Using a Satellite DNA Family for Species Identification of Commercial Oysters

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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 angulata (Portuguese oyster) or Crassostrea gigas (Pacific oyster). We 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. angulata, and, conversely, upholds the contention that C. angulata or C. gigas 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; Amezcuia, 1999). To this end the use of rapid-evolulion 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 HindIII satellite DNA from O. edulis. The sequence length of the monomers was 166 bp. Sequence analysis was used for the design of specific primers for PCR amplification of HindIII sequences of O. edulis, C. angulata and C. gigas by PCR. The sequence length of the monomers was 167 bp in C. angulata (5 monomers) and 168 bp in 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 C. angulata. 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. angulata by taxonomic affinity strongly separated from a grouping of mixed sequences from C. angulata and C. gigas (Figure 2).

DISCUSSION

The HindIII satellite DNA results are a very useful marker between species of the Ostrea genus. At the larval 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 (Amezcuia et al., 1999). This similarity poses a serious problem when the wild sp. O. edulis is collected for farming. Some molecular data (allozymes and mitochondrial DNA) (Amezcuia, 1999; Comesaña et al., 2001) have begun to provide tools to solve this problem. The HindIII 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. Ecophysiologically 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).

REFERENCES


Figure 1. Aligned HindIII satellite DNA sequences of the Ostrea and Crassostrea species studied. (A) The alignment of the sequences from O. edulis and O. stentina shows 13 diagnostic positions (marked with *) which allow the molecular identification of each species. (B) No taxonomic differences exist between C. angulata and C. gigas sequences. Inference analysis was performed using DNAstar package and MEGA2 program.

Figure 2. Sequences tree of the Ostrea and Crassostrea species studied using their HindIII satellite DNA sequences. The dendrogram was obtained from the analysis of all sequences shown in Figure 1 using neighbor-joining method. The branches represent genetic distance deduced from 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 is strongly supported (100%). Grouping of C. angulata and C. gigas sequences are not taxonomic neither population origin.

Table 1. Information concerning the oyster species analysed.