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Preliminary selection study of potential probiotic bacteria from aquacultural area in Tunisia

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Abstract - In order to test the ability to produce antibacterial substances within marine bacteria, prior to select potential probiotics for use in shellfish farming, we targeted a large collection of bacterial isolates (132 strains), brought from the clam *Ruditapes decussatus* and 37 reference strains. First, we proceeded to their biochemical identification and the screening of antibiotic resistance profiles. Else, we investigated their inhibitory activity *in vitro* against several fish and shellfish pathogens, using two methods: the double-layer agar and the direct simultaneous antagonism methods. The results showed high frequencies of inhibitory producing bacteria (IPB) within the isolates. These bacteria (25%) were aerobic mesophylic bacteria belonging to various bacterial groups: 33.7% oxidase-positive Gram-negative bacteria, 7.4% *Enterobacteriaceae* and 28% lactic acid bacteria. Besides this group, nine strains produced strong inhibition effect. These bacteria belonged to : *Aeromonas hydrophila, Aeromonas sobria*, *Pseudomonas cepacia*, *Vibrio* sp, *Serratia liquefaciens* and *Lactobacillus rhamnosus.* They were active against pathogenic bacteria belonging to the genera: *Aeromonas, Pseudomonas* and *Vibrio*. These potential probiotics were submitted to further investigations prior to their introduction in larval shellfish farming.

Key words: aquaculture; antibacterial activity; probiotic.

INTRODUCTION

High larval mortality is one of the most serious problems affecting fish and shellfish farming worldwide. These losses are generally attributed to the effects of opportunistic pathogenic bacteria, which may spread rapidly through the population (Skjermo and Vadstein, 1999). Control of these aquatic diseases was focused on the use of vaccines and antimicrobial substances. Unfortunately, the frequent use of antibiotics led to the emergence of antibiotic-resistant strains of several fish and shellfish pathogens and had thus epidemiological consequences (Gram *et al*., 1999). Vaccination against specific pathogens has been used, but is time-consuming, labour-intensive and in some instances, requires sedation of fish (Gibson, 1999). Vaccination in shellfish has not been reported. For these reasons, use of probiotic bacteria to prevent or reduce diseases has received increasing attention as an alternative method (Gatesoupe, 1999; Gomez-Gil *et al*., 2000; Verschuere *et al.,* 2000). Therefore, evaluation of the effectiveness of probiotic bacteria in aquaculture is an area of intense research and its application shown a remarkable effectiveness in several countries (Hjelm *et al*., 2004).

In Tunisia, as for the Mediterranean basin countries, the development of aquaculture was affected by microbial infections causing high mortalities at larval stages, resulting in a very low production. In addition, widespread use of antibiotics created an ecological problem for the coastal ecosystems due to the emergence of antibiotic resistant pathogen bacteria (Bouamama, 2001; Dellali, 2001; El Bour *et al*., 2001). Therefore, selection and use of probiotic bacteria capable of inhibiting pathogenic bacteria without destroying the marine ecosystem would be a welcome solution for our farming problems.

With this in mind, and primarily for their *in vivo* study as probiotics, we isolated and identified a large collection of marine bacteria isolated from shellfish *Ruditapes decussatus* (the bivalve of highest national economic importance) and investigated and compared their antagonistic activity with reference strains of marine bacteria, on the main species of fish and shellfish pathogens.

MATERIALS AND METHODS

Sampling of bivalve. Twenty-two samples of *Ruditapes decussatus* were collected from different coastal shellfish areas in Tunisia (10 zones, Fig. 1). Each sample (5 clams) was rinsed successively with sterile distilled water and alcohol 95% before opened aseptically using a sterile scalpel. The whole content of the shell (flesh and intravalvair liquid) was crushed in a stomacher, using phosphate buffered saline (PBS, pH 7.2). The bivalve samples were serially

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diluted 10-fold in physiological saline buffer up to 10^{-6} and appropriate dilutions were spread on various types of selective agar media.

FIG. 1 - Localisation of different sampling areas: B: Bizerte; T: Tunis Lake; SN: Sfax Northern zone; SC: Sfax Center; SS: Sfax Southern zone; GS: Gabes Southern zone; MN: Maghraoua; LB: Lagoon of Bougrara; MS: Hassi Jerbi; JS: El Biban.

Bacterial isolation and biochemical identification. The isolation of the different kinds of bacteria was realised using selective growth media: Desoxycholate and MacConkey agar for the selection of *Enterobacteriaceae*, King B agar for the isolation of the genus *Pseudomonas,* Man Rogosa and Sharpe (MRS) agar for the lactic acid bacteria and the Thiosulphate-Citrate-Bile Salt agar (TCBS) for the selection of *Vibrionaceae.* Tryptone Soya broth and Tryptone Soya agar supplemented with 2% NaCl (TSBS, TSAS) were used as general growth media for preparation of the inoculum for bioassays.

A total of 132 isolates were selected and picked up from the different media agar after incubation at 20 °C for 72 h. Then, the isolates were characterised on basis of colony shape, cell morphology, motility and Gram staining. Further biochemical identification was made using conventional tests (catalase and oxidase production) and rapid identification systems (e.g. the API systems: API20E, 20NE, 20Staph and 50CH, Biomerieux, France). The inoculation of the API strips was performed according to manufacturer's instructions.

A further 37 bacterial reference strains were used in this study: *Vibrio hunge* (1 strain), *Vibrio tapetis* (5), *Vibrio* sp. (5), *Vibrio logei* (1), *Vibrio alginolyticus* (1), *Vibrio hings* (1), *Vibrio haloplensis* (1), *Vibrio haliolytica* (1), *Vibrio menticus* (1) from IFREMER-Brest, France and *Aeromonas hydrophila* (5), *Aeromonas caviae* (1), *Aeromonas sobria* (1) and *Aeromonas salmonicida* (1) from the Royal Veterinary and Agricultural University, Copenhagen, Denmark (RVAU), *Vibrio proteolyticus* ATCC 15338^T, *Vibrio vulnificus* ATCC 27562^T, *Vibrio tapetis* CECT 4600^T, *Vibrio anguillarum* ATCC 12964^T, *Vibrio alginolyticus* ATCC 17749^T, *Vibrio pectidine* CIP 105190^T, *Vibrio splendidus* ATCC 33125^T, *Lactobacillus plantarum* ATCC 27700, *Lactobacillus helveticus* ATCC 15009, *Lactobacillus rhamnosus* ATCC 7449, *Lactobacillus lactis* ATCC 602 and the probiotic bacteria *Pseudomonas fluorescens* AH2 (Gram *et al*., 1999) as positive control from the Danish Institute of Piscicultural Research.

Antimicrobial susceptibility testing. The antibacterial sensitivity was determined by the agar diffusion method according to Chabbert (1982), using 15 antibiotics representing the most commonly used drugs in aquaculture (penicillin G, amoxicillin, oxacilin, cefoxitin, ceftriaxone, streptomycin, tobramycin, neomycin, chloramphenicol, tetracyclin, oleandomycin, nitrofurantoin, trimethoprim-sulphonamide, rifampicin and oxolinic acid). Inoculum of the strain to be tested was swabbed onto the surface of a Muller-Hinton agar plates, then the disks impregnated with antimicrobial agents are placed on the agar. After overnight incubation at 20 °C, the diameter of the zone of inhibition of bacterial growth around each disk was measured. Based on the zones of inhibition a qualitative report of "susceptible", "intermediate" or "resistant" can be determined for the tested bacteria according to French national guidelines (Comité de l'Antibiogramme de la Société Française de Microbiologie, 1996).

Screening for the inhibitory producer substances bacteria (IPB). Two methods were used to test the production of antibacterial substances within bacterial strains: the deferred and the direct antagonism assays. For these tests, we cross-reacted the whole isolates, which were also used as indicators.

The direct antagonism test. The method used was as described by Maduwe *et al.* (1999) modified as follows: colonies of the strains to be tested were stabled into TSAS plates previously overlaid with a TSAS soft agar (0.9%) lawn of the indicator culture. Indicator lawns were prepared by adding 0.25 ml of a 10^{-1} dilution from an overnight culture to 10 ml of TSAS soft agar. The contents of the tube were gently mixed and poured over the surfaces of prepared TSAS agar plates. After stabling with multiple strains to be tested, plates were incubated at 20 °C for 24 h. After incubation, the indicator lawns were examined for zones of inhibition surrounding each producer stab.

