IDENTIFICATION OF STRAIN-SPECIFIC DNA-BASED MARKERS FOR THREE STRAINS OF RAINBOW TROUT

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ABSTRACT

Phenotypically similar, but genetically different, strains of rainbow trout *Oncorhynchus mykiss* are routinely used in recreational stocking programs by natural resource agencies. In South Dakota, three strains in particular, Erwin, McConaughy, and Shasta, have been stocked into the same water bodies. To enable fisheries managers to determine the strain of unmarked or untagged rainbow trout, we used RAPD (Rapid Amplification of Polymorphic DNA) primers to identify strain-specific markers. Using DNA extracted from fin tissue of known-strain fish, we tested 30 RAPD primers. Although the majority of the 30 RAPD primers tested had virtually identical PCR (Polymerase Chain Reaction) profiles among all three strains, one specific marker was identified for each strain. The RAPD technique is an accurate, low-cost, and relatively quick method to differentiate between these three strains, particularly compared to more intensive molecular techniques such as microsatellites, or traditional marking and tagging methods.

Keywords

Rainbow trout, *Oncorhynchus mykiss*, DNA, strains, RAPD

INTRODUCTION

Stocking of hatchery-reared rainbow trout *Oncorhynchus mykiss* is the primary fisheries management strategy for lakes in the Black Hills of South Dakota (Erickson and Galinat 2005). Over 200,000 rainbow trout of three different strains, Erwin, McConaughy, and Shasta, were stocked in 2004, sometimes in the same water body (Cooper et al. 2005). These strains are used because they are either available for hatchery production at different times (Erwin strain eggs are available in the fall, whereas Shasta and McConaughy are available in late

winter) or vary in the degree of domestication (Barnes et al. 2006). Unless these fish are marked prior to stocking, either by differential fin removal (Gunnes and Refstie 1980), tagging (Wydoski and Emery 1983), or chemical marking (Hall 1991), strains cannot be easily identified after their release into recreational fishing waters. Thus, if unexpected phenomena occur after stocking, such as excessive death rates or previously unobserved natural reproduction, the only option available to determine the strain(s) of fish involved is extensive and relatively expensive genetic analysis, such as with microsatellite analysis (Liu and Cordes 2004).

Random Amplified Polymorphic DNA (RAPD) analysis could be used for strain identification if specific genetic markers can be found that are associated with each strain (Williams et al. 1990; Bardakci 2001). RAPD markers are DNA fragments generated by Polymerase Chain Reaction (PCR) amplification of random segments of genomic DNA using a single, short primer of arbitrary nucleotide sequence. The RAPD method is based on PCR amplification of DNA sequences that may vary randomly, and is relatively quick, inexpensive, and yet powerful (Ramos et al. 2008). RAPD markers also exhibit Mendelian inheritance (Foo et al. 1995; Stott et al. 1997). The RAPD technique has been used to differentiate fish species (Bardakci and Skibinski 1994; Partis and Wells 1996; Callejas and Ochando 2001; Asensio et al. 2002), strains (Bardakci and Skibinski 1994; Dinesh et al. 1995; Naish et al. 1995; Bielawski and Pumo 1997; Dahle et al. 1997; Cagigas et al. 1999; Hatanaka and Galetti, Jr. 2003), and hybrids (Elo et al. 1997; Jug et al. 2004).

There is no published information regarding RAPD strain-specific markers for Erwin, McConaughy, and Shasta strain rainbow trout. Thus, the objective of this research was to identify unique markers that could be derived through the RAPD technique and subsequently used to differentiate among these three strains.

METHODS

Fin clips were obtained from Erwin, McConaughy, and Shasta strain rainbow trout reared at McNenny State Fish Hatchery, rural Spearfish, South Dakota. McNenny hatchery obtained these strains as eggs from Ennis National Fish Hatchery, Ennis, Montana. DNA from clipped fin tissue was extracted and purified using a Qiagen DNAeasy kit according to the manufacturer's recommendations. RAPD primers (10-mers) were obtained from Westburg (Pasadena, CA). PCR amplification was performed using a Qiagen HotStar kit. Reaction volumes (20 ul) included 5 picomoles of a single RAPD primer, and 40-50 ng of template DNA. All PCR were performed using a Perkin-Elmer 9700 thermocycler, and conditions were 95 °C (1 min), 40 °C (1 min), 72 °C (2 min) for 40 cycles. PCR products were fractionated in 2.2% (ultrapure) agarose gels with TBA buffer, and visualized by staining with ethidium bromide.

Ultrapure, molecular biology grade agarose (Fisher Scientific) yielded the best results for sharp resolution of DNA products. Higher relative agarose concentrations also yielded sharper DNA banding patterns that were easier to resolve

and compare. For routine screening, 2.2% agarose concentration was the best overall concentration for the RAPD primers used in this study.

After some minor modifications to the manufacturer instructions, the Qiagen DNAeasy kit was extremely effective for DNA extraction from fin clips and muscle tissue. The modified extraction protocol included dicing the tissue (fin or muscle) into smaller sections no larger than about 1.0 mm^3, and incubating 3-4 of these tissue pieces overnight (12-18 hrs) in manufacturer's lysis buffer at 50 °C with gentle rotation. For fin tissue especially, it was important to centrifuge the lysate for 4-5 minutes to pellet unlysed, mucous-like debris before transferring the supernatant to a new tube. From this point on, the manufacturer's recommended protocol was followed. It should be noted that although brief vortex steps are included in the manufacturer's instructions, at no point during DNA extraction were the samples vortexed so as to avoid the possible shearing of the large weight DNA molecules. The entire procedure, after overnight lysis, took approximately 3.5 hours for preparation and actual running of the gel.

