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DNA extraction from *Ascaris suum* muscle tissue

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Abstract A new method for the extraction of DNA from *Ascaris suum* muscle has been developed. It combines a standard SDS-based extraction with a plant DNA extraction procedure. The use of SDS and proteinase K allows the elimination of proteins, while CTAB and polyclar AT eliminate glycogen and polyphenols. The DNA thus obtained can easily be digested by endonucleases and amplified by PCR.

Due to improved breeding conditions in many industrialised countries, pigs are now less infected by *A. suum*. This drastically limits the availability of *Ascaris*. DNA for molecular studies is usually isolated from visceral tissues like gonads and intestine, whereas muscle and hypodermis are normally not used. Muscle tissues are relatively rich in glycogen and poor in DNA, whereas polyphenols are found in the cuticle to which the hypodermis is attached. DNA extraction has been difficult in some plant species such as grapevine (Lodhi et al. 1994), due to the presence of contaminants such as polyphenols and polysaccharides. Such DNA is not susceptible to digestion with endonucleases and cannot be used for PCR amplification. These problems were solved *using* acetyltrimethylammonium bromide (CTAB)-based extraction and a Polyclar AT (water-insoluble polyvinylpyrrolidone) purification (Lodhi et al. 1994). We used this technique and combined it with classical extraction procedures (Ausubel et al. 1995) to obtain reasonably pure DNA from *Ascaris* muscles and hypodermis.

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The gonads and gut were removed from two adult *A suum*, and the remains of the body were cut into pieces of 5 cm. The cuticle was eliminated as described by Betschart et al. (1990). Muscle and hypodermis were placed in a 50 ml tube and 10 ml of SDS-extraction buffer (100 m*M* NaCl, 10 m*M* Tris-Cl pH 8, 25 m*M* EDTA pH 8, 0.5% SDS) and 10 µl of proteinase K (Boehringer; 0.5 mg/ml) were added. The sample was incubated at 55 °C for 18 h, inverting the tube three or four times during the first 2 h. Then, 10 ml of phenol was added and mixed by inverting the tube gently for 10 min. After centrifugation at 3500 *g* for 15 min, the aqueous phase containing the DNA was extracted a second time with 10 ml of phenol:chloroform:isoamyl-alcohol (49.5:49.5:1). The white aqueous phase was transferred into a new tube and an equal volume of cold (−20 °C) 100% ethanol was added. The DNA was precipitated for 20 min at −80 °C, and spun at 3500 *g* for 10 min. The supernatant was discarded and the pellet washed with cold 70% ethanol. The ethanol was removed and the pellet resuspended in 5 ml of TE buffer (10 m*M* Tris-Cl pH8, 1 m*M* EDTA pH 8). Then 15 ml of prewarmed (60 °C) CTAB-extraction buffer (2% CTAB, 100 m*M* Tris-Cl pH 8, 20 m*M* EDTA pH 8, 1.4 m*M* NaCl, 0.2% of β-mercaptoethanol) and 50 mg of Polyclar AT (Serva, Heidelberg Germany) was added. After incubation for 30 min at 60 °C, cooling at room temperature and extraction with chloroform (v/v), the sample was spun for 5 min at 3500 *g*, the pellet was resuspended with 5 ml TE buffer and a second CTAB extraction was performed. TE buffer was added (about 30 ml), and a ultracentrifugation at 90 000 *g* for 1 h was carried out in order to sediment glycogen. The supernatant was transferred into a new tube and 2 vol cold (−20 °C) 100% ethanol were added. The DNA was precipitated for 20 min at −80 °C, the pellet was washed with 70% ethanol and resuspended in 300 µl TE buffer. The sample was then treated with RNAse (Boehringer, Mannheim Germany) for 30 min at room temperature and the DNA was quantified in a spectrophotometer. DNA was stored at −70 °C.

