# ISOLATION AND SCREENING FOR PROTEASE ACTIVITY BY MARINE MICRO-ORGANISMS

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**ABSTRACT**

The aim of this study was to isolate and identify marine yeast strains from seawater, sediments seaweed, and fish/shrimp coproducts. Over six-different identified species of marine yeast, *Yarrowia lipolytica* strain having a proteolytic activity. Enzyme extracts showed that the relative optimal enzymatic activity was reached at pH = 9.0 and temperature of 45.0° C.

**RESUME**

Au cours de cette étude, des souches de levures marines ont été isolées et identifiées à partir d'eau/sédiment marins, des coproduits de poissons et d'algues marines. Les cultures ont révélé sept espèces différentes dont une, *Yarrowia lipolytica* a montré une activité protéolytique. L'extraction de l'enzyme a montré une activité enzymatique relative maximale à un pH = 9.0 et à une température 45.0°C.

## INTRODUCTION

Marine yeasts are divided into obligate and facultative groups (Kutty and Philip, 2008) with the capacity of producing various bioactive substances such as lipase (Chi at al. 2009), probiotic (Gatesoupe, 2007) and alkaline protease (Ma et al., 2007; Chi et al., 2009)

Fungal proteases have been shown to have many practical applications in detergent production, leather processing, silver recovery, medical purposes, food processing, feeds, chemical industry as well as waste treatment (Kumar and Takagi, 1999). They also contribute to the development of valuable platform applications or products by using the enzyme-aided digestion of proteins from different sources (Chi and Liu, 2006). This study aims to screen and isolate of marine yeasts with protease activities that can be used to hydrolyze fish co-products in an ecologically friendly way.

## MATERIALS AND METHODS

Different samples of seawater, sediments, seaweed (*Posidonia oceanica* and *Zostera marina*) were collected from the North of Tunisia and seafood coproducts (*Dicentrarchus labrax*, *Sparus aurata* and *Parapenaeus longirostris*) were used for yeast isolation. The yeast was cultivated in different media (YPD and Minimal-N Media) for 3-4 days as described by Chi et al., (2007). DNA extraction from each strain was done according to the method of Sambrook et al., (1989). Common primers used for the amplification of PCR were ITS1 and NL4 (Josefa et al., 2004). The amplified DNA obtained above was aligned by using BLAST analysis (http://www.ncbi.nlm.nih.gov/BLAST). The protease activity was determined as indicated by Lowry et al. (1951), Laemmli (1970), Hartee (1972), Bradford (1976) and Vijayaraghavan and Vincent (2013). Data were analyzed using *Tukey* test in *SPSS© 17.0* software.

## RESULTS AND DISCUSSIONS

From the 42 cultured strains, DNA sequencing showed only 6 different species of marine yeast which were also identified microscopically (fig. 1).

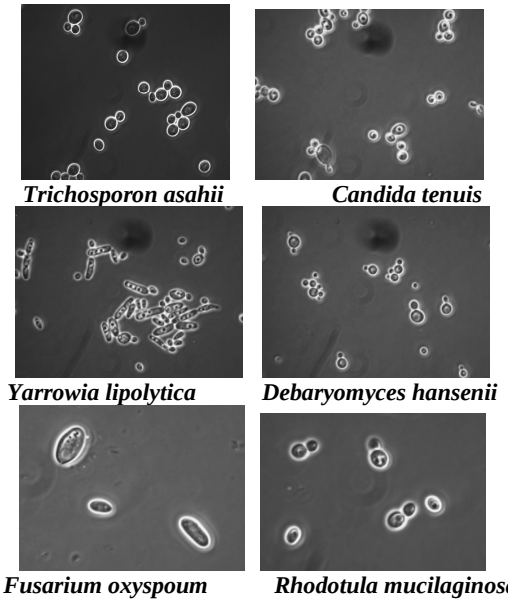


**Figure 1:** Microscopic observation of isolated strain of 6 identified species (X: 100)

Te casein plate agar assays allowed principally for qualitative determination of protease activities (fig. 2) (Alnahdi, 2012). The incubation of the isolated strains on Petri plates containing AZCL-Casein showed that only one strain *Yarrowia lypolitica* has a protease activity.

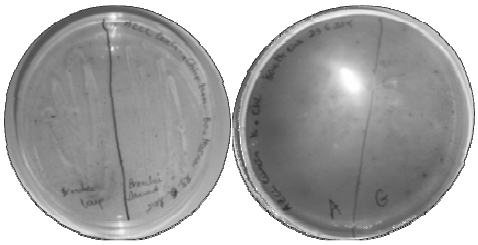

**Figure 2:** Color dispersion indicates protease activity: the left plate

This strain was then cultivated during 5 days and the crude enzyme was extracted.

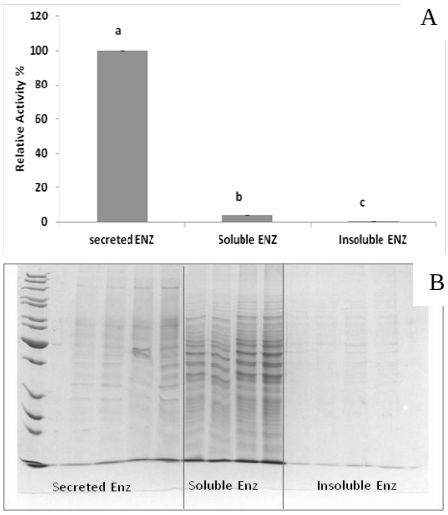


**Figure 3:** Relative activity (A) and the SDS-PAGE (B) of the crude enzyme at pH =9.0 and T= 45.0°C

The relative protease activity measured at different temperature (15.0 to 60.0°C) and pH (6.0-10.0) showed that the optimal activity was obtained at pH 9 and 45.0°C. These results suggested that the extracted enzyme was an alkaline protease (Anwar et Saleemuddin, 1998).

## CONCLUSION

This preliminary study allowed the identification of 6 different species and showed the marine yeast *Yarrowia lypolitica* isolated only from fish coproducts (*Sparus aurata* and *Dicentrarchus labrax*) produce a protease which is active at pH =9 and 45°C. A kinetic study will be performed after purification of the enzyme and determination of the protein profile.

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