The deferred antagonism test. This test was realised as described by Dopazo *et al*. (1988). TSAS plates were spot inoculated with 10 µl of overnight cultures in TSBS of each bacterial strain to be tested. After incubation at 20 °C for 24 h, the colonies were exposed to chloroform vapour for 45 min. These plates were then overlaid using 6 ml of TSAS soft agar (0.9%), containing 0.1 ml of a 1/10 dilution of 16 h culture of the indicator strain. Plates were read after 24 h of incubation at 20 °C, and each isolate that showed an inhibition zone of at least 5 mm was considered inhibitory to the indicator strain. Control plates without macrocolonies of the tested strains were included to evaluate the possible effect of chloroform on the growth of target bacteria. No effect was observed.

The producers strains were classified as "weak" antagonists if the zone of inhibition did not exceed 10 mm, "medium" antagonists strains if the diameter was between 10 and 15 mm and "strong" antagonists if the diameter exceeds 15 mm.

RESULTS

Identification of the natural bivalve flora

A total of 132 strains of bacteria were isolated from *R. decussatus,* and classified into 15 taxonomic groups as follow: *Aeromonas hydrophila* (43 strains), *Pseudomonas cepacia* (23), *Vibrio alginolyticus* (17), *Klebsiella pneumoniae* (9), *Enterobacter cloacae* (8), *Serratia liquefaciens* (8), *Pseudomonas fluorescens* (6), *Vibrio parahaemolyticus* (4), *Lactobacillus delbrueckii* (3), *Aeromonas sobria* (2), *Enterobacter sakazaki* (2), *Bacillus firmus* (2), *Staphylococcus xylosus* (2), *Staphylococcus sciuri* (2), and *Staphylococcus epidermidis* (1).

The oxidase-positive Gram-negative organisms were predominant. Familly *Vibrionaceae* (*Aeromonas* spp. and *Vibrio* spp.) was the commonest isolates (50%), whereas 20% of the isolates belonged to the *Enterobacteriaceae* (*Klebsiella pneumoniae, Enterobacter cloacae, Serratia liquefaciens* and *Enterobacter sakazaki)*. The Gram-positive bacteria were less frequent (7.5%) and represented by *Staphylococcus* sp., *Bacillus* and *Lactobacillus* sp.

Antimicrobial susceptibility

According to the results obtained, we noted that all the strains tested were multiresistant to more than three different antibiotics. Resistance to the beta-lactams (oxacillin and cefoxitin), macrolids (oleandomycin) and aminoglycosides (streptomycin) was common (Fig. 2). In contrast, trimethoprim-sulphonamide, rifampicin and nitrofurantoin were the most active antibiotics against the majority of the bacterial isolates. Nevertheless, it's well known by now that administration of nitrofuran was forbidening in fish and shellfish farming.

Screening for the inhibitory producer substances bacteria (IPB)

The results of the screening for IPB were summarised in Table 1. Forty-two strains (25%) exhibited *in vitro* antibacterial activity.

The IPB strains belonged essentially to the Gram-negative oxidase-positive group (33.7%), in particular to *Aeromonas* spp. (24.2%), *Pseudomonas* spp. (7.3%) and *Vibrio* spp. (6.3%).

Among the *Aeromonas* species, 5 strains: named as O62, O70, O103, R29 and R31 showed an inhibitory effect towards a wide range of the indicators strains with large zones of inhibition (12 to 25 mm). The strain *P. cepacia* O95 showed most important antibacterial effect against all the pathogenic species tested with inhibition zones often exceeding 40 mm. In comparison, the probiotic reference strain *P. fluorescens* (AH2) gave only a weak antagonistic activity against two species of the indicator strains (*V. haloplensis* and *A. salmonicida*). Among the vibrios, the strain *Vibrio* sp. R5 produced a high inhibitory effect, since it generated inhibitory zones with diameters between 12 and 25 mm. It's active against 40% of the indicators strains.

