Vol. 124: 85–90, 2017
https://doi.org/10.3354/dao03103

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published March 30

NOTE

Isolation of *Lacinutrix venerupis* strains associated with disease outbreaks in sea bream *Sparus aurata* and European sea bass *Dicentrarchus labrax*

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ABSTRACT: Four Gram-negative bacterial isolates were recovered from 2 disease outbreaks that occurred in 2013 affecting European sea bass *Dicentrarchus labrax* fry and sea bream *Sparus aurata* adults. Main symptoms were erratic swimming, eroded fins and, in the sea bream outbreak, haemorrhages on the body surface; bacteria were always recovered from internal organs, almost in pure culture. On the basis of phenotypic characterization and 16S rRNA gene sequence analysis, the isolates were identified as *Lacinutrix venerupis*, a bacterium not previously reported as a fish pathogen. The highest 16S rDNA sequence similarities were recorded with the type strain of this species (99.9–100% similarity), while other species showed similarities below 97%, the closest relative being *L. mariniflava* (96.3% similarity). Phenotypic characterization showed some discrepancies with the *L. venerupis* type strain (mainly in BIOLOG GN profile); however, DNA–DNA hybridization assays with *L. venerupis* and *L. mariniflava* type strains confirmed that these isolates belong to the former species (levels of DNA relatedness were 98–100% and 38–50%, respectively). Finally, a virulence evaluation of the isolates using Senegalese sole *Solea senegalensis* fry was also performed; significant mortalities (80–100% mortality within 4 d) were recorded after intraperitoneal injection, but only with high doses of bacteria (10^7 colony forming units $fish^{-1}$). Further studies will be necessary to determine the importance of this species as a fish pathogen.

KEY WORDS: Virulence · Aquaculture · Fish pathogen · Bacteria · Characterization

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INTRODUCTION

The family *Flavobacteriaceae* includes many species that have been isolated from marine organisms, either as commensals or as opportunistic pathogens (Lasa et al. 2015). Some members of this family are well known fish pathogens, including *Flavobacterium psychrophilum*, which is responsible for 'cold water disease' in salmonids (Starliper 2011), *F. columnare*, which causes 'columnaris disease' in freshwater fish (Declercq et al. 2013), and *Tenacibaculum maritimum*, described in a variety of marine fish as being responsible for 'black patch necrosis' or 'flexibacteriosis' (Toranzo et al. 2005, Avendaño-Herrera et al. 2006). Many more species of this family have been associated with disease in fish, some of which have been described only recently, such as *Chryseobacterium piscicola* and *T. dicentrarchi* in salmonids (Ilardi et al. 2009, Starliper 2011, Piñeiro-Vidal et al. 2012, Avendaño-Herrera et al. 2016) or *T. soleae*, associated with disease in a number of flatfish species (Piñeiro-Vidal et al. 2008, López et al. 2010).

The genus *Lacinutrix*, a member of the family *Flavobacteriaceae*, was defined by Bowman & Nichols (2005) with the description of *L. copepodicola*, recovered from the copepod *Paralabidocera antarctica*. To date, this genus comprises 10 species, all of them isolated from marine environments or marine organisms

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(algae, clams, copepods, flounder), although never associated with disease outbreaks. Here we report the first description of *L. venerupis*, a species recently described from apparently healthy clams in Spain (Lasa et al. 2015), associated with disease in marine fish.

MATERIALS AND METHODS

Bacterial isolation

During April and December 2013, 2 epizootic outbreaks, occurring in 2 marine farms located in southwestern Spain, affected European sea bass *Dicentrarchus labrax* and sea bream *Sparus aurata* cultures, respectively. In the first case, mortalities occurred in fry-sized fish, with high mortality rates; water temperature was 16–20°C, and the main symptoms were erratic swimming and eroded fins. In the second case, mortalities occurred in adult fish, with moderate mortalities; water temperature was 14°C, and the main symptoms were erratic swimming, eroded fins and haemorrhages on the body surface (Fig. 1). Samples for bacterial isolation were collected from external lesions, liver and kidney of moribund fish, and cultured on *Flexibacter maritimus* medium (FMM) (Pazos et al. 1996) at 20°C for 24 to 96 h. Four isolates (a72, a722, a726 and a727) were obtained from these samples. For long-term preservation, strains were frozen at −80°C in sterile seawater supplemented with 20% (v/v) glycerol.

