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Full Length Research Paper

Phenotypic characterization and enterobacterial repetitive intergenic consensus polymerase chain reaction (eric-pcr) of *Aeromonas* spp. and *Vibrio* spp. strains isolated from *Sparus aurata* fish farm (Khenis, Tunisia)

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This study characterises the *Aeromonas* and *Vibrio* strains isolated from seawater and sediments out of *Sparus aurata* marine farm. The bacterial strains were isolated on Thiosulfate-Citrate-Bile-Sucrose (TCBS) agar plates and described using different bacteriological tests and using standardised micro-methods "API 20 NE strips". The exoenzymes production and antibiotics susceptibility were also investigated. The enterobacterial repetitive intergenic consensus (ERIC)-PCR was used to evaluate the genetic diversity of the isolated strains. Two dominant genera of bacteria were found (*Aeromonas* and *Vibrio*). *Aeromonas hydrophila* strains were the dominant bacteria (56.5% of total isolates) followed by *V. alginolyticus* strains (26.1%). Most of the studied strains were β-haemolytic, hydrolyze the DNA and produce many exoenzymes such as lecithinase, caseinase, amylase and lipase. All tested strains were resistant to at least three antimicrobial agents. The antibiotic resistance index was 0.683 for *V. alginolyticus* and 0.711 for *A. hydrophila* strains. The ERIC-PCR profiles among the isolated bacteria were generally heterogeneous, showing a high polymorphism between *Aeromonas* strains.

Key words: *Aeromonas* spp., *Vibrio* spp., seawater, genetic diversity, virulence properties.

INTRODUCTION

In Tunisia, and since many years, several programs have been developed to study the phenotypic and genetic properties of environmental *Vibrio* and *Aeromonas* strains isolated from different fish and mollusks farms where *Sparus aurata* and *Dicentrarchus labrax*, and *Mytilus edulis* and *Crassostrea gigans* are reared (Bakhrouf et al., 1992; 1995; Snoussi et al., 2006; 2008a; 2008b; 2010; Ben Kahla-Nakbi et al., 2007b; Snoussi). These strains can adhere to several biotic and abiotic surfaces (Snoussi et al., 2008c; 2009; Ben et al., 2009a) and survive as a VBNC forms (Ben Kahla-Nakbi, et al., 2007a; Ben Kahla-Nakbi et al., 2008; Ben Abdallah et al., 2009a, b). These properties allow these bacteria to survive, proliferate and persist in marine biotopes (Snoussi et al., 2009).

Gilthead sea bream (*S. aurata* L.) is one of the most important species reared in marine fish farms installed along Tunisian coastal areas. Members of the Vibrionaceae and Aeromonadaceae families are responsible for fish and aquatic animal pathologies (Martin and Phelepp, 1983; De Kinkelin et al., 1985). These bacteria

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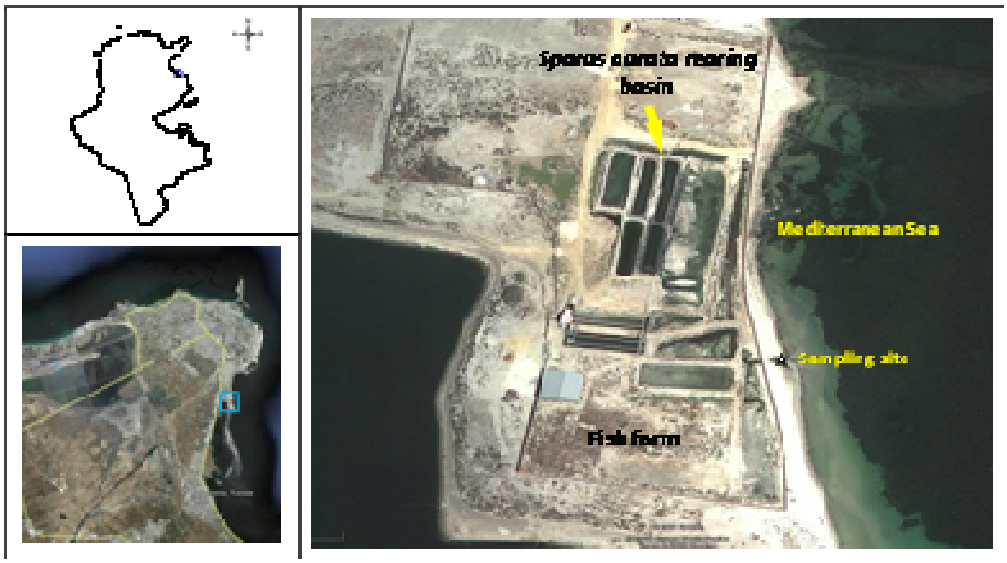

Figure 1. Localization of the site of study.

cause several economic losses and high mortality in larvae of many species during the first days following hatching: *S. aurata* and *D. labrax* (Bakhrouf et al., 1992; Snoussi et al., 2006; Ben Kahla-Nakbi et al., 2007b).

