DOES DEGRADATION OF DNA IN DEAD *TRICHOGRAMMA* INDIVIDUALS LEAD TO FAILURE OF THEIR IDENTIFICATION USING ITS2 PCR ?

Américo I. Ciociola Jr., Raul P. de Almeida & Richard Stouthamer

Department of Entomology, Wageningen Agricultural University
P.O. Box 8031, 6700EH Wageningen, The Netherlands

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Summary

Trichogramma is a small wasp less than 1 mm and difficult to identify. Correct identification of *Trichogramma* is important for the success of biological control programs of lepdopteran species. Therefore, samples of *Trichogramma* need to be sent to a taxonomist for identification. *Trichogramma* species can be identified using DNA characters. When the *Trichogramma* samples are sent alive they may arrive to the taxonomist or a molecular lab already dead. DNA degradation takes place after the wasps have died. Here we study the rate of DNA degradation in wasps to determine how many days after *Trichogramma* death we can extract enough DNA to sequence the ITS2 region of the rDNA.

INTRODUCTION

Parasitoids of the genus *Trichogramma* are minute wasps, less than 1 mm in length. They are the most important egg parasitoids used in biological control programs against lepidopteran pests (Parra, 1997). The taxonomy of this wasp is difficult because of their size, and they used to be identified only by colour and setation (Stouthamer *et. al.*, 1999). Nagarkatti and Nagaraja (1971) discovered the value of male genitalia for the identification of different *Trichogramma* species, and this was the beginning of the modern taxonomy of this genus. The number of species identified using these morphological characters is approaching two hundred (Pinto, 1999). Despite the undeniable value of the morphological identification, some problems remain in species identification. Most notably the presence of parthenogenetic forms in this genus which can not be identified because no males are known.

To simplify the identification and to make it possