OCCURENCE OF LACTIC ACID BACTERIA (LAB) IN FARMED SEA BASS (DICENTRARCHUS LABRAX);AND SEA BREAM (SPARUS AURATA) SOUTHERN OF TUNISIA

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Abstract
Lactic acid bacteria (LAB) collection was identified from enteric organs of healthy sea bass and sea bream cultured in tunisia. Thus we charecterized mainly Enterococci group with predominance of *E. faecium* (53%) and *E. faecalis* (21%). *E. sanguinicola, E. casseliflavus, E. mundtii, E. gallinarum, E. pseudoavium, lactococcus lactis, Aerococcus viridans* and *Carnobacterium sp*. were also identified by 16S rDNA sequencing and comparing RAPD-PCR profiles. Further, we obtained clear discrimination within species which were sensitive to vancomycin and resistants to others antibiotics tested.
Keywords: Aquaculture, Bacteria, Fishes

Introduction
LAB were described elsewhere as part of normal intestinal flora of fish and reported acting as probiotics since they can be harmless bacteriocin-producing bacteria and therefore may reduce the use of antibiotics in aquaculture [1]. The *Enterococcus* were LAB of primarily human and animal gastrointestinal flora which were introduced in farmed animals as biological control agents [2]. Here, we present investigation results of LAB isolated from healthy farmed sea bream and sea bass in Tunisia in order of to test their introduction as probiotics for the most valuable local cultured fish species.

Materials and methods
Sixty healthy fish specimens of sea bass and sea bream were collected from the greatest fish farm in southern zone of Tunisia (Monastir). Samples of skin patches and intestinal contents were sterily removed, homogenised and diluted in 0.9% saline solution and spread on MRS and M17 plates (Oxoid) before isolation and phenotypic characterization by mean of standard tests (colony and cell morphology, Gram stain, mobility, production of oxidase and catalase) and miniaturized API50CH biochemical tests (BioMérieux, France). Genetic characterization was performed using PCR-ribotyping of 16S rRNA sequences using universal primers p8FPL(5'-AGTTTGATCCTGGCTCAG-3') and p806R(5'-GGACTACCAGGGTATCTAAT-3'). Further genetic intra-specific characterization was performed by RAPD-PCR using M13 primer (5´-GAGGGTGGCGGTTCT-3´). In addition we test antibacterial sensitivity of all the strains by classical agar diffusion method [3].

Results and discussion
From eighty-four LAB strains isolated of fish samples : 32 strains were from skin and 52 from gut of both sea bream and sea bass. *E. faecium* was the most frequent (53%), followed by *E. faecalis* (21%). *E. sanguinicola* (8 strains), *E. casseliflavus* and *Aerococcus viridans (each* 3 strains) and *E. mundtii*, E. *gallinarum*, *E. pseudoavium*, *Lactococcus lactis* and *Carnobacterium sp*. (each one strain). The results obtained highlighted the first indigenous fish flora description of *E. faecalis, E. faecium* and other enterococci not previously considered [4]. Also, RAPD-PCR discrimination results were in agreement with phylogenetic analysis based on 16S rRNA sequences. From another view, *Enterococcus* isolates were sensitive to vancomycin and resistant to up to 10 ATB tested. Increasing use of antibiotics should be considered as principal cause of emergence for such resistance since supported by transfer of plasmids and transposable elements within marine ecosystem [6]. Present results should be considered in further to LAB investigation in fish both for probiotic assessments and antibacterial resistance treatment in aquaculture.

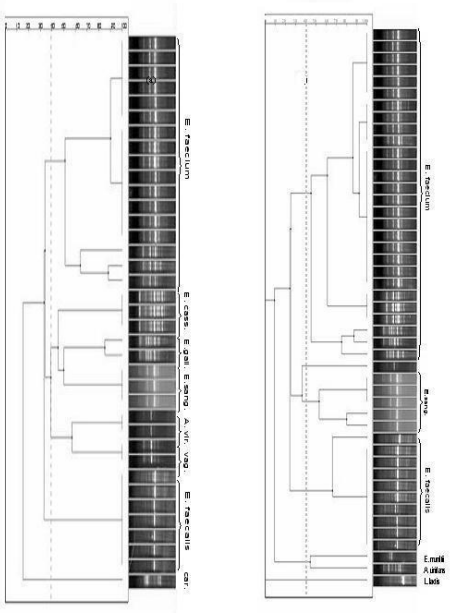

Fig. 1. RAPD patterns of the sea bream (a) and sea bass (b) isolates obtained by using the primer M13, and dendrogram obtained by UPGMA of correlation value of merged normalised RAPD patterns.

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