Vibrio and *Aeromonas* genera are widespread of tropical and temperate waters around the word and they colonise the costal waters of the Mediterranean Sea (Caruso et al., 1998; Zanetti et al., 2000; Robert-Pillot et al., 2002; Snoussi at al., 2008b). *Vibrio* spp. strains are very normal inhabitants in estuarine and marine environments and are frequently isolated from bathing area as free-living bacteria and associated to different surfaces (Tamplin et al., 1990; Barbieri et al., 1999; Baffone et al., 2005). These strains were always associated with gastrointestinal and extra-intestinal troubles related to the consumption of insufficiently cooked seafood or deep contact with contaminated seawater (Ottaviani et al., 1995; Croci et al., 2001; Thompson et al., 2004).

Aeromonas hydrophila is a Gram-negative bacterium that infects a wide range of hosts including amphibians, reptiles, avians and mammals such as cows and man (Popoff, 1984), but it is most well known as a fish pathogen. It causes motile aeromonad septicemia (MAS), which results in great economic losses in fresh water fish farming worldwide. The pathogenesis of *A. hydrophila* is multifactorial. A variety of virulence factors may contribute to the overall virulence of this bacterium. These include extracellular products (Ljungh and Wadstrom, 1982; Leung and Stevenson, 1988; Anglka et al., 1995), S-layer (Dooley and Trust, 1988) and adhesins. It is suggested that *A. hydrophila* is entero-pathogenic, using disease by adhesive and entero-toxic mechanisms (Quinn et al., 1993).

In this study, we reported the isolation, characterisation, and ERIC 2-PCR typing of 23 *Aeromonas* spp. and *Vibrio* spp. strains isolated from seawater and sediments near a *S. aurata* rearing Farm (Monastir). The presence of *Vibrio cholerae* virulence genes toxR, toxS, toxRS and toxT genes were sought in the genome of all tested strains using PCR technique. Exoenzymes production and antimicrobial resistance have also been investigated.

MATERIALS AND METHODS

Sampling site

The area of study (Figure 1) is localized on the eastern littoral of Tunisia (Monastir). This bay is localised between the latitudes 35°40 and 35°40 and the longitudes 10°48 and 10°54. The Monastir-Sayada seacoast is characterized by an algal bloom distribution during all the year especially during the three months of the summer related to an increase in the temperature with a rarefaction of fish capture. Seawater and sediment from the aquaculture farm installed along the seacoasts of Khenis (Monastir, Tunisia) were collected during the summer 2006. In the laboratory, *Aeromonas* spp. and *Vibrio* spp. research was carried out by filtering the water samples (500 ml) through 0.45 µm pore size filter membranes. These membranes were incubated in alkaline peptone water (1% NaCl, pH 8.6) for 24 h. The enrichments were then streaked onto Thiosulfate-citrate-bile salt-sucrose agar (TCBS agar, Biorad) and incubated for 18 to 24 and 30°C. Twenty five grams of sediment were diluted in Stomacher packet each containing 225 ml of alkaline peptone water supplemented with 1% NaCl. The homogenate was incubated at 30°C for 18 to 24 h.

Characterization of bacterial strains

Bacterial strains growing on TCBS plates were subjected to standard morphological, physiological and biochemical plate and tube tests using the procedures described previously by Snoussi et al. (2006, 2008a). A commercial miniaturized API 20 NE system (Bio Mérieux, Marcy l'Étoile, France) was used to identify the strains isolated. For exoenzymes productions, all strains were tested for

Table 1. Selected primers of four *Vibrio cholerae* virulence genes investigated in this study.

Target genes	PCR primer sequence (5'- 3')	Size of PCR product
toxR	R1 : TGGTTTGGCGTGAGCAAGGTTT R2 : GGTTATTTTGTCCGCCAGTGG	595 pb
toxS	S1 : CCACTGGCGGACAAAATAACC S2 : AACAGTACCGTAGAACCGTGA	640 pb
toxT	T1 : TTGCTTGGTTAGTTA TGAGAT T2 : TTGCAAACCCAGACTGATAT	581 pb
toxRS	R0 : GAGTCATATTGGTACTTAAATT S2 : AACAGTACCGTAGAACCGTGA	1397 pb

lipase on a medium including Tween 80, haemolysin (Human blood agar, BIO-RAD, France) and DNA hydrolysis (DNase Agar, Scharlau Microbiogy, Spain). The enzymes amylase, caseinase and lecithinase were detected on media prepared with Phosphate Buffer Saline (PBS) supplemented respectively with 0.5% casein peptone, 5% skim milk powder and 5% egg yolk emulsion (Snoussi et al., 2009).

Susceptibility to several antimicrobials agents was determined using the Kirby-Bauer method and Muller Hinton agar plates supplemented by 1% NaCl as described by Ottaviani et al. (2001). The followings antibiotics were selected for this study from previous reports (Ben Kahla-Nakbi et al*.,* 2006; Snoussi et al., 2006) including: ampicillin (10 µg), Gentamicin (10 µg), tetracycline (30 µg), trimethoprim-sulphamethoxazole (1.25 + 23.7 µg), oxolnic acid (20 µg), nitrofurantoin (300 µg), chloramphenicol (30 µg), norfloxacine (5 µg), erythromycin (15 µg), ciprofloxacin (5 µg), cefotaxime and nalidixic acid (30 µg). After incubation at 37°C for 18 to 24 h, the diameter of the inhibition zone was measured with 1mm flat rule and the diameters were interpreted according to (Cavallo et al*.,* 2006). After 24 h of incubation at 37°C, organisms were classified as sensitive (S), intermediate (I), or resistant (R) upon the diameters of inhibition zone obtained on MH-1% NaCl (Snoussi et al., 2008). Disk diffusion susceptibility test against the vibriostatic agent O/129 (2, 4-diamino-6, 7-diisopropylpteridine, OXOID, England) was performed on Mueller Hinton agar plates supplemented with 1% NaCl using (10 and 150) µg diagnostic disk as described by the manufactures.

The antibiotic resistance index (ARI) of each bacterial population was determined using the following formula: ARI = y/nx, where y was the actual number of resistance determinants recorded in a population of a size n and x was the total number of antibacterial tested for in the sensitivity test. Based on the occurrence of resistance to more than three antibiotics, the isolates were grouped as multiple antibiotic resistant isolates (Manjusha et al., 2005).

The multiple antibiotic resistance (MAR) index of the isolates was done as referred to described by Krumperman (1983). The MAR index was defined as a/b where a represents the number of multiple antibiotics to which the particular isolate is resistant and b as the number of multiple antibiotics to which the particular isolates were exposed.

Enterobacterial repetitive intergenic consensus-PCR (ERIC2-PCR)

All strains of *A. hydrophila* (n = 13), *A. salmonicida* spp. *salmonicida* (n = 2), *V. alginolyticus* (n = 6) and *V. vulnificus* (n = 2) were grown overnight at 30°C on LB agar plate (Sigma). One colony was suspended in 5 ml of LB broth (Sigma, St. Quentin, France) for 24 h at 30°C. Chromosomal DNA was extracted using a Wizard Genomic purification Kit (Promega, USA) following the instructions of the manufacture's. ERIC 2-PCR was performed in a final volume of 25 µl containing 3 µl of extracted DNA, 5 µl green Go Taq buffer (5x), 200 µM of each deoxynucleoside triphosphates (dNTP), 25 pmol of ERIC-2 primer 5'-AAGTAAGTGACTGGGGTGAGCG-3' and 1 U of GO Taq DNA polymerase (Promega, USA). ERIC 2-PCR parameters consists of 35 cycles having the following parameters: denaturation at 95°C for 30 s, hybridisation at 52°C for 45 s and primer extension at 72°C for 1.5 min. PCR products (5 µl) were analyzed on 1% agarose gel stained with ethidium bromide (0.5 µg/ml), which was visualized under UV transillumination and photographed using Gel Doc XR apparatus (Biorad, USA).

The gels were viewed under ultraviolet light and photographed by the Gel Doc (Bio-Rad, USA). The number and size of DNA fragments were evaluated by visual inspection and using Gel Pro Analyser 3.1 software. Dendrogram based on the unweighted pair group method of arithmetic averages (UPGMA) and Jaccard's correlation coefficient on the basis of ERIC-2 patterns was built using the MVSP 3.1 software (Snoussi et al., 2008a).

Distribution of toxR, toxT, toxS and toxRS genes

Primers for the amplification of the *tox*RS genes were designed to be complementary to the 5' and 3' ends of the *t*oxR and the 5' and 3' ends of toxS. For toxT amplification, primers toxT1 and toxT2 were designed. All primers used in this study were summarized in Table 1. Amplification reactions were performed in a 25 µl reaction mixture contained 3 µl of genomic DNA, 5µl of green Go Taq buffer (5x), 200 µM of each deoxynucleoside triphosphates (dNTP), 25 µM of each primer and 1U of GO Taq DNA polymerase (Promega, USA). The mixtures were incubated for 5 mn at 94°C, followed by 35 cycles of amplifications. Each cycle consisted of a step of denaturation at 94°C for 40 s, annealing for 40 s, and primer extension for 1 mn at 72°C. The mixtures were kept at 72°C for 10 mn between two cycles. The annealing temperatures were: 58°C for toxR, toxS, toxT and toxRS.

All PCR-positive strains had the presence of the virulence genes confirmed by repeating the PCR three times independently. All bands produced were normalized by comparing molecular weight marker (Hyper Ladder II DNA molecular weight marquer, Bioline) between different gels. The amplification products were visualized by staining the gel with ethidium bromide after electrophoresis at 90 V for 90 min in 1% agarose gel (FMC, Bio products). All DNA amplifications were performed in a DNA thermal cycler (PTC-100,

Table 2. Biochemical activities of *Aeromonas* spp. and *Vibrio* spp. strains isolated from seawater and sediment tested using the API 20 NE strips.

Strain characterization	*A. hydrophila* (n= 13)	*A. salmonicida* spp. *salmonicida* (n=2)	*V. alginolyticus* (n= 6)	*V. vulnificus* (n=2)
Gram staining (KOH method)	+	+	+	+
Motility	+	+	+	+
Oxidase	+	+	+	+
Catalase	+	+	+	+
Na+ required for growth				
- Growth at 0 % NaCl	0	0	0	0
- Growth at 3 % NaCl	100	100	100	100
- Growth at 8 % NaCl	100	100	100	100
- Growth at 10 % NaCl	0	0	100	100
Vibriostatic Agent				
- O/129 (10 µg)	R	R	R	R
- O/129 (150 µg)	S	S	S	S
Activities on API 20NE Strips (%)				
-$N0_3$: Reduction of nitrates	100	100	100	100
-TRP : Indole production	84.61	0	100	100
-GLU : Glucose fermentation	84.61	100	100	100
-ADH : Arginine dihydrolase	23.07	50	0	0
-URE : Urease	61.53	0	0	0
-ESC : Hydolysis of esculin	100	100	100	100
-GEL : Hydolysis of gelatin	92.3	100	100	100
-PNG : β-galactosidase	53.84	100	16.66	100
-GLU : Assimilation of D-glucose	84.61	100	66.66	0
-ARA : Assimilation of L-arabinose	61.53	0	0	0
-MNE : Assimilation of D-mannose	76.92	0	50	0
-MAN : Assimilation of D-mannitol	84.61	100	83.33	0
-NAG : Assimilation of N-acetyl-glucosamine	100	100	50	0
-MAL : Assimilation of D-maltose	92.3	100	83.33	0
-GNT : Assimilation of potassium gluconate	92.3	100	83.33	0
-CAP : Assimilation of capric acid	38.46	0	0	0
-ADI : Assimilation of adipic acid	53.84	0	0	0
-MLT : Assimilation of malic acid	92.3	50	100	100
-CIT : Assimilation of trisodium citrate	84.61	0	0	0
-PAC : Assimilation of phenylacetic acid	61.53	0	0	50
Exoenzymes activities (%)				
Protease	100	100	100	100
Gelatinase	100	100	100	100
Lipase	100	100	100	100
DNase	100	100	100	100
Amylase	84.61	100	100	100
Lecithinase	100	100	100	100
Haemolysis on Human blood agar	100	100	100	100

USA).

RESULTS AND DISCUSSION

A total of 13 *A. hydrophila* strains, two *A. salmonicida* spp. *salmonicida,* two *V. vulnificus* and six *V. alginolyticus* strains were isolated from all samples tested in this work (Table 2). In fact, eight strains (34.78 %) were isolated from seawater and 15 strains from the sediment (65.2 %).

The biochemical activities of the *Aeromonas* spp. (15

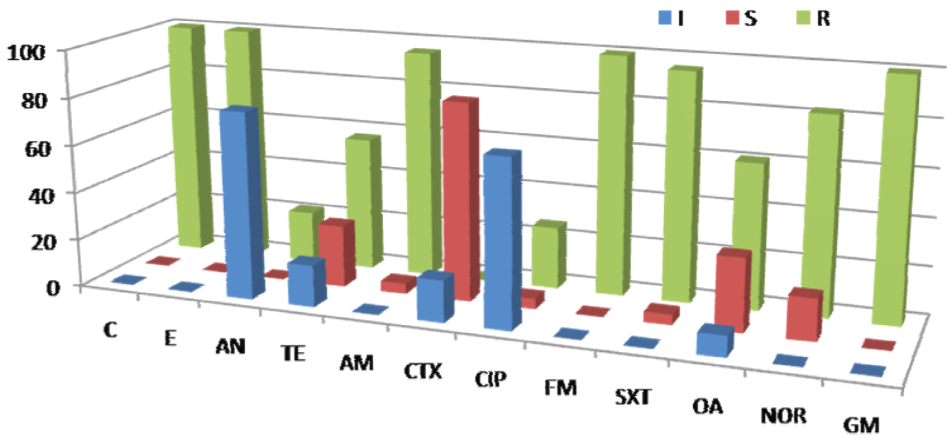

Figure 2. Percentage of resistance to 11 antibiotics of the 23 strains of *Aeromonas* spp. and *Vibrio* spp. identified from seawater and sediment during summer 2006. Antibiotics tested are as follow: C: Chloramphenicol (30 µg); E: Erythromycin (15 µg); AN: Nalidixic Acid (30 µg); TE: Tetracycline (30 UI); AM: Ampicillin (10 µg); CTX: Cefotaxim (30 µg); CIP: Ciprofloxacin (5 µg); FM: Nitrofurantoin (300 µg); SXT: Trimethoprim–sulphamethoxazole 25 µg (1.25+23.75); OA: Oxolinic acid (10 µg); NOR: Norfloxacin (5 µg); GM: Gentamycin (10UI).

strains) and *Vibrio* spp. (8 strains) tested on API 20 NE strips demonstrated the heterogeneity of the two populations studied (Table 2). In fact, out of 13 *A. hydrophila* strains tested, eleven biotypes were identified and five biotypes were identified out of six *V. alginolyticus* strains tested. All *A. hydrophila* strains were identified as Gram-negative motile fermentative bacilli, producing enzymes like catalase and oxidase, susceptible to Vibriostatic compound O/129. In this study, the identification procedure of environmental *Vibrio* and *Aeromonas* strains using the API 20 NE strips was modified in order to inoculate a loopful of a bacterial suspension prepared on sterile saline water (2.5% NaCl). All strains tested with this modification and using the API Web software were successfully identified with a high level of acceptance. In previous studies, we reported the isolation and characterization of large populations of *V. alginolyticus* strains isolated from the same area associated with a high mortality affecting *S. aurata* larvae using API 20 E and RapID^{TM} NF plus strips (Snoussi et al., 2008a; 2008b).

On API 20 NE strips, all strains were positive for the following tests: reduction of nitrates, hydrolysis of esculin and assimilation of N-acetyl-glucosamine. All strains grew in peptone water prepared, respectively with 3 and 8% of NaCl. The two *A. salmonicida* spp. *salmonicida* strains were positive for: reduction of nitrates, glucose fermentation, hydrolysis of esculin and gelatine, β-galactosidase and assimilation of the following substrates: D-glucose, D-maltose, D-mannitol, β-galactosidase, potassium gluconate and N-acetyl-glucosamine. All *V. alginolyticus* strains (n = 6) and *V. vulnificus* strains (n = 2) grew in peptone water prepared respectively with 3, 8 and 10% of NaCl. These vibrios were positive for: nitrate reductase, indole production, glucose fermentation, hydrolysis of esculin and gelatine, and assimilation of malic acid.