Among *Enterobacteriaceae*, 7.4% of the strains produced inhibitory effect and the active strains belonged to the species *Serratia liquefaciens* and *Enterobacter cloacae.* The strain *Serratia liquefaciens* (O121) presented a strong antagonistic activity since it gave large zones of inhibition (from 12 to 24 mm) and it's active against all the species tested.

For the lactic acid bacteria tested, two strains (*Lactobacillus plantarum* ATCC 27700 R33 and *Lactobacillus rhamnosus* ATCC 7449 R35) gave inhibitory zones with diameters not exceeding respectively 10 and 18 mm. This activity was against mainly species of the indicators strains tested. In addition, these IPB strains selected demonstrated a resistance to the effect of their own inhibitory substances and did not inhibit each other's growth.

DISCUSSION

The emergence of multi-antibacterial resistant bacteria has become a major problem for Tunisian fish and shellfish farming during the last decades. Despite this, there has not been any investigation into the use of probiotics as an alternative to antibiotics. First to the probiotic study, we selected inhibitory producing substances bacteria (IPB) against different species of major pathogens in aquaculture systems.

Thus, we identified 132 strains isolated from clams (*R. decussatus*), wich the majority were oxidase-positive Gram-negative bacteria (*Aeromonas* and *Vibrio).* These results are in agreement with those of Martinez (1984) and Prieur (1984) who found that bacteria associated with adult and larval molluscs were predominantly Gram-negative fermentative organisms, frequently belonging to the family of *Vibrionaceae.*

In addition, antibiotic multiresistance observed for the most isolates is also consistent with previous reports (Bhattacherjee *et al.,* 1988; Pathak *et al.,* 1993; Goni-Urriza *et al*., 2000, Rhodes *et al.,* 2000). According to these studies, the increasing use of antibiotics in the medical and agricultural fields as well as the sea water discharge were the cause of enrichment of the aquatic ecosystem by the resistant bacteria. Also, the appearance of such resistant bacteria to many antibiotics is supported by the transfer of plasmids of resistance between the bacteria.

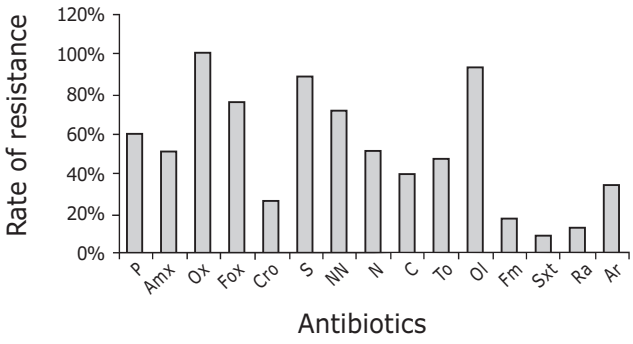

FIG. 2 - Profile of resistance obtained for the different isolates tested to antibiotics of different families. P: penicillin G; Amx: amoxicillin; Ox: oxacillin; Fox: cefoxitin; Cro: ceftriaxon; S: streptomycin; NN: tobramycin; N: neomycin; C: chloramphenicol; Te: tetracyclin; OL: oleandomycin; FM: furans; Sxt: trimethropin-sulfamid; Ra: rifampicin; Ar: oxolinic acid.

