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EVALUATION OF THE USE OF API-STAPH IN THE IDENTIFICATION OF FISH PATHOGENIC STAPHYLOCOCCI

Özgür ÇANAK
Istanbul University, Faculty of Aquatic Sciences, Department of Fish Diseases, Istanbul TURKEY
ocanak@istanbul.edu.tr

Abstract: *In this study, the use of API-Staph rapid identification kit, which is a miniaturized biochemical identification kit previously developed for the clinically important medical bacteria, was evaluated for the identification of staphylococci recovered from moribund fish samples and mostly successful results were achieved.*

Keywords: *Staphylococcus*, fish pathogenic bacteria, API-Staph

Introduction: Modern marine aquaculture activities in the Mediterranean have started in the 1970's and showed a rapid development in the following decades in terms of produced species and production amount (Pavilidis & Mylonas, 2011). Bacterial diseases are among the main obstacles of this production (Pavilidis & Mylonas, 2011). Staphylococci are Gram-positive bacteria that are widespread around the world. They can survive in water, soil and air and some of them are opportunistic pathogens of human and animal including fish (Schleifer & Bell, 2009). As they became medically important, rapid identification schemes and kits were developed and previously used for the identification of human and animal isolates (Kloos&Schleifer, 1975; Brun *et al.*, 1978; Kloos&Wolfshohl, 1982). The aim of this study is the evaluation of a rapid identification kit which is developed for staphylococci, in the identification of staphylococci recovered from moribund fish samples.

Material & Methods: Marine cage, off-shore marine cage, inland pool and hatchery units of 13 different fish farms located in İzmir, Aydın, Muğla, Samsun, Ordu and Trabzon was visited between October 2013 and August 2014. A total of 63 fish samples of gilthead sea bream, European sea bass, meagre, sharpsnout sea bream, dentex, rainbow trout and Blacksea trout weighing between 10 g and 2,5 kg were sampled bacteriologically. Staphylococci were recovered from the internal organs (liver, kidney, hearth and spleen) of fish samples (Roberts, 2012). They were identified by their phenotypic and biochemical characters at the species and sub-species level according to Schleifer&Bell (2009). API-Staph rapid identification kit (Biomerieux, France) was also used according to the directions of the manufacturer. Identification at the genus level was confirmed with the *tuf*-PCR developed by Martineau *et al.*, (2001).

Results & Discussion: After the inoculations made from the visceral organs of moribund gilthead sea bream, sea bass and sharpsnout sea bream samples obtained from 7 fish farms, mixed infections with *Vibrio spp.* in 6 farms and a motile *Aeromonas sp.* in 1 farm besides the Gram-positive bacteria were detected. Of the 12 Gram-positive bacteria with cocci shaped cells that are cytochrome oxidase negative, catalase positive and facultative anaerobic isolates, 4 isolates were identified as *S. capitis* subsp. *capitis*, 3 isolates were identified as *S. epidermidis*, 2 isolates were identified as *S. lentus* and others were identified as *S. aureus*, *S. hominis* subsp. *hominis* and *S. sciuri* subsp. *sciuri* according to Schleifer&Bell (2009). Identification of the bacterial isolates according to their phenotypic and biochemical characters and API-Staph profile were summarized in Table 1. All isolates were confirmed by the use of *Staphylococcus*

genus-specific *tuf* based PCR. All isolates generated a single band of 370 bp and hence the identification at the genus level was confirmed.

The API-Staph rapid identification kit identified *S. epidermidis* (97,9 %) (Fig. 1) and *S. lentus* (98%4) successfully and *S. aureus* (85,9 %) in an acceptable level. Bacterial isolates identified as *S. capitis* subsp *capitis* at the sub-species level according to Schleifer & Bell (2009) were identified as *S. capitis* with the API-Staph kit with a similarity of 88,8 %. Bacterial isolates identified as *S. sciuri* subsp. *sciuri* at the sub-species level according to Schleifer & Bell (2009) were identified as *S. sciuri* with the API-Staph kit with a similarity of 71 %. Bacterial isolates identified as *S. hominis* subsp. *hominis* at the sub-species level according to Schleifer & Bell (2009) could not be identified with the API-Staph kit. In conclusion, the results of this study showed that this kit may provide successful identification of fish pathogenic staphylococci at the species level, but detailed routine laboratory tests are needed for a more precise and reliable identification especially at the sub-species level.

Table 1. Bacteria recovered from moribund fish samples and their identification by using biochemical characters and API-Staph identification kit

Farm	Type	Fish	Bacterial idendifitcation (Schleifer & Bell, 2009)	API-Staph Profile	API identification (Similarity %)
1 – İzmir	Off-shore	*D. labrax*	*S. epidermidis*	6706113	*S. epidermidis* (97.9 %)
2 – Muğla	Land-pool	*S. aurata*	*S. capitis* subsp. *capitis*	6122000	*S. capitis* (88.8 %)
3 – Muğla	Land-pool	*S. aurata*	*S. aureus*	6716173	*S. aureus* (85.9 %)
7 – Samsun	Off-shore	*D. labrax*	*S. capitis* subsp. *capitis*	6122000	*S. capitis* (88.8 %)
7 – Samsun	Off-shore	*D. labrax*	*S. lentus*	6732670	*S. lentus* (98,4 %)
7 – Samsun	Off-shore	*D. labrax*	*S. hominis* subsp. *hominis*	2214710	Not identified
8 – Samsun	Off-shore	*D. labrax*	*S. capitis* subsp. *capitis*	6122000	*S. capitis* (88.8 %)
11 – Trabzon	Off-shore	*D. puntazzo*	*S. capitis* subsp. *capitis*	6122000	*S. capitis* (88.8 %)
12 – Muğla	Hatchery	*S. aurata*	*S. sciuri* subsp. *sciuri*	6136110	*S. sciuri* (71 %)



Figure 1: API-Staph profile coded 6706113 of *S. epidermidis*

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