For exoenzymes production, all *Aeromonas* spp. (n = 15) and *Vibrio* spp. (n = 8) strains produced several hydrolytic enzymes including: protease, gelatinase, lipase, DNase and lecithinase. All these strains were β-haemolytic on Human Blood agar. All these data were in accordance with our previous findings regarding *V. alginolyticus* and *A. hydrophila* strains isolated from different Tunisian marine biotopes (Ben Kahla-Nakbi et al., 2006, Ben Kahla-Nakbi et al., 2007b, Ben Kahla-Nakbi et al., 2009; Snoussi et al., 2006; 2008a, 2008b, 2008c). In fact, several virulence-associated factors including adhesins, enterotoxins, hemolysins, proteases, DNases, lipases and other extracellular enzymes, have been identified in *Aeromonas* spp. Some of these bacterial products cause tissue damage and aid in establishing an infection by overcoming host defenses and by providing nutrients for bacterial proliferation (Agarwal et al., 1998).

Figure 2 represents the percentage of resistance to the selected antibiotics tested in the present study. The 23 strains of *Aeromonas* and *Vibrio* were resistant to chloramphenicol, erythromycin, nitrofurantoin and gentamycin while 95.65% of strains were resistant to ampicillin and cefotaxime. The lowest percentages of resistance were noted when acid nalidixic (21.73%) and ciprofloxacin (26.08 %) were tested.

All, the isolates were multi-resistants, defined as resistance to at least three different antibiotics with multiple resistance index ranging from 0.5 to 0.75 to *V. alginolyticus*, from 0.583 to 0.833 for *Aeromonas hydrophila* strains. The antibiotic resistance index was 0.683 for *V. alginolyticus* and 0.711 for *A. hydrophila*

Table 3. MAR index and distribution of four *V. cholerae* virulence genes in the genome of *Aeromonas* spp. and *Vibrio* spp. strains.

Strains or origin	MAR index	*V. cholerae* virulence genes			
		toxR	toxT	toxS	toxRS
A. hydrophila					
5: Sediment	0.583	+	-	-	-
6: Sediment	0.667	-	-	-	-
7: Sediment	0.667	-	-	-	-
8: Sediment	0.583	-	-	-	-
10: Sediment	0.833	-	-	-	-
11: Seawater	0.667	-	-	-	-
12: Sediment	0.75	-	-	-	-
14: Seawater	0.75	-	-	-	-
15: Sediment	0.75	-	-	-	-
17: Seawater	0.833	-	-	-	-
18: Seawater	0.667	-	-	-	-
24: Sediment	0.75	-	-	-	-
25: Sediment	0.75	-	-	-	-
A. salmonicida spp. *salmonicida*		-	-	-	-
13: Seawater	0.75	-	-	-	-
16: Sediment	0.833	-	-	-	-
V. vulnificus		-	-	-	-
9: Seawater	0.667	-	-	-	-
4: Sediment	0.75	-	-	-	-
V. alginolyticus		-	-	-	-
1: Seawater	0.583	-	-	-	-
2: Sediment	0.667	-	-	-	-
3: Sediment	0.5	-	-	-	-
19: Sediment	0.667	+	-	-	-
21: Sediment	0.667	-	-	-	-
22: Seawater	0.75	+	-	-	-
% of positive tests	13.04		0	0	

strains. The high value of AR index was recorded for the two *A. salmonicida* sp. *salmonicida* strains (0.791). All these data are summarized in Table 3. In a previous study, we studied the resistance of 28 strains isolated from the seawater of the seacoast of Khenis (Monastir) to 18 antibiotics including those tested in the present work (Snoussi et al., 2008a), and we founded different values of MAR indexes for *V. alginolyticus* strains ranging from 0.3 to 1 and a high value of antibiotic resistance index (ARI = 0.856). While, in another marine biotope (Lac of Bizerte), the multiple antibiotic resistance index of the 31 *V. alginolyticus* strains was ranging from 0.25 to 0,833 and with a low value of ARI = 0,449 (Snoussi et al., 2010).

In a recent study, Devi et al. (2009) founded that the multiple antibiotic resistance (MAR) index was found to be lower (0.2) indicating the meager resistance acquired by the isolates. Parveen et al. (1997) was the first to propose the MAR index for differentiating the sources of antibiotic pollution. The occurrence of MAR among the bacterial species could be a problem associated with transfer or resistance to other organisms of human or veterinary significance. As evident in the present study, *V. parahaemolyticus* isolates have not so far developed resistance to life saving drugs like tetracycline, nalidixic acid followed by chloramphenicol, trimethoprim and nitrofurantoin, although in a very few number of isolates, resistance was noticed towards gentamycin indicating the safety of seafood products. The significance of MAR in different ecosystem was studied by Kaspar et al. (1990) and the results of the present study are in agreement with their study.

ERIC-2 finger printing

Figure 3 represents the patterns obtained with ERIC 2 primer tested with *A. hydrophila* and *Vibrio* spp. strains isolated from seawater and sediment of the fish farm in Khenis (Monastir). This technique applied to the 23

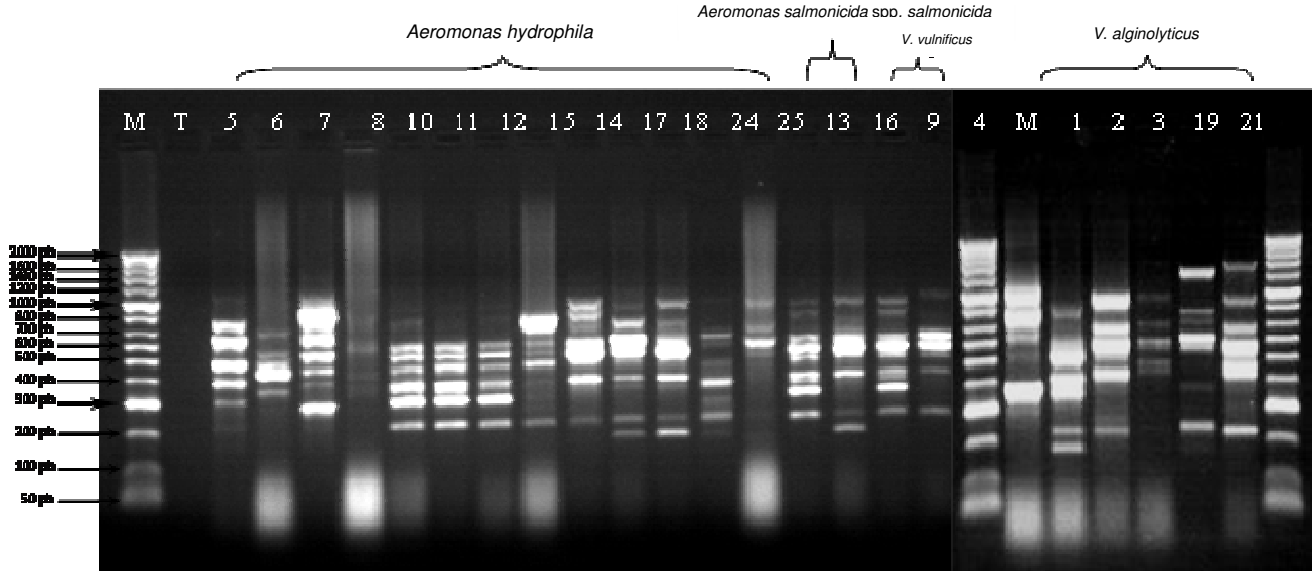

Figure 3. DNA fingerprints generated on 1% agarose gel by ERIC 2-PCR analysis of *A. hydrophila* and *Vibrio* spp. strains isolated from seawater and sediment. (M): Hyper Ladder II DNA molecular weight marquer (Bioline) and (T) negative control.

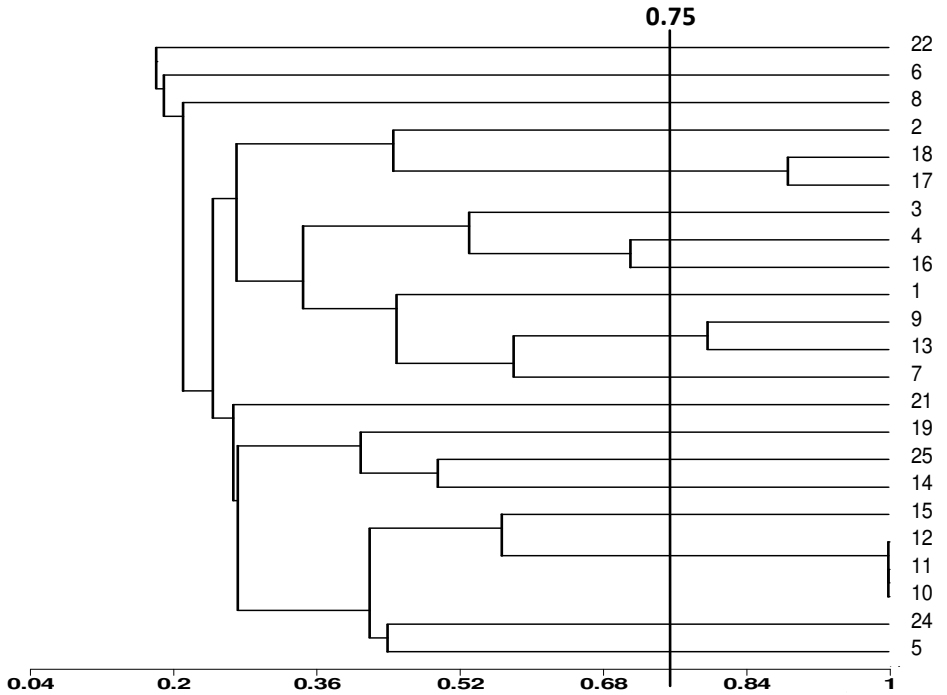

Figure 4. Dendrogram based on the unweighted pair group method of arithmetic averages and Jaccard's correlation coefficient on the basis of ERIC-2 patterns for the 23 strains of *Aeromonas* spp. and *Vibrio* spp. isolated from seawater and sediment from the aquaculture installation in Khenis. Numbers on the horizontal axis indicate the percentage of similarity.

isolates showed a reproducible patterns consisting of 4 to 9 bands ranging from 200 pb to 971 pb for *Aeromonas* spp. strains and from 181 pb to 1480 pb for *Vibrio* spp. strains estimated using the Gel Pro Analyzer 3.1 software. The phylogenetic analysis of patterns generated by ERIC 2-PCR (Figure 4) based on UPGMA method (Jaccard's coefficient) has shown that both *Vibrio* and *Aeromonas* strains exhibit high amount of heterogeneity not only according to the origin (seawater, sediment) but also within the same samples. We found in this study that

strains isolated from the same origin were genetically different as they were grouped in different clusters. Moreover, strains originated from the same sample were genetically heterogeneous as they fell into different clusters. Eleven genotypes of *Aeromonas* spp. were identified and only tree *A. hydrophila* strains originated from two different samples (seawater and sediment) were grouped into the same cluster and were considered as closely related strains (100% of similarity). The six *V. alginolyticus* strains originated from seawater and sediment were genetically heterogeneous as they were grouped in different clusters.

In a previous studies, we used the same technique (ERIC 2- PCR) to type different populations of *V. alginolyticus* strains isolated from different Tunisian marine biotopes (Snoussi et al., 2006; 2008a; 2010). In a previous study, Ben Kahla-Nakbi et al. (2006) used ERIC2-PCR fingerprinting method to type *V. alginolyticus* strains isolated from internal organs and external lesions of diseased gilthead sea bream and sea bass and their growing area harvested from a Tunisian farm and reported a high level of genomic diversity. In fact, 19 genotypes out 34 strains studied were reported by Ben Kahla-Nakbi et al. (2006) with patterns including 2 to 7 bands and there is no correlation between genotypes and season of recovery was signaled.

Distribution of virulence genes

The virulence genes toxR, toxS, toxT and toxRS were sought in the genome of all isolates by PCR (Table 3). The expected toxRS fragment was amplified from the chromosome of only two strains of *V. alginolyticus* out of six tested and interestingly from one *A. hydrophila* strain (isolate 5). All strains were negative for the amplification of toxR, toxS, and toxT virulence genes. In a previous study, we reported a large distribution of *V. cholerae* genes among the *Vibrio* spp. isolated from mussels and their growing area from the lac of Bizerte (Snoussi et al., 2010) and from Monastir-Sayada lagoon (Snoussi et al., 2008b). It is now clear that in aquatic biotopes, *V. alginolyticus* might be continually undergoing genetic change by the acquisition of DNA originated from *Vibrio cholerae* strains (Sechi et al. 2000; Deriu et al., 2002; Baffone et al., 2005; Xie et al., 2005; Snoussi et al., 2008b; 2010; Ben Kahla-Nakbi et al., 2009;).

Conclusion

In this study, we found a large distribution of *Aeromonas* and *Vibrio* strains in the marine fish farm installed in Khenis (Lagoon of Monastir-Sayada) with several virulence factors (lipolytic, proteolytic and haemolytic properties) and we confirm the use of the ERIC 2-PCR as an easy and rapid tool for the genetic typing of *Aeromonas* and *Vibrio* strains.

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