

MARINE *CYTOPHAGA*-LIKE BACTERIA (CLB) ISOLATED FROM DISEASED REARED SEA BASS (*DICENTRARCHUS LABRAX* L.) FROM FRENCH MEDITERRANEAN COAST

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During the spring of 1993, an epizootic outbreak occurred in a sea bass farm, located on the French Mediterranean coast. The disease appeared after a change of cage. The sea temperature was about 12°C and fish weight was 200 to 300 g. The diseased

fish exhibited a loss of appetite. The cumulative mortality reached 25%. The most consistent external signs were yellow-edged, pale skin lesions, with abundant yellowish mucus, scale losses, dorsal and caudal fin rot. In severe cases a muscle

Table 1. Characteristics of the *Cytophaga*-like bacteria obtained from diseased sea bass.

Gram stain	—	Degradation of :	
Cell morphology	Long rod	Aesculin	—
Motility	Gliding +	Cellulose	—
Cytochrome oxidase	+	Chitin	—
Metabolism	Oxidative	Starch	—
O/F glucose	—	APY ZYM / 12 h :	
API 20 E / 4 days :		Alkaline phosphatase	+
Voges Proskauer	+ (weak)	Esterase (C4)	+
Gelatinase	+	Esterase lipase (C8)	+
Other API reactions	—	Lipase (C14)	+
Nitrate reduction	—	Leucine arylamidase	+
Catalase	+	Valine arylamidase	+
Growth at :		Cystine arylamidase	+
15°C	+	Trypsin	+
20°C	+	Chymotrypsin	+
25°C	+	Phosphatase acid	+
Sensitivity to ¹ :		Naphthol-AS-B1-phosphohydrolase	+
Ampicillin	S	Other API reactions	—
Bacitracin	R		
Chloramphenicol	S		
Erythromycin	S		
Tetracyclines	S		
Trimethoprim-sulpha	S		
O/129 (150µg)	S		

- ¹Antibacterial sensitivity : Marine Agar, heavy inoculation, Pasteur tablets.

- Symbols used are : +, positive reaction; —, negative reaction; R, resistant; S, sensitive.

erosion was observed.

Presumptive diagnosis was accomplished through phase contrast examination of wet mount preparations of mucus and lesions. Microscopic examinations revealed filamentous bundles of flexuous bacteria which exhibited gliding motility, associated with motile *Vibrio* spp. These clinical observations are similar to those described for marine species by Hikida (1979) and Demoury (1987).

For bacterial isolation, some samples were taken by surface sterilising lesion surfaces with 70% ethanol and obtaining inocula from deep within lesions (Kent, 1988). Skin, spleen and kidney samples were inoculated onto Marine Agar plates (Difco) supplemented with 10% of calf serum (Sigma), and incubated at 20°C for up to 5 days. Bacterial growth was obtained from all skin inocula. There was no growth from the internal organs which meant that septicaemic vibriosis was unlikely.

The isolated organisms produced typical colonies characteristic of gliding bacteria, 5 mm in diameter, grey-yellowish in colour, sticky and spreading with irregular edges. Isolates were gram-negative oxidative rods 4-8 µm long and 0.2-0.3 µm wide, oxidase, catalase and gelatinase positive. Biochemical and enzymatic tests (API, Bio-Mérieux) were performed according to Bernardet & Grimont (1989). Acid was not produced from any of a range of carbohydrates. Further characters are presented in Table 1.

For histological examination, tissues were fixed in Davidson's solution and processed using standard techniques. After Mann Dominici staining, sections of skin lesions were seen to have large numbers of CLB on the epithelial surface of scale pocket and in the stratum spongiosum. The epidermis was partially damaged and bacteria were infiltrated within the dermis and the underlying muscle.

Pathogenicity tests were performed by intraperitoneal and subcutaneous infection of sea bass with 0.1 ml of a bacterial suspension containing 10⁸ cells/ml in 0.9% (w/v) saline solution. A group of fish was inoculated with cells from fresh colonies by direct contact after scarification. Sea bass (average weight=30g) were maintained in small tanks with flowing sterile sea water at 13°C. Fish were examined daily for 15 days, dead and moribund animals were subjected to bacterial and histological examination. Intraperitoneally challenged fish did not exhibit signs of the disease. After 5 days, all the others challenged animals died within 15 days. Both affected groups, fish infected by scarification and fish infected by subcutaneous inoculation, showed pale skin necrotic lesions around infection point. The inoculated strain was recovered in pure culture from all dead or moribund fish, but only from skin lesions. The isolated bacteria shows the main characteristics of the CLB, further work is needed to identify the present strain. It may be interesting to define its relations with *Flexibacter maritimus* identified in marine fish elsewhere in Europe, *Solea solea* (Bernardet *et al.*, 1990).

Summary

During spring of 1993, an epizootic outbreak occurred in a sea bass farm, located on the French Mediterranean coast. The cumulative mortality reached 25%. First examination of cutaneous lesions revealed filamentous bundles of flexuous bacteria which exhibited gliding motility. Bacterial growth was obtained only from skin lesions. The biochemical characteristics of isolates are described. Pathogenicity tests of representative strains were carried out. Until fuller data is available, the pathogen here reported was provisionally identified as a *Cytophaga*-like bacterium (CLB).

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References

- Bernardet, J.F. and Grimont, P A D (1989). Deoxyribonucleic acid relatedness and phenotypic characterisation of *Flexibacter columnaris* sp. nov., nom. rev., *Flexibacter psychrophilus* sp. nov., nom. rev., and *Flexibacter maritimus* Wakabayashi, Hikida and Masumura 1986. *International Journal of Systematic Bacteriology* **39**, 346-354.
- Bernardet, J.F., Campbell, A.C., Buswell, J.A. (1990). *Flexibacter maritimus* is the agent of 'black patch necrosis' in Dover sole in Scotland. *Dis. Aquat. Org.*, **8**, 233-237.
- Demoury, M. (1987). Fonctionnement du centre aquacole de Gravelines, étude de la maladie nécrosante des bars sur ce site. *Thèse de Docteur Vétérinaire, Ecole Nationale Vétérinaire de Nantes*.
- Hikida, M., Wakabayashi, H., Egusa, S., Masumura, K. (1979). *Flexibacter* sp., a gliding bacterium pathogenic to some marine fishes in Japan. *Bull. Jap. Soc. scient. Fish.* **45**, 421-428.
- Kent, M.L., Dungan, C.F., Elston, R.A., Holt, R.A. (1988). *Cytophaga* sp. (Cytophagales) infection in seawater pen-reared Atlantic salmon *Salmo salar*. *Dis. Aquat. Org.* **4**, 173-179