16S rDNA sequencing and phylogenetic analysis

Partial 16S rRNA gene sequences were obtained using the universal primers 20F and 1500R, capable of amplifying nearly full-length 16S rDNA (Weisburg et al. 1991). DNA was extracted from pellets of bacterial cells by boiling for 5 min in distilled water, and PCR amplification was carried out according to López et al. (2009). PCR products were purified with the commercial kit Illustra ExoProStar 1-step (GE Healthcare) following the manufacturer's instructions. Direct sequencing of purified PCR products was performed by Secugen (Madrid). The sequences were analysed using Chromas LITE and BioEdit programs and subjected to BLAST (https://blast.ncbi.nlm.nih.gov) and EzTaxon (www.ezbiocloud.net) searches to retrieve the most closely related sequences. Sequence similarities were calculated using the SIAS software (http://imed.med.ucm.es/Tools/sias). The 16S rDNA sequences of the 4 isolates were aligned with those of related species using Clustal Omega software, and a phylogenetic tree was constructed according to the neighbour-joining method (Saitou & Nei 1987) by using the program MEGA 3. The accuracy of the resulting tree was measured by bootstrap resampling of 1000 replicates.

Phenotypic characterization

Phenotypic characterization was performed according to Bernardet et al. (1990) and Avendaño-Herrera et al. (2004). The Gram reaction was determined according to the KOH method proposed by Buck (1982) and by the Gram staining method. Temperature tolerance was tested by checking growth on Marine Agar 2216 (Difco) at 4, 15, 25, 30, 35, 40 and 45°C for 10 d, and tolerance to salinity was tested by growth on basal medium (neopeptone 4 g l^{-1}; yeast extract 1 g l^{-1}; agar 15 g l^{-1}) supplemented with 0, 3, 6, 8, 10 and 12% (w/v) NaCl for 3 d. Growth on thiosulphate–citrate–bile salts–sucrose (TCBS) agar (Difco) was also tested. All tests were incubated aerobically at 20°C. Commercial miniaturized API 20E, API 20NE and API ZYM galleries (bioMérieux) and Biolog GN2 Micro-Plates were also used according to the manufacturers' instructions, except that sterile seawater was used as the dilutent and 20°C as the incubation temperature. The type strain of *Lacinutrix venerupis* Cmf 20.8^T was characterized together with the isolates under study with the same methodology.

Preparation of fatty acid methyl esters (FAMEs) from strain a722, grown at 20°C on Marine Agar 2216 plates, was performed according to the instructions of the Microbial Identification System (MIDI) as described by Sasser (1990). FAMEs were analysed by gas chromatography in an Agilent 6850 system, using

Fig. 1. External symptoms of sea bream *Sparus aurata* infected by *Lacinutrix venerupis*, showing (a) eroded fins and (b) haemorrhages in fins and body surface

the MIDI operating system and the aerobic bacteria library TSBA6 (MIDI 2008).

DNA–DNA hybridization

For DNA–DNA hybridization assays, DNA from isolates a72, a722, a726 and a727, and from *L. venerupis* Cmf 20.8^T and *L. mariniflava* AKS432^T, was extracted with a NucleoSpin Tissue kit (Macherey-Nagel), and the concentration and purity of each sample were determined by measuring A_{260} and the A_{260}:A_{280} ratio, respectively. DNA–DNA hybridization assays were performed by the plate method proposed by Ziemke et al. (1998), combining the hydroxyapatite method with non-radioactive detection of released DNA. The hybridization temperature was 55°C. DNA–DNA hybridization assays were done in duplicate for all strains tested.

Pathogenicity assays

To investigate the pathogenicity of the isolates, 2 isolates, 1 recovered from sea bass (a72) and 1 recovered from sea bream (a722), were selected. Experimental infection assays were performed by intraperitoneal injection of 3 different doses (10^5, 10^6 and 10^7 colony-forming units [cfu] fish^{-1}) in Senegalese sole *Solea senegalensis* fry with an average weight of 5 g. Two groups of 10 fish were used for each dose. Bacterial doses were prepared according to López et al. (2009): bacterial concentration was estimated by absorbance at 600 nm wavelength; doses were confirmed by colony enumeration of serial dilutions on FMM plates. A control group of 10 fish was included in each virulence assay. After bacterial challenge, experimental and control fish were kept without feeding in 18 l tanks at the appropriate temperature (18°C for isolate a72 and 14°C for isolate a722) in continually flowing seawater, and mortalities were recorded daily for 10 d. Dead fish were removed and subjected to standard bacteriological examination.

RESULTS

Bacterial isolation

Yellow colonies suspected to belong to the family *Flavobacteriaceae* appeared on FMM plates inoculated with liver and kidney samples, almost in pure culture. Four *L. venerupis* isolates — 1 from the sea bass outbreak (a72) and 3 from the sea bream outbreak (a722, a726 and a727) — were selected for identification. Two other isolates, one belonging to the *Vibrio* genus and one to the *Pseudoalteromonas* genus, were also recovered.

16S rDNA sequencing and phylogenetic analysis

Almost complete 16S rDNA sequences were obtained from strains a72, a722, a726 and a727 (accession numbers in GenBank/EMBL/DDBJ: LT616973 to LT616976, respectively) and used for BLAST and EzTaxon homology searches to retrieve the most closely related species. On the basis of sequence analysis, the isolates were included in the genus *Lacinutrix*, within the family *Flavobacteriaceae*. The highest sequence similarity for the sequence of isolate a722 (1396 bp) was recorded with the *Lacinutrix venerupis* type strain (Cmf 20.8^T; 100% similarity), followed by the type strain of *L. mariniflava* (AKS432^T; 96.3%). Sequence similarities between the 4 isolates under study ranged from 99.78 to 100%. The phylogenetic tree derived from these sequences illustrates the position of the isolates recovered from sea bass and sea bream, clearly grouped with *L. venerupis* strains and separated from other *Lacinutrix* species (Fig. 2).

Phenotypic characterization

Colonies of the investigated isolates were bright yellow, did not adhere to agar, and consisted of Gram-negative, non-fermentative rods. All isolates were cytochrome oxidase and catalase positive. Growth was observed from 4 to 35°C, but not at 40°C. All strains grew in 3% NaCl, but none grew in 0% or 6–12%. No isolate was able to grow in TCBS, absorbed Congo red or produced flexirubin-type pigments. Hydrolysis of Tween 20 and Tween 80 were positive, but starch, casein and lecithin were not hydrolysed. In API 20E and 20NE galleries, only Voges-Proskauer and hydrolysis of gelatin and esculin gave positive results. The enzymatic profiles in API ZYM galleries of the 4 isolates were similar and showed positive results for the following activities: alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, alpha-chymotrypsin, acid phosphatase and phosphohydrolase.

Similar results were displayed by the type strain of *L. venerupis* for all of these tests, except for the Voges-Proskauer test, which was negative, and the ability to

