FIRST CONFIRMED RECORDS OF THE BROWN RING DISEASE (BRD) IN THE CLAM (*RUDITAPES DECUSSATUS*) FROM TUNISIAN WATERS (CENTRAL MEDITERRANEAN)

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Abstract

In this paper, the authors present the first confirmed records of the brown ring disease in wild populations of *Ruditapes decussatus* collected in 15 sampling sites from marine and brackish Tunisian waters. Prevalence rates ranged from 0% to 20%. By contrast, a prevalence rate of 40% was recorded for one sampling site located in southern Tunisia. The prevalence rates assessed were not related to mortality in clams populations investigated. Moreover, no strain of *V. tapetis* has been isolated.

Keywords : Bivalves, Bacteria, Diseases, Coastal Waters, Western Mediterranean.

Introduction

The brown ring disease (BRD) was reported in clam species such as *Ruditapes philipinarum* and *Ruditapes decussatus* collected in France, England, Spain, Italy, Eire and beyond until Southern Korea [2-3, 5].

The BRD disturbs shell calcification process, reduces growth rate and induces shell deformation, concomitant to occurrence of a brown organic deposit on inner surface of shell, located between pallial line and shell margin [5]. This infection was frequently due to clam infestation by the bacterial species *Vibrio tapetis* or Vibrio P1 [1, 4].

In the present study, we report and comment the first BRD records observed in adult wild populations of *R. decussatus* collected in both marine and brackish Tunisian waters.

Materials and Methods

Sampling and processing:

Wild clams were collected from 15 different areas in the Tunisian inshore during December 2000. Further survey was realized for clams harvested monthly from four northern sites (northern Lake of Tunis, Canal of navigation and lagoon of Bizerte) for the period of January to October 2001.

Determination of the brown ring disease (BRD) prevalence:

Diagnosis of brown ring was realized depending on the external observations of shells (brownish organic deposit that adheres to the internal surface of the valves).

Bacterial numeration:

For all the sites, we proceeded to counting total mesophyllic charge and total *Vibrionaceaes* charge present in clams by the method of plate counting agar (PCA), using Zobell agar (ZA) and Zobell mannitol agar (ZMA).

Phenotypic characterization of pathogenic bacterial isolates:

The bacterial isolates, were selected on Zobell Mannitol Agar medium and TCBS agar and identified by standard morphological, physiological and biochemical tests including : Gram staining, motility and sporulation feature, sensitivity test to vibriostatic agent O129, oxydase test, growth temperature and using the Api systems (Api 20E, Api 20NE and Api 50CHE) and Biolog system (based on ninety six different metabolic tests). Else, the strain *V. tapetis* (CECT 4600 from IUEM - Brest collection) was used in the identification tools of ours isolates.

Results and discussion

The prevalence rates obtained for the BRD investigations in wild clams populations taken, were lower than 10% in almost areas. Nevertheless, a prevalence of 40% was noted for one southern site (O. Chabaa).

The monthly survey of BRD prevalence rates in four northern stations (northern lake of Tunis, channel of navigation, Faroua and Menzel Jemil), showed that BRD infection was more frequent in the summer than in the winter for almost stations investigated (Fig.1). All the clams investigated presented high bacterial charges that varied from 1.10^5 ufc/mg to 8.10^5 ufc/mg (for heterotrophic and mesophyllic bacteria). The phenotypic characterization (based on more than 24 different tests), allowed us to identify thirty five bacterial strains originated from BRD clams. All the isolates were Gram negative, motile non sporulating curved rods that were oxidase positive, sensitive to vibriostatic agent O129 and for almost didn't metabolize sucrose and mannitol. All these isolates were obtained for temperature incubation lower than 25°C. According to results obtained at first by the Api system profiles, five Vibrio species were identified: *V. alginolyticus* (3 strains), *V. proteolyticus* (1 strain), V . *harveyi* (1 strain), *V. vulnificus* (1 strain), *V. carchanium* (1 strain). Nevertheless, we failed to type twenty six bacterial strains by Api system and to specify the BRD bacterial agent (V.tapetis).

Although the report of the BRD in the two species of clams in northern Mediterranean countries, in Tunisia, data about bacterial disease in shellfish were lacking. Preliminary results obtained here, showed presence of this infection at very low rates. Else, prevalence rates obtained were more important in the spring period than in winter nor in summer. As this infection was observed mainly in the northern European countries (French Atlantic coast, Norway and Scotland), it could be classified as a cold water disease.

Although the *Vibrio tapetis* was incriminated in studies of tracking for the illness, it shouldn't be considered as the only condition of development of the brown ring at the bivalves [1, 5]. The failure to isolate a *Vibrio tapetis* of the infested samples prospected. This was probably due to that a recovery state. Further studies incriminated biochemical method to be not sufficient to identify this bacterial species [4, 5]. It will be useful to employ antigenic and molecular methods jointly to biochemical methods.

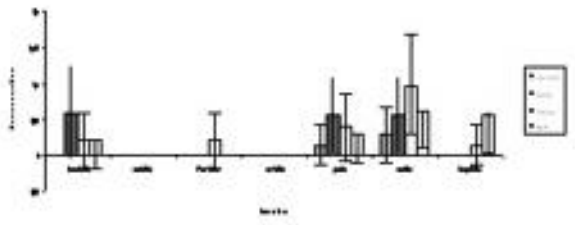

Fig. 1. BRD prelevance rates recorded in the four northern sites.

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