Using a Satellite DNA Family for Species Identification of Commercial Oysters

I. López-Flores[1], R. de la Herrán[1], M.A. Garrido-Ramos[1], P. Boudry[2], C. Ruiz-Rejón[1], M. Ruiz-Rejón[1]

[1]Departamento de Genética, Facultad de Ciencias, Universidad de Granada. 18071, Granada.
[2]IFREMER, Laboratoire de Génétique et Pathologie, 17390 La Tremblade, France

ABSTRACT

The cultivation of oyster presents some problems related with the identification and differentiation of species as *Ostrea edulis* (European flat oyster) with respect to *Ostrea stentina* (Provence oyster) or *Crassostrea angulata* (Portuguese oyster) *vs. Crassostrea gigas* (Pacific oyster). We have characterized a satellite DNA family and used it as a molecular marker for genetic differentiation between commercial and non-commercial oyster species present in Europe. This marker clearly supports a high degree of differentiation between *O. edulis* and *O. stentina*, and, conversely upholds the contention that *C. gigas* and *C. angulata* are the same species.

INTRODUCTION

Despite numerous morphological studies our knowledge concerning the taxonomic status of the most commercially important species of oysters remains incomplete. The shape of their shells varies enormously according to the peculiarities of their habitat, even within the same species, and this makes it very difficult to identify the species of any wild individual by its morphological characteristics alone. Due to the confusion which often arises from the morphological characteristics of these bivalves, molecular biological techniques have been employed in recent years in an attempt to identify individuals on the basis of various different genetic markers (Boudry *et al.*, 1998; Amezcua, 1999). To this end the use of rapid-evolution sequences, such as satellite DNA, may contribute to the identification of very closely related oyster species.

RESULTS

We analysed four oyster species obtained from different locations (Table 1). *C. angulata* and *C. gigas* were genetically characterised previously (Boudry *et al.*, 1998).

We obtained a total of 8 monomeric units of the *Hind*III satellite DNA from *O. edulis*. The sequence length of the monomers was 166 bp. Sequence information was used for the design of specific primers for PCR amplification of *Hind*III sequences of *O. stentina*, *C. angulata* and *C. gigas* by PCR. The sequence length of the monomers was 167 bp in *O. stentina* (5 monomers) and 168 bp in *C. angulata* (4 monomers) and *C. gigas* (6 monomers) (Figure 1).

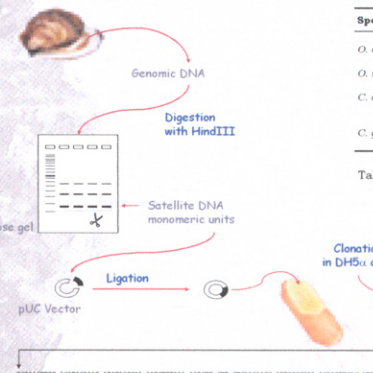
Multiple alignments of the sequences showed diagnostic positions which clearly differentiate the sequences belonging to each species except in the case of *C. angulata* compared to *C. gigas*. Between the sequences of these latter species there are no differences allowing species identification (Figure 1). Sequence analysis showed that the intraspecific variability was lower than interspecific variability for *O. edulis* and *O. stentina*. Nevertheless when the sequences of *C. angulata* and *C. gigas* were compared, interspecific variability was lower or very close to intraspecific variability. These results are reflected in a sequence tree as a grouping of sequences from *O. edulis* and *O. stentina* by taxonomic affinity strongly separated from a grouping of mixed sequences from *C. angulata* and *C. gigas* (Figure 2).

DISCUSSION

The *Hind*III satellite DNA results are a very useful marker between species of the *Ostrea* genus. At the larva stages *O. edulis* (the European flat oyster) is very difficult to distinguish from *O. stentina*, a species without commercial interest but co-existing in the wild with the former (Amezcua *et al.*, 1999). This similarity poses a serious problem when the wild spat of *O. edulis* are collected for farming. Some molecular data (allozymes and mitochondrial DNA) (Amezcua, 1999; Comesaña *et al.*, 2001) have begun to provide tools to solve this problem. The *Hind*III satellite DNA provides an additional marker for their taxonomic identification because these sequences are highly differentiated between *O. edulis* and *O. stentina*.

The taxonomic status of *C. angulata* and *C. gigas* has been debated for a long time. Ecophysiological characteristics (see Haure *et al.*, 2003) and genetic differences in mtCOI support the idea that they are two different taxa (Boudry *et al.*, 1998). Our results based on rapidly evolving satellite DNA support the acceptance of *C. angulata* and *C. gigas* as a single species according to morphological, genetic and experimental hybridization data (see Huvet *et al.*, 2004).

MATERIAL AND METHODS



Species	Common name	Origin	Population
O. edulis	European flat oyster	Huelva, Spain	Hatchery
O. stentina	Provence oyster	Huelva, Spain	Wild
C. angulata	Portuguese oyster	Cádiz, Spain Kaoshiung, Taiwan	Wild
C. gigas	Pacific oyster	Arcachon, France Hiroshima, Japan	Wild

Table 1.- Information concerning the oyster species analysed

Figure 1. Aligned *Hind*III satellite DNA sequences of the *Ostrea* and *Crassostrea* species studied. (A) The alignment of the sequences from *O. edulis* and *O. stentina* shows18 diagnostic positions (mark with *) which allow the molecular identification of each species. **(B)** No taxonomic differences exist between *C. angulata* and *C. gigas* sequences. Sequence analysis was performed using DNAstar package and MEGA2 program.

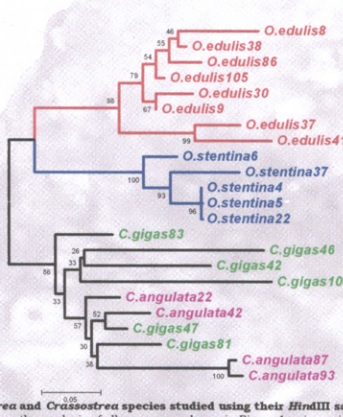


Figure 2. Sequences tree of the *Ostrea* and *Crassostrea* species studied using their *Hind*III satellite DNA sequences. The dendrogram was obtained from the analysis of all sequences shown in Figure 1 using neighbor-joining model. The bar represents genetic distance d=0.05 of pairwise comparisons calculated according to Kimura's two-parameter method. Bootstrap values (1000 replicates) supporting each node are presented to the left of the branches. Taxonomic grouping of *O. edulis* and *O. stentina* sequences are strongly supported (100%). Grouping of *C. angulata* and *C. gigas* sequences are not taxonomic neither population origin.

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