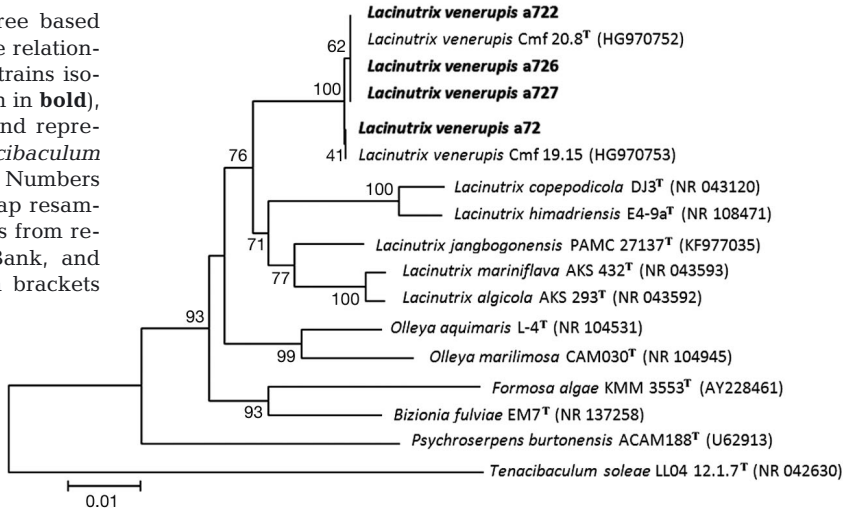

Fig. 2. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships between the *Lacinutrix venerupis* strains isolated from marine fish in this study (shown in **bold**), other members of the genus *Lacinutris* and representatives of related genera. The *Tenacibaculum soleae* sequence was used as an outgroup. Numbers at the nodes indicate the levels of bootstrap resampling based on 1000 replicates. Sequences from related species were obtained from GenBank, and their accession numbers are indicated in brackets

grow in 6% NaCl, which was positive. Some discrepancies were observed with the data provided by Lasa et al. (2015) for the type strain of *L. venerupis*, even though the same media and culture conditions were employed; in our study, this strain was able to grow at 35°C (weakly), but was unable to grow in 8–10% NaCl.

Data obtained from GN MicroPlates (Biolog) for isolates a72, a722 and a727 and for the *L. venerupis* type strain showed differences between the characterized strains. All strains utilized the following carbon sources: α-cyclodextrin, dextrin, D-fructose, α-D-glucose, α-D-lactose, maltose, sucrose, D-trehalose, turanose, xylitol, L-alaninamide, L-aspartic acid, L-glutamic acid, glycyl-L-aspartic acid, glycyl-L-glutamic acid, L-ornithine and L-serine. No strain used Tween 40, Tween 80, adonitol, L-arabinose, D-mannose, methyl piruvate, acetic acid, cis-aconitic acid, citric acid, formic acid, D-glucosaminic acid, γ-hydroxybutyric acid, p-hydroxy phenylacetic acid, itaconic acid, α-keto butyric acid, propionic acid, sebaric acid, bromo succinic acid, glucuronamide, D-alanine, L-histidine, hydroxy L-proline, D-serine, phenyethylamine, putrescine and 2-aminoethanol. Variable results were found in the remaining 52 tests (Table 1).

The FAME analyses showed that the major cellular fatty acids (>5% of the total) in strain a722 were iso-$C_{15:1}$G (27.9%), iso-$C_{15:0}$ (16.9%), iso-$C_{15:0}$3OH (15.98%), iso-$C_{16:0}$3OH (7.7%), iso-$C_{17:0}$3OH (12.36%) and summed feature 3 (as defined by MIDI; 6.76%). These results agreed with the FAME profile published for *L. venerupis* (Lasa et al. 2015).

DNA–DNA hybridization

DNA–DNA hybridization experiments with the type strains of other *Lacinutrix* species, selected on the basis of phenotypic traits and 16S rRNA gene sequence analysis, were done in duplicate and confirmed the results obtained previously. Levels of DNA reassociation between strain a722 and the type strain of *L. venerupis* ranged from 98.2 to 100%, whereas the DNA–DNA hybridization values with the other 3 isolates under study ranged from 95 to 100%. In contrast, levels of DNA reassociation with the type strain of *L. mariniflava* were clearly lower (38.2–50.2%).

