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TECHNICAL NOTE

Applications of 5S rDNA in Atlantic salmon, brown trout, and in Atlantic salmon × brown trout hybrid identification

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The members of the salmonid family form perhaps the most economically important group of the world's fish species. In Europe the most important species employed in fish farming are the introduced rainbow trout (*Oncorhynchus mykiss*) and the Atlantic salmon (*Salmo salar*), whereas interest in the brown trout (*Salmo trutta*) is based on its ecological diversity and sport fishing. These two *Salmo* species are very difficult to distinguish using morphological characters, not only during their first months of life (eggs and alevins) but also at the returning adult stage. Isoenzyme genetic analysis, which has usually been used for their identification, has often demonstrated that anglers have confused adults of brown trout with Atlantic salmon, or with salmon × trout hybrids (Leaniz & Verspoor 1989). Caution must therefore be taken in enhancement programmes since the artificial spawning of adults caught in the river could lead to restocking with salmon × trout hybrids. Another area of interest is the identification of manufactured products of these species (i.e. smoked). Genetic analysis potentially provides the only reliable method to unambiguously determine their species identity. In addition, the genetic marker employed should be a nuclear marker if interspecific hybrids among these species have to be recognized. From a practical point of view, the procedure chosen must be fast, extremely robust, and be able to utilize tiny quantities of any tissue. This last requirement excludes most isoenzyme techniques. At present, PCR-based methodologies constitute the most reliable techniques available for this purpose. Most of these approaches are, however, based on the use of conserved mitochondrial DNA primers (for the cytochrome *b* gene) and sequencing of the amplified fragment (Pääbo *et al.* 1989; Bartlett & Davidson 1992). Instead, we have focused on the 5S ribosomal RNA genes as suitable candidates for the genetic identification of related species, since in higher eukaryotes the 5S ribosomal DNA (5S rDNA) comprises a 120 bp highly conserved coding sequence (5S rRNA) and a variable nontranscribed spacer (NTS). This unit is tandemly repeated and usually arranged head to tail. The length of this NTS does not exceed the length of the PCR amplification range (up to 2 kb, in humans) and is usually species-specific (Pendás *et al.* 1994a).

We have recently designed a set of primers, based on the conserved region of the 5S rRNA of *O. mykiss*, to amplify one unit of any tandemly arranged 5S rDNA of the salmon, brown trout and rainbow trout (Pendás *et al.* 1994a). Only one out of the two 5S rDNA amplified loci of both *Salmo* species differed in the length of the NTS, whilst the rainbow trout presented a more complex pattern. In the present work, this set of primers (A 5′-TACGCCCGATCTCGTCCGATC-3′ and B 5′-CAGGCTGGTATGGCCGTAAGC-3′, corresponding to nucleotides 1–21 and 24–45 of the 5S coding region) have been tested to amplify the whole 5S rDNA (coding + NTS) using salmon and trout DNA templates from different sources and to further examine the absence of genetic polymorphisms within species. All the PCR amplification reactions were performed in a total volume of 50 µL with 1 unit of Taq DNA polymerase and 10 ng to 50 pg of template DNA. Thirty standard amplification cycles were performed at an annealing temperature of 64 °C. Total DNA was extracted following the miniprep protocol of Taggart *et al.* (1992). As shown in Fig. 1, Atlantic salmon, brown trout and rainbow trout are clearly distinguished by running directly onto agarose gel (2%) the 5S rDNA amplification products obtained. Atlantic salmon × brown trout hybrids were easily identified since THEIR PCR amplification product includes both parental patterns. This set of primers has also been used to amplify the 5S rDNA of nonsalmonid fish species (eel, tench, carp, etc.) and nonfish species such as mussel.

