A SUMMARY DESCRIPTION OF THE MAIN BACTERIAL SPECIES PRESENT IN TUNISIAN COASTAL AREAS

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Abstract
Monitoring different bacterial populations in coastal and lagoonal areas in Tunisia allowed us to distinguish more than 600 bacterial strains from seawater, sediments, shellfish and moribund fishes. Thirty-six different aerobic and anaerobic bacterial species were isolated. *Aeromonas hydrophila* was the most dominant species isolated during the last decade (75%). Biotyping methods were used to characterize 52 different biotypes within this species. Antimicrobial typing showed that multi-resistant strains were common among *A. hydrophila*. Experimental infection assays indicated negative effect of *A. hydrophila* on the mussel (*Mytilus galloprovencialis*). Studies are currently conducted to determine the effect of oligotrophic stress upon the biochemical, molecular and virulence characteristics of *A. hydrophila*.

Keywords : bacteria, aeromonads, biotyping, survival, pathogenesis.

Introduction

By using a biomonitoring approach for several coastal regions in Tunisia, we found that different bacterial populations were widespread in these areas [1]. Aerobic and anaerobic bacterial populations showed high levels of heterogeneity [2]. The present work describes features of the main bacterial species (*Aeromonas hydrophila)* present in Tunisian coastal areas.

Materials and Methods

Sampling and isolation: different bacterial strains were isolated from costal and lagoonal Tunisian ecosystems originating from the water column, sediment, shellfish and moribund fishes during the period 1994-2001. All the bacterial strains (mesophilic and psychrophilic aerobic and anaerobic populations) were isolated on basis of growth on selective media [3s] and morphological features.

Biotyping and antibacterial sensitiveness analysis: the characterization tools included: production of catalase and oxydase enzymes and biochemical Api system profiles (Api 20E, Api 20NE, Api 50CHE and Api 32Ana) besides the Biolog test of Micromer. The antibacterial sensitivity profiles for all the bacteria isolated were detected by the standard antibiogram method.

Oligotrophic stress analysis: The ability of *Aeromonas hydrophila* strains to survive in seawater was experimentally tested in microcosms inoculated and incubated at ambient temperature (about 20°C) in the dark without shaking. Growth and survival of bacteria were followed periodically by viable counts and measurements of optical densities at wavelength (at 620nm).

Experimental infection assays of M. galloprovencialis: These assays were done to test effect of *A. hydrophila* species on mussels maintained under farming conditions. Thus, we inoculated samples of mussels with suspension of *A. hydrophila* (suspension title = 5.10^7 cells/ml). Biological modifications of *M. galloprovincialis* were detected using the index condition measured periodically.

Results and Discussion

About 600 bacterial strains (87% aerobic species and 13% anaerobic species) were isolated from diverse habitats including water, sediments, shellfish and moribund fishes. The biochemical characterization profiles indicated 11 anaerobic species and 25 aerobic species.

A. hydrophila was the most dominant species isolated (75%). According to the enzymatic and metabolic tests, we distinguished 52 different biochemical profiles within the species *A. hydrophila*. The main profiles (15 biotypes) are listed in Table 1.

The results of antibiotic sensitivity tests showed that, multi-resistance patterns were common to more than one biochemical profile of *A. hydrophila* (Fig. 1). No specific correlation was found between biotypes and antibiotic resistance profiles obtained.

Under oligotrophic conditions, *A. hydrophila* strains grew slowly during the initial 8 d of incubation. Subsequently, the viable counts decreased and no growth was detected after 158 d (Fig. 2).

Mussels infected by *A. hydrophila* showed structural modifications of gonads. The index condition measured was influenced by the *A. hydrophila* strains injected.

Conclusion

Aerobic mesophilic bacteria were abundant in coastal ecosystems. Within these populations, *A. hydrophila* was the dominant species. Its growth seems to be influenced by the oligotrophic conditions of the seawater. Bivalves harboured different kinds of aerobic mesophilic bacteria. It seems that shellfish were affected by high concentration of mesophilic aeromonads.

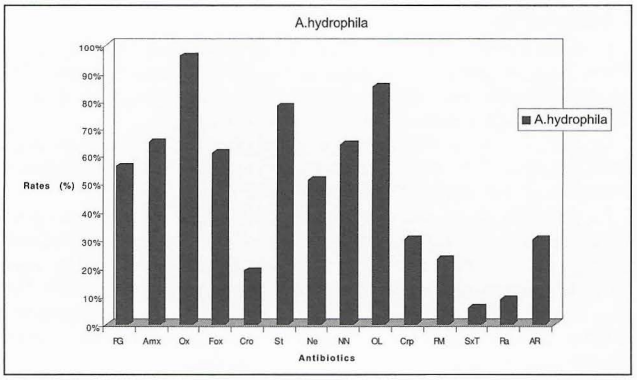

Fig. 1 : Antibacterial resistance profiles of Aeromonads *(A. hydrophila)*
Legend : PG : penicillin **G,** Amx : amoxicillin, **Ox** : oxacillin, **Fox** : cefoxitin, **Cro** : ceftriaxon, **St** : Streptomycin, **Ne** : neomycin, **AR** : flumequin, **NN** : tobramycin, **OL** : olendomycin, **C** : Chloramphenicol, **FM** : furans, **SXT** : Trimethoprim-sulfamids, **Ra** : Rifamycin

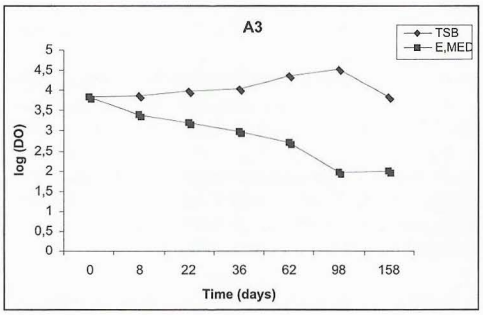

Fig. 2 : Survival curves of *A. hydrophila* in Trypcase Soja Broth (TSB) and in sea water

Table I : The main biotypes described within the species *Aeromonas hydrophila*

Référence	NO3	TRP	GLU	ADH	URE	ESC	GEL	PNG	GLU	ARA	MNE	MAN	NAG	MAL	GNT	CAP	ADI	MLT	CIT	PAC	OX	CAT
B1	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+
B6	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
B3	+	+	+	-	-	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	+	
B10	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+
B47	+	-	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+
B5	+	+	+	-	-	+	+	-	+	+	+	+	+	+	+	-	-	+	+	-	+	+
B33	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	+
B9	+	+	+	-	-	+	+	-	+	-	+	+	+	+	+	-	-	+	+	-	+	+
B14	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+
B7	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+
B11	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	-	+	+	-	+	+
B13	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+	-	-	+	+	-	+	+
B15	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	-	-	+	+	-	+	+
B39	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+
B42	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+

Legend : (+) : positive reaction to the enzymatic or metabolic test, (-) : negative reaction to the test. (No3): nitrate reduction, (TRP) : Tryptophane, (ADH) : Arginine dihydrolase, (Ure) : Urease, (ESC) : Esculine, (GEL) : Gelatinase, (PNG) : Beta galactosidase, (Ara) : Arabinose, (Mne) : Mannose, (Man) : Mannitol, (Nag) : N-acetylglucosamine, (Mal) : Maltose, (Gnt) : Gluconate, (Cap) : Caprate, (Adi) : Adipate, (Mlt) : Malate, (Cit) : Citrate, (Pac) : Phenyl acetate, (Ox) : oxydase, (Cat) : Catalase.

References

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