Pathogenicity tests

Strains a72 and a722, isolated from sea bass and sea bream, respectively, were selected for experimental infection. Mortalities of Senegalese sole were observed only at the highest of the 3 doses employed (10^7 cfu fish^{-1}), and were 100% for isolate a72 and 80–100% for isolate a722, within the first 4 d after exposure to the pathogen. The inoculated strains were recovered from most of the dead fish. The *Vibrio* and *Pseudoalteromonas* isolates caused no mortality even at the highest dose. None of the control fish died during the assays.

DISCUSSION

This work reports the first isolation of a bacterium of the genus *Lacinutrix* associated with disease in marine fish in 2 different outbreaks. These outbreaks affected cultures of the most important marine fish species for aquaculture in Spain, viz. sea bass and sea bream, with a considerable difference in size and age (fry and adults), at a temperature that ranged from 14 to 20°C. The main symptoms (eroded fins, external

haemorrhages) were similar to those observed with other marine fish pathogens belonging to the family *Flavobacteriaceae*, such as *Tenacibaculum soleae* (López et al. 2010), although no ulcers were observed as typically occur with *T. maritimum* (Toranzo et al. 2005).

The phenotypic and genotypic characterization allowed the identification of the isolates as *L. venerupis*. Phenotypic profiles of the isolates under study were quite similar to those of both the *L. venerupis* and *L. marinifl ava* type strains; hence, species assignation was mostly based on the genotypic characterization. Analysis of 16S rDNA sequences showed that only *L. venerupis* shared similarity values above the limit of intraspecific variability (98.7%) proposed by Stackebrandt & Ebers (2006). On the other hand, levels of DNA reassociation of strain a722 with the type strain of *L. venerupis* and with the other isolates were 98.2–100% and 95–100%, respectively; these values are above the threshold value for species delineation of 70% proposed by Wayne et al. (1987), proving that all of these strains belong to a unique species. In contrast, levels of DNA reassociation with the type strain of *L. marinifl ava* (38.2–50.2%) were clearly below the threshold value.

Phenotypic characterization based on the Biolog GN system showed a variety of profiles between *L. venerupis* strains. Variable results were found in 52 tests, although in most of these tests (n = 37), most strains (75%) shared the same result. On the other hand, the differences could be related to the origin of the strains; for instance, strain a72, isolated from sea bass, displayed differences compared to all other *L. venerupis* strains in 16 tests; similarly, type strain Cmf 20.8^T, isolated from clams, showed differences compared to all other strains in 12 tests.

Finally, experimental infection tests, carried out by intraperitoneal injection, clearly demonstrated the pathogenic potential of these isolates. Whereas 2 isolates belonging to the genera *Vibrio* and *Pseudoalteromonas*, recovered from the same samples, were unable to cause mortalities, high mortality rates (80–100%) were observed with the 2 *L. venerupis* strains assayed; however, these mortalities were registered only with the highest dose (10^7 cfu fish^{-1}), a matter that could indicate that these strains act as opportunistic pathogens, i.e. organisms that are present in healthy hosts as symbionts or are present in the environment, and only become pathogenic under certain

Table 1. Differences in carbon compound utilization between *Lacinutrix venerupis* strains obtained in this work from sea bass *Dicentrarchus labrax* (a72) and sea bream *Sparus aurata* (a722, a727; isolate a726 was not tested due to logistical constraints), and the type strain of this species (Cmf 20.8^T), determined using the BIOLOG GN system

