora have already adapted to the chilled temperatures. During ice storage, bacteria double in approximately one day and, within 2-3 weeks, reach 10^8 - 10^9 cfu/g flesh or cm^2 skin. During ambient storage, a level of 10^7 - 10^8 cfu/g is reached within

24 hours. The bacteria on fish caught in tropical waters often pass through a lag phase of 1-2 weeks if the fish are stored in ice, after which exponential growth begins (Gram, 1990; Gram et al., 1990).

From the point of view of marketing and aquaculture and based on previously reported studies, we conclude that the microbial flora of cultured eel change depending on environmental factors but are the same as wild forms. Because eel aquaculture is based on the catching of elvers and small eels after they relocate to freshwater basins, it is a pity that no information exists about the skin microflora of eel from different sources or geographical areas. Further research is required before final conclusions can be drawn.

References

- Beri H.K., James M.A. and K.K. Solanki**, 1989. Bacterial flora of fishes of Maharashtra and Saurashtra coasts (India). *J. Fd. Sci. Technol.*, 26(6):318-321.
- Cahill M.M.**, 1990. Bacterial flora of fishes: A Review. *Microb. Ecol.*, 19:21-41.
- Deelder H. and K. Anders**, 1986. *Diseases and Parasites of Marine Fishes*. Verlag Möller, Kül. 365 pp.
- Frimodt C.**, 1995. *Multilingual Illustrated Guide to the World's Commercial Coldwater Fish*. Fishing News Books, Osney Mead, Oxford, England. 215 pp.
- Fujioka R.S., Tenno K. and S. Kansako**, 1988. Naturally occurring fecal coliforms and fecal streptococci in Hawaii's freshwater streams. *Toxic Assess.*, 3:613-630.
- Gram L.**, 1990. *Spoilage of Three Senegalese Fish Species Stored in Ice and at Ambient Temperature*. Paper presented at SEAFOOD 2000, May 12-16, 1990, Halifax, Canada.
- Gram L., Wedell-Neergaard C. and H.H. Huss**, 1990. The bacteriology of spoiling Lake Victorian Nile perch (*Lates niloticus*). *Int. J. Food Microbiol.*, 10:303-316.
- Holt J.G., Krieg N.R., Sneath P.H.A., Staley J.T. and S.T. Williams** (eds.), 1994. *Bergey's Manual of Determinative Bacteriology, 9th Ed.* Williams and Wilkins, Baltimore.
- Keith P., Allardi J. and B. Mantan**, 1992. Livre rouge des espèces menacées de poissons d'eau douce de France et bilan des introductions. Museum National d'Histoire Naturelle, Secretariat de la Faune et del a Flore, Conseil Supérieur de la Peche, Cemagref & Ministère de l'Environnement. 111 pp.
- McClave J.D., Brickley P.J., O'Brien K.M., Kistner B.A., Wong M.W., Gallagher M. and S.M. Watson**, 1988. Leptocephali of the European eel swim to reach continental waters. Status of the question. *J. Mar. Biol. Assoc., UK*, 78(1):285-306.
- Nieto T.B., Toranzo A.E. and J.L. Barja**, 1984. Comparison between the bacterial flora associated with fingerling rainbow trout cultured in two hatcheries in the north-west of Spain. *Aquaculture*, 42:193-206.
- Ringo E.**, 1993. The effect of chromic oxide (Cr₂O₃) on aerobic bacterial populations associated with the intestinal epithelail mucosa of arctic charr, *Salvelinus alpinus* (L.). *Can. J. Microbiol.*, 39:1169-1173.
- Ringo E. and E. Strom**, 1994. Microflora of arctic charr, *Salvelinus alpinus* (L.): gastrointestinal microflora of free-living fish and effect of diet and salinity on intestinal microflora. *Aquacult. Fish. Management*, 25:623-629.
- 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. on Handling, Processing and Marketing of Tropical Fish*. Tropical Products Inst., London.
- Tesch F.W.**, 1977. *The Eel Biology and Management of Anguillid Eel*. John Wiley and Sons, New York. 434 pp.
- Wellcomme R.L.**, 1988. *International Introductions on Inland Aquatic Species*. FAO Fish Tech. Pap. No. 294:3189.