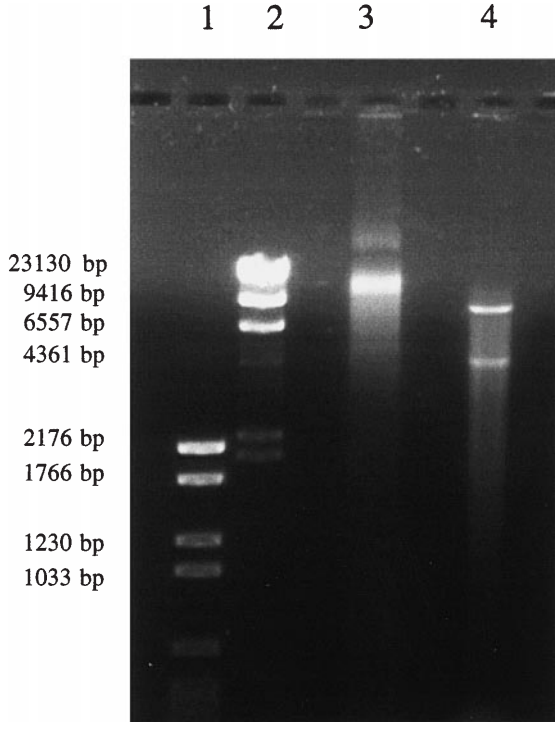

Fig. 1 *Eco*RI digestion of Ascaris suum DNA from muscle *lanes 1*, *2* markers (Boehringer: markers VI and II), *lane 3* DNA from muscle, lane 4 DNA from muscle digested with *Eco*RI). Approximately 1 μg DNA was used in each case. Samples were separated by electropphoresis (1% agarose, 0.5 × TBE) and stained with ethidium bromide

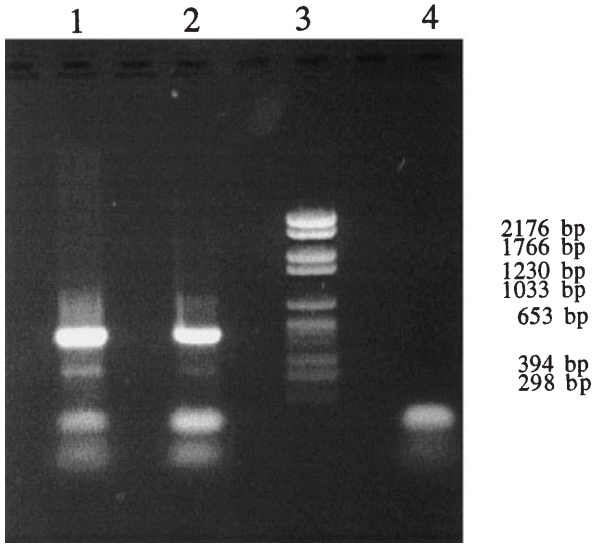

Fig. 2 PCR amplification of DNA from A. suum. DNA from gonads (*lane 1*) and muscle (*lane 2*) amplified with the following 23- and 21-base nucleotides: 5′-CAGTAGTCATATGCTTGTCTCAG-3′; 5′-TCCTTTAAGTTTCAGCTTTGC-3′. *Lane 3* DNA marker (Boehringer: marker VI). *Lane 4* negative control. Approximately 1 μg DNA was amplified in each case. Samples were separated by electrophoresis (0.8% agarose, 0.5 × TBE) and stained with ethidium bromide

DNA extracted from muscle tissue was compared to DNA isolated from gonads. The yield of DNA per worm was ~406 μg for muscle tissue and ~600 μg for gonads. DNA from muscle showed a strong band around 20 000 bp. DNA digested with *Eco*RI separated into two fragments of approximately 9000 and 6000 bp, indicating the absence of blocking substances (Fig. 1). The quality of DNA isolated from muscle tissues was compared with that isolated from gonads by PCR amplification using random primers (Fig. 2). The amplification products after electrophoresis on agarose could not be distinguished and showed an identical quantity and quality.

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