Characteristic	Positive strains	Characteristic	Positive strains
Glycogen	All except Cmf 20.8^T	α-keto valeric acid	Cmf 20.8^T
N-acetyl-D-galactosamine	Cmf 20.8^T	D,L-lactic acid	All except a72
N-acetyl-D-glucosamine	All except a727	Malonic acid	a722, Cmf 20.8^T
D-arabitol	a72	Quinic acid	a722, Cmf 20.8^T
D-cellobiose	a722	D-saccharic acid	All except a72
I-erythritol	a72, a722	Succinic acid	a722
L-fucose	All except Cmf 20.8^T	Succinamic acid	a722, a727
D-galactose	a72, Cmf 20.8^T	L-alanine	All except a72
Gentiobiose	All except a72	L-alanyl-glycine	All except a72
M-inositol	a722, Cmf 20.8^T	L-asparagine	a72
Lactulose	All except a72	L-leucine	a72, a727
D-mannitol	All except Cmf 20.8^T	L-phenylamine	Cmf 20.8^T
D-melibiose	a72, a722	L-proline	a727, Cmf 20.8^T
β-methyl-D-glucoside	a72	L-pyroglutamic acid	a722, Cmf 20.8^T
D-psicose	Cmf 20.8^T	L-threonine	All except a72
D-raffinose	a727, Cmf 20.8^T	D,L-carnitine	a722
L-rhamnose	a722, Cmf 20.8^T	γ-amino butyric acid	a72
D-sorbitol	All except a72	Urocanic acid	a722
Mono-methyl succinate	a722	Inosine	All except a727
D-galactonic acid lactone	Cmf 20.8^T	Uridine	All except Cmf 20.8^T
D-galacturonic acid	Cmf 20.8^T	Thymidine	a72
D-gluconic acid	Cmf 20.8^T	2,3-butanediol	a72
D-glucuronic acid	Cmf 20.8^T	Glycerol	a722, Cmf 20.8^T
α-hydroxybutyric acid	Cmf 20.8^T	D,L-α-glycerol phosphate	All except a72
β-hydroxybutyric acid	a722, Cmf 20.8^T	Glucose-1-phosphate	All except a727
α-keto glutaric acid	All except a72	Glucose-6-phosphate	All except a727

circumstances (Brown et al. 2012). Opportunistic diseases are triggered when hosts are stressed, and temperature also plays an important role because of its influence on the prevalence of the pathogen and on the immune response in fish; on the other hand, high population densities in aquaculture facilities not only cause stress, but also contribute to the spread of the infection (Hurst 2016). An example of an opportunistic pathogen within the family *Flavobacteriaceae* is *T. maritimum*, which displays an increased prevalence and severity at temperatures above 15°C, with low water quality, stressful conditions (high density, poor feeding, excess of UV light) or with bad skin conditions (Avendaño-Herrera et al. 2006). On the other hand, since intraperitoneal injection is not a natural route of infection, more work is needed to investigate the susceptibility of sea bass and sea bream to *L. venerupis* under more natural conditions.

In summary, this work represents the first report of bacteria of the genus *Lacinutrix* associated with disease in marine fish, and allows the number of potential pathogens that affect sea bass and sea bream to be extended.

Acknowledgements. This study was funded by project BONAQUA (0433_BONAQUA_5_E POCTEP). J.R.L. holds a postdoctoral grant from IFAPA supported by the European Union FEDER program. We thank Dr. J. L. Romalde for providing the type strain of *L. venerupis.*

LITERATURE CITED

Avendaño-Herrera R, Magariños B, López-Romalde S, Romalde JL, Toranzo AE (2004) Phenotypic characterization and description of two major O-serotypes in *Tenacibaculum maritimum* strains from marine fishes. Dis Aquat Org 58:1–8

Avendaño-Herrera R, Toranzo AE, Magariños B (2006) Tenacibaculosis infection in marine fish caused by *Tenacibaculum maritimum*: a review. Dis Aquat Org 71: 255–266

Avendaño-Herrera R, Irgang R, Sandoval C, Moreno-Lira P and others (2016) Isolation, characterization and virulence potential of *Tenacibaculum dicentrarchi* in salmonid cultures in Chile. Transbound Emerg Dis 63:121–126

Bernardet JF, Campbell AC, Buswell JA (1990) *Flexibacter maritimus* is the agent of 'black patch necrosis' in Dover sole in Scotland. Dis Aquat Org 8:233–237

Bowman JP, Nichols DS (2005) Novel members of the family *Flavobacteriaceae* from Antarctic maritime habitats including *Subsaximicrobium wynnwilliamsii* gen. nov., sp. nov., *Subsaximicrobium saxinquilinus* sp. nov., *Subsaxibacter broadyi* gen. nov., sp. nov., *Lacinutrix copepodicola* gen. nov., sp. nov., and novel species of the genera *Bizionia, Gelidibacter* and *Gillisia.* Int J Syst Evol Microbiol 55:1471–1486