TABLE 1 - Antibaterial activity of strains tested

Antagonistic bacteria		Indicator strains																														
		O13 *P. cepacia*	O16 *P. cepacia*	O18 *P. fluorescens*	O19 *P. fluorescens*	O21 *P. fluorescens*	R10 *V. hings*	R12 *V. sp.*	R15 *V. alginolyticus*	R17 *V. haloplensis*	R21 *V. anguillarum*	O25 *V. alginolyicus*	O26 *V. alginolyicus*	O28 *V. alginolyicus*	O37 *V. parahaemolyticus*	O39 *V. parahaemolyticus*	O42 *A. hydrophila*	O44 *A. hydrophila*	O49 *A. hydrophila*	O50 *A. hydrophila*	O53 *A. hydrophila*	O55 *A. hydrophila*	O56 *A. hydrophila*	O57 *A. hydrophila*	O58 *A. hydrophila*	O59 *A. hydrophila*	O60 *A. hydrophila*	O61 *A. hydrophila*	O69 *A. hydrophila*	O75 *A. sobria*	R32 *A. salmonicida*
P. cepacia	O3	+	-	+	-	+	-	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. cepacia	O9	+	-	+	-	-	-	-	-	-	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. cepacia	O10	++	+	++	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
P. cepacia	O95	++	-	+++	-	-	++	-	-	-	+++	-	-	+++	++	+++	+++	+++	+++	+++	+++	-	++	-	+++	-	+++	++	+++	-	++
P. cepacia	O14	+	+	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. fluorescens	O19	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. fluorescens	AH2	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
V. sp.	R5	++	++	+++	-	-	-	++	-	-	+++	++	++	++	++	++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+++
V. sp.	R11	+	-	-	-	-	-	-	++	-	++	+	+	++	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
V. sp.	R12	+	-	+	-	-	-	-	+	-	-	-	-	+	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+
V. tapetis	R9	+	-	+	-	-	-	++	-	-	++	++	-	++	-	++	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
V. hings	R10	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	+
V. haloplensis	R17	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
V. anguillarum	R21	+	-	-	-	-	+	-	+	-	-	-	-	+	-	+	-	+	+	+	+	-	-	-	+	-	-	-	-	-	-	-
A. hydrophila	O44	+	-	+	-	-	-	-	-	-	+	-	-	-	-	+	+	-	+	+	-	-	-	-	+	-	+	-	+	-	-	+
A. hydrophila	O50	-	-	+	-	-	-	-	-	-	+	+	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
A. hydrophila	O56	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+	-	-	-	-	-	-	-	+	-	-	+
A. hydrophila	O57	+	-	+	-	++	-	-	-	-	+	-	-	++	-	+	+	+	-	-	-	+	-	-	-	+	-	-	-	-	-	+
A. hydrophila	O58	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	+	+	+	+	+	-	+	-	-	-	-	-	-	-	-	+
A. hydrophila	O60	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	++	+	+	+	+	-	-	-	-	-	-	-	+	-	-	+
A. hydrophila	O61	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	+
A. hydrophila	O62	++	-	-	-	-	++	-	-	-	++	-	-	++	++	++	+++	+++	+++	+++	+++	-	+	-	+	-	+	++	++	-	-
A. hydrophila	O64	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
A. hydrophila	O65	+	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
A. hydrophila	O68	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
A. hydrophila	O69	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-
A. hydrophila	O70	++	-	++	-	++	-	-	++	++	++	-	-	++	-	++	+++	+++	+++	+++	+++	-	-	-	-	++	-	-	-	-	-	-
A. hydrophila	O71	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-
A. hydrophila	O72	+	-	-	-	-	-	-	-	-	+	-	-	+	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
A. hydrophila	O103	+	+	-	-	-	++	-	-	-	++	-	-	++	++	-	++	+	++	+	+	-	+	-	+	-	+	-	-	-	-	-
A. hydrophila	O73	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	+
A. hydrophila	R28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. hydrophila	R29	++	-	++	-	++	-	-	+	++	++	-	-	++	-	++	+++	++	++	+++	+++	-	-	-	-	+	-	-	-	-	-	+
A. sobria	O74	+	-	+	-	-	-	-	-	-	+	-	-	+	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
A. sobria	O75	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
A. sobria	R31	++	-	++	-	++	-	-	-	-	++	-	-	+++	-	+++	++	+++	++	+++	+++	++	-	-	-	-	-	-	+	-	++
L. plantarum	R33	+	-	+	-	-	-	-	-	-	+	-	-	+	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+
L. rhamnosus	R35	+++	-	+++	-	++	-	-	-	-	++	-	-	++	-	++	++	-	++	++	++	+	-	-	-	+	-	-	-	-	-	++
E. cloacae	O119	+	-	+	-	+	-	-	-	-	+	-	-	+	-	-	+	+	+	+	+	+	-	-	-	+	-	-	-	-	-	+
S. liquefaciens	O121	+	-	-	-	-	-	-	-	-	++	-	-	-	++	+	++	+	+	+	++	-	+	-	-	-	+	-	-	-	-	++

(-): negative response; (+): diameter < 10 mm; (++): diameter between 10 and 15 mm; (+++): diameter > 15 mm.