The tissue analyzed was collected as muscle from 7 to 10 cm trout or fin clips from larger fish. Tissue was placed immediately in 100% ethanol, and stored from a few days to many months before DNA extraction was performed. All ethanol solutions were replaced with fresh solutions every week for the first three weeks of storage. Fin clips stored for over a year in ethanol also yielded high quality DNA with no apparent degradation or pattern differences in PCR amplification results.

RESULTS AND DISCUSSION

Primer A6 (sequence: 5'-GTG GGC TGA C-3') produced distinct bands for Erwin and Shasta strains (Figure 1). McConaughy strain determination was possible through Primer A8 (5'-CCA AGC TGC C Y-3') (Figure 2). The use of these primers and techniques during RAPD analysis allow for relatively quick and inexpensive strain determination. One limitation to the RAPD based

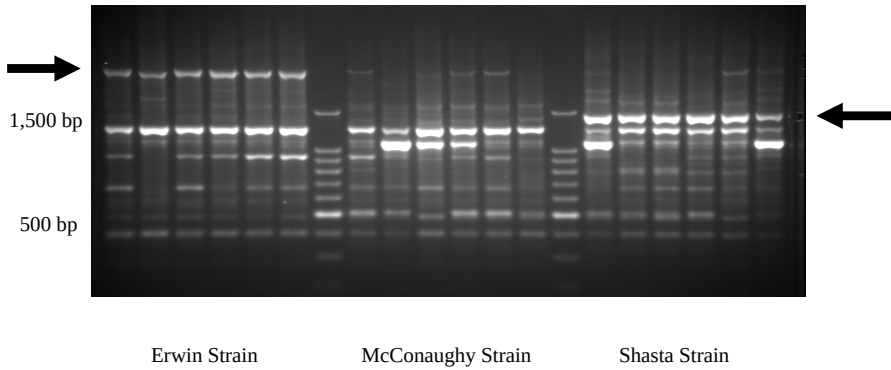

Figure 1. Banding pattern produced by primer A6 for the Erwin, McConaughy, and Shasta strains rainbow trout. Arrows denote unique bands for the Erwin and Shasta strains.

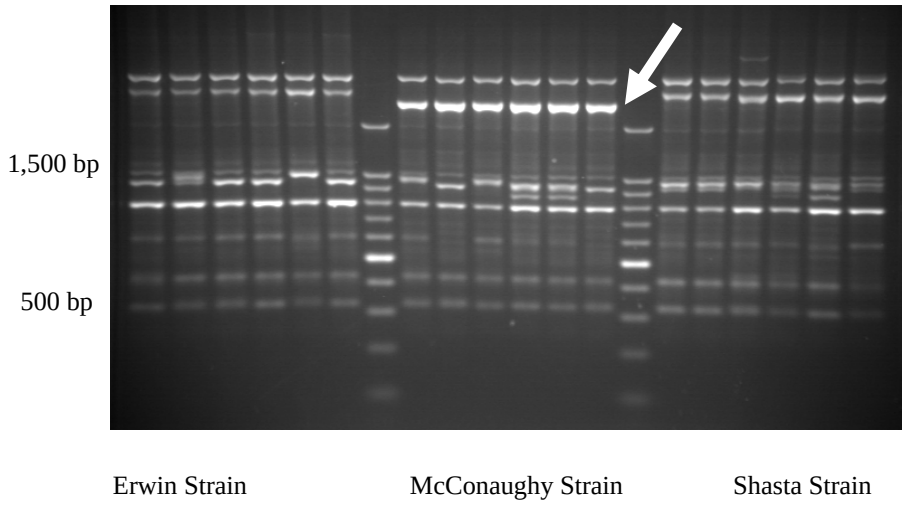

Figure 2. Banding pattern produced by primer A8 for the Erwin, McConaughy, and Shasta strains rainbow trout. Arrow denotes unique band for the McConaughy strain.

approach is that it is not possible to distinguish whether the genomic source of an amplified DNA fragment from a given locus is heterozygous (1 copy) or homozygous (2 copies), since nearly all RAPD markers are dominant (Stott et al. 1997). However, this technical limitation has negligible impact with regard to the applications reported here involving strain-specific identification.

The RAPD technique has been criticized because of poor repeatability and the production of spurious bands (Jones et al. 1997; Pérez et al. 1998; Rabouam et al. 1999; Bagley et al. 2001). However, these criticisms can be overcome with consistent laboratory procedures (Penner et al. 1993; Mitchell et al. 1994) and adequate sample sizes (Ravelo et al. 2003; Jug et al. 2004; Ramos et al. 2008).

For most of the post-stocking rainbow trout strain evaluations conducted, differential fin removal has been the predominate method used by the South Dakota Department of Game, Fish and Parks. This method has two important limitations: (1) considerable bias can be introduced due to the highly variable and differential mortality from clipping different fins (Nicola and Cordone 1973; Wydoski and Emery 1983; Pennel et al. 2001), and (2) fin-clipping is a labor intensive procedure. In addition, fins from clipped fish can also grow back (Eipper and Forney 1965), and workers that record which fins are clipped can easily make mistakes regarding fin location (e.g. right or left side of the fish; pectoral or pelvic) (Gunnes and Refstie 1980). In 2005 alone, an estimated 580 hours of labor were required for fin-clipping trout for strain evaluations by staff of the South Dakota Department of Game, Fish and Parks.

Using relatively inexpensive genetic markers and the RAPD technique described to identify rainbow trout strains eliminates the bias, inconsistencies, and labor-intensiveness associated with the standard fin clipping approach. Management decisions regarding which strains to stock in particular lakes would be greatly improved, leading to greater efficiencies and cost-savings during hatchery

production. Genetic strain identification would also produce substantial labor savings, thereby allowing for the re allocation of personnel into other resource management areas.

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