Identification of *Flexibacter maritimus* or *Tenacibaculum maritimum* from post-larvae of *Litopenaeus vannamei* ?. Comment on Mouriño et al. (2008)

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Recently, Mouriño et al. (2008) reported the first record in Brazil of the filamentous bacteria *Flexibacter maritimus* (Wakabayashi et al., 1986) from the hatching of *Litopenaeus vannamei* post-larvae (Boone, 1931). In our view, a generalized lack of clarity about this identification has occurred and the discussion of some issues raised in the work is beyond the scope of this commentary.

As Mouriño et al. argued, the identification of this isolate as *F. maritimus* was influenced by the tests of Wakabayashi et al. (1986) and Chen et al. (1995), who had misclassified this microorganism within the *Flexibacter* genus. In fact, Suzuki et al. (2001), in a phylogenetic analysis and taxonomic study, demonstrated that *Flexibacter maritimus* is distantly related to *Flexibacter flexilis*, the type species of the genus *Flexibacter*. Therefore, Suzuki et al. (2001) concluded that this strain constitutes a new bacterial species in the genus *Tenacibaculum*, distinct from *F. maritimus*, named *T. maritimum*. Fourteen members are currently classified within this genus, including *T. litopenaei* isolated from shrimp in Taiwan (Sheu et al., 2007). Therefore, to avoid confusion with other bacteria, this note will use the name *T. maritimum*.

Even though the bacterium reported by Mouriño et al. (2008) could be *T. maritimum*, one of the major constraints on the detection of this pathogen is the lack of methods to distinguish this microorganism from others that are phenotypically similar and phylogenetically related species, particularly those of the genus *Flavobacterium* and *Cytophaga* (Suzuki et al., 2001). The morphological, physiological and biochemical characteristics useful in the identification of *Tenacibaculum maritimum* have been detailed by several authors (see review Avendaño-Herrera et al., 2006a), and must contain at least a limited number of data (see Table 1). However, as Mouriño et al. show in their paper, only thirteen biochemical assays were done to propose *T. maritimum* as the cause of massive mortality of *L. vannamei* post-larvae. Some of these tests are not necessary for the identification of this pathogen as growth on

Table 1. Biochemical characteristics of filamentous bacteria in *Litopenaeus vannamei* and *Tenacibaculum maritimum* reference strains. Data are from Wakabayashi et al. (1986), Gosink et al. (1998), Suzuki et al. (2001) and Avendaño-Herrera et al. (2004a). +, positive; –, negative; W, weakly positive; ND, not determined; NG, no growth in the presence of NaCl. These bacteria are Gram-negative, rod-shaped and positive for catalase and oxidase.

Characteristic	Isolated strain	*Tenacibaculum maritimum* (n = 2)
Origin	Post-larvae of shrimp, Brazil	Diseased red sea bream fingerling, Japan
Cells size (µm)	15 x 0.4 – 0.5	2 – 30 x 0.5
Colony morphology		
Shape	Rhizoid	Uneven edge
Colour	Creamy	Pale yellow
Gliding motility	ND	+
Congo red absorption	+	+
Flexirubin pigment	ND	–
Temperature range (ºC)	ND	15 – 34
Optimal temperature (ºC)	ND	30
Salinity range (%)		
Seawater [a]	ND	NG
NaCl [b]	ND	30 – 100
pH range	ND	5.9 – 8.6
Growth on:		
Casamino acids	ND	+
Sucrose	–	– [†]
D-Ribose	ND	– [†]
DL-Aspartate	ND	–
L-Proline	ND	–
L-Glutamate	ND	W [†]
L-Leucine	ND	–

Table 1. Continued...

Characteristic	Isolated strain	*Tenacibaculum maritimum* (n = 2)
Degradation of:		
Starch	–	–
Gelatin	+	+ ††
Tween 80	ND	+
Tyrosine	+	+
Agar	ND	–
Carboxymethyl cellulose	ND	–
Cellulose	ND	–
Chitin	ND	–
Esculin	ND	–
Nitrate reduction	+	+ ††
H_2S production	–	–

[a]100 = full-strength seawater; [b]Percentage of NaCl in the medium; [†]Data from Gosink et al. (1998); a different result was obtained by Suzuki et al. (2001); and [††]Data from Wakabayashi et al. (1986); a different result was obtained by Suzuki et al. (2001).

TCBS. In addition, as can be seen from Table 1, variable results have been reported for gelatine and nitrate reactions, while Avendaño-Herrera et al. (2004a) also reported difference in the hydrogen sulphide tests. These three reactions were included in Mouriño et al. and as suggested by Suzuki et al. (2001), the employment of different basal media could account for this variability.

On the other hand, as can be seen from the photomicrograph of filamentous bacteria (Figure 2, see Mouriño et al.), the spores found by these authors are not a typical characteristic of *T. maritimum* (Suzuki et al., 2001). In fact, Chen et al. (1995) did not observe cysts or microcysts neither from the microorganism isolated from marine fishes of California, nor from the reference strains, in contradiction with Mouriño et al. Our studies have demonstrated that the morphology of the typical slender rod shaped *T. maritimum* cells appear shorter and tend to become spherical, about 1 µm diameter, after 3 days of incubation (Avendaño-Herrera et al., 2006b).

To date, two PCR primer pairs have been designed for the detection of *T. maritimum* using the 16S ribosomal RNA gene as target (Toyama et al. 1996; Bader and Shotts 1998). Comparing the specificity of the 2 PCR protocols demonstrated that the sequences of both primer pairs were species-specific for *T. maritimum*, and no amplification products were obtained from chromosomal DNA of other non-*T. maritimum* (Avendaño-Herrera et al. 2004b). However, despite the potential of the PCR detection, Mouriño et al. did not test any of these primer sets. We think that these molecular tools could shed light on the confirmation of the presumptive biochemical identification of the filamentous bacterium *T. maritimum* in Brazil, and thus presence cannot be excluded.

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