Prior to the choice of a species-specific genetic marker suitable for routine application, the absence of intraspecific variability must be confirmed. For this purpose, 24 individuals from 10 Atlantic salmon populations (includ-

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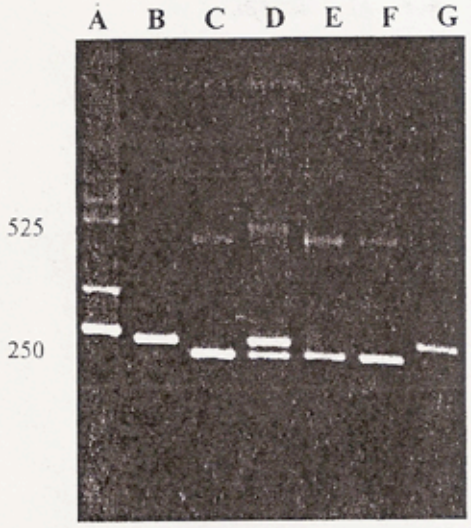

Fig. 1 Agarose gel electrophoresis (2%) of amplification products from total DNA extracted from well preserved muscle (A–D) of rainbow trout (lane A), brown trout (lane B), Atlantic salmon (lane C) and salmon × brown trout hybrid (lane D) and from partially degraded DNA (E–G) extracted from commercial smoked salmon (E), from a salmon atlas bone found in a wild otter faeces (lane F) and from a 4-year-old brown trout scale (lane G). Primers used were designed back to back to amplify the whole 5S rDNA tandem repeat (Pendás *et al.* 1994a). Molecular weights of the amplified bands are indicated in base pairs.

ing three populations from North America) and 20 individuals from 10 brown trout populations, including populations from the Mediterranean and both Atlantic races, have been screened. No differences in the size of the amplified fragments were found among the different populations within each species. Moreover, it was not even possible to detect sequence polymorphism by digesting the PCR product with ten different restriction enzymes: *Nde*II, *Eco*RI, *Bam*HI, *Ava*II, *Xba*I, *Pal*I, *Hin*dIII, *Alu*I, *Taq*I, *Dra*I and *Bgl*I. These results suggest that the 5S rDNA sequences of salmon and brown trout, although including nontranscribed sequences (NTS), are highly conserved in both species, probably due to the existence in the NTS of sequences involved in the control of transcription by RNA polymerase III (Pendás *et al.* 1994a).

The second aspect concerning the routine application of species-specific DNA markers is related to obtaining suitable DNA from small quantities of different tissues. We have applied standard protocols (i.e. Taggart *et al.* 1992) to extract amplifiable DNA from any fresh, frozen or alcohol preserved tissue, fin or bones, the results being satisfactory even when the tissue was not properly stored. Moreover, degraded DNA obtained from salmon scales preserved dry at room temperature for more than four years, from commercial smoked salmon, and from small bones, was also satisfactorily amplified (Fig. 1). Since the 5S rDNA is repeated in the genome and its NTS is quite short (less than 600 bp), the PCR amplification of small amounts of partially degraded DNA (down to 10 pg) is extremely robust and provides sufficient resolution for the identification of salmonids and brown trout × Atlantic salmon hybrids. As an example, we have recently determined by this procedure one previously supposed returning salmon from the river Eo (Spain) to be a salmon × brown trout hybrid. This result was also confirmed by isoenzyme analysis (GPI and GPM) and by the analysis of an RFLP in the histone DNA cluster (Pendás *et al.* 1994b). We have also determined by this procedure the species identity of small atlas bones from salmon and trout juveniles found in wild otter faeces.

The 5S rDNA polymorphism constitutes a novel species-specific nuclear genetic marker, and its PCR amplification is probably the simplest procedure for identifying fish species as compared to other classical techniques such as isoenzyme or RFLP analysis. This opens up the prospect of performing retrospective studies of the incidence of natural hybridization between salmon and brown trout in wild populations, as well as determining the animal origin of wild samples (small bones) when this is not possible by conventional means. In addition, we believe that the observed heterogeneity in the 5S rDNA might provide a very useful tool in taxonomic and evolutionary studies.

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