Brown SP, Cornforth DM, Mideo N (2012) Evolution of virulence in opportunistic pathogens: generalism, plasticity, and control. Trends Microbiol 20:336–342

Buck JD (1982) Nonstaining (KOH) method for determination of Gram reactions of marine bacteria. Appl Environ Microbiol 44:992–993

Declercq AM, Haesebrouck F, Van den Broeck W, Bossier P, Decostere A (2013) Columnaris disease in fish: a review with emphasis on bacterium-host interactions. Vet Res 44:27

Hurst CJ (ed) (2016) The Rasputin effect: when commensals and symbionts become parasitic. Advances in environmental microbiology, Vol 3. Springer, Berlin

Ilardi P, Fernández J, Avendaño-Herrera R (2009) *Chryseobacterium piscicola* sp. nov., isolated from diseased salmonid fish. Int J Syst Evol Microbiol 59:3001–3005

Lasa A, Diéguez AL, Romalde JL (2015) Description of *Lacinutrix venerupis* sp. nov.: a novel bacterium associated with reared clams. Syst Appl Microbiol 38:115–119

López JR, de la Roca E, Núñez S, de la Herran R and others (2009) Identification of *Vibrio harveyi* isolated from diseased cultured wedge sole *Dicologoglossa cuneata.* Dis Aquat Org 84:209–217

López JR, Piñeiro-Vidal M, García-Lamas N, de la Herran R, Navas JI, Hachero-Cruzado I, Santos Y (2010) First isolation of *Tenacibaculum soleae* from diseased cultured wedge sole, *Dicologoglossa cuneata* (Moreau), and brill, *Scophthalmus rhombus* (L.). J Fish Dis 33:273–278

MIDI (2008) Sherlock Microbial Identification System operating manual, version 6.1. MIDI Inc., Newark, DE

Pazos F, Santos Y, Macias AR, Nuñez S, Toranzo AE (1996) Evaluation of media for the successful culture of *Flexibacter maritimus.* J Fish Dis 19:193–197

Piñeiro-Vidal M, Carballas CG, Gómez-Barreiro O, Riaza A, Santos Y (2008) *Tenacibaculum soleae* sp. nov., isolated from diseased sole (*Solea senegalensis* Kaup). Int J Syst Evol Microbiol 58:881–885

Piñeiro-Vidal M, Gijón D, Zarza C, Santos Y (2012) *Tenacibaculum dicentrarchi* sp. nov., a marine bacterium of the family *Flavobacteriaceae* isolated from European sea bass. Int J Syst Evol Microbiol 62:425–429

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids. MIDI Tech Note 101. MIDI Inc., Newark, DE

Stackebrandt E, Ebers J (2006) Taxonomic parameters revisited: tarnished gold standards. Microbiol Today 33: 152–155

Starliper CE (2011) Bacterial coldwater disease of fishes caused by *Flavobacterium psychrophilum.* J Adv Res 2: 97–108

Toranzo AE, Magariños B, Romalde JL (2005) A review of the main bacterial fish diseases in mariculture systems. Aquaculture 246:37–61

Wayne LG, Brenner DJ, Colwell RR, Grimont PAD and others (1987) Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. Int J Syst Bacteriol 37:463–464

Weisburg WG, Barns SM, Pelletier DA, Lane DJ (1991) 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 173:697–703

Ziemke F, Höfle MG, Lalucat J, Rosselló-Mora R (1998) Reclassification of *Shewanella putrefaciens* Owen's genomic group II as *Shewanella baltica* sp. nov. Int J Syst Bacteriol 48:179–186

Editorial responsibility: Andrew Barnes, Brisbane, Queensland, Australia

Submitted: September 6, 2016; Accepted: January 12, 2017
Proofs received from author(s): March 14, 2017