As expected, ours results revealed the presence of IPB in shellfish samples. A number of earlier studies have also shown that several bacteria produce inhibitory substances that inhibit the bacterial pathogens in aquaculture systems (Nogami and Maeda, 1992; Austin *et al*., 1995; Rengpipat *et al*., 1998; Gram *et al*., 1999). The use of such bacteria to inhibit pathogens by release of antimicrobial substances is now gaining importance in fish and shellfish farming as a better and more effective alternative than administering antibiotics to manage the health of these organisms (Vijayan *et al*., 2006). Results in this study have shown that the inhibition activity rate was 25% of the whole collection tested. These results were similar to those described by Westerdahl *et al.* (1991), and by Jayanth *et al.* (2001) for marine bacteria isolates. According to these authors, the relatively significant rate of antagonistic marine bacteria could be due to the unfavourable conditions of their growth in marine environments. In contrast, Sugita *et al.*

(1996) reported that among the 304 strains isolated from the intestinal fish tract, only 3.2% exhibited antibacterial activities towards other organisms. Also, Riquelme *et al.* (1997), showed that 2.2% of the isolates of the Chilean scallop *Argopecten purpuratus* demonstrated antibacterial activity and Spanggaard *et al.* (2001) found that only 5% of the indigenous microflora of rainbow trout that were able to inhibit the growth of *V. anguillarum*. According to Nair and Simidu (1987), the absence or the low inhibitory activity of the bacteria was due to the trophic state of these bacteria, which tended to moderate the production of inhibitory substances.

In keeping with results from previous studies, ours results revealed that antagonism was common in the *Aeromonas* group (Bergh, 1995; Sugita *et al*., 1996; Gibson *et al*., 1998, 1999; Messi *et al*., 2003). Also, several studies reported the antagonistic activities of marine *Pseudomonas* (Bergh 1995; Sugita *et al*., 1996, Gram *et al*., 1999). In this study, we just isolated one strain *P. cepacia* O95 who's the most active IPB. For *Vibrio* species, several previous studies reported their antagonistic activities (Pybus *et al*., 1994; Austin *et al*., 1995; Bergh, 1995; Riquelme *et al*., 1997, 2000; Gatesoupe, 1997).

There does not appear to be any previous description of antibacterial activity associated with *Serratia* spp., as it was revealed by ours findings for *Serratia liquefaciens* O121.

In other hands, from the lactic acid bacteria tested, two strains (*Lactobacillus plantarum* R33 and *Lactobacillus rhamnosus* R35) showed an inhibitory activity against different bacterial species. Previous studies described the capacity of lactic acid bacteria to produce inhibitory compounds. In the marine environment, the majority of these bacteria belong to the genus *Carnobacterium* (Gatesoupe, 1991, 1994; Jöborn *et al.,* 1997; Gildberg and Mikkelsen, 1998; Robertson *et al.*, 2000). Spanggaard *et al*. (2001) reported that such antagonism was the most influential factor preventing the establishment of the exogenous bacteria and suggested that the antagonistic components of an indigenous flora may make a significant contribution to the control of unwanted (pathogenic) bacteria.

CONCLUSION

This preliminary report provides the starting point for investigating the use of probiotic bacteria in aquaculture in Tunisia. Potential probiotic bacteria with a broad antibacterial activity were *A. hydrophila* (4 strains)*, A. sobria* (1)*, P. cepacia* (1)*, Vibrio* sp. (1)*, Serratia liquefaciens* (1) and *Lactobacillus rhamnosus* (1)*.* An important characteristic of these strains is that they are antagonistic to several bacterial species such as *A. hydrophila,* A*. salmonicida, V. anguillarum* and *V. alginolyticus*. These species are the most common pathogenic bacteria isolated from the marine environment, causing high mortalities of fish and shellfish. These bacteria do not inhibit each other growth, so they can be used in a mixture, enhancing their effectiveness.

However, further investigations are required to study the assimilation of this potential probiotics and determination of their spectrum of action on various species and to ascertain whether or not this *in vitro* activity could be translated to the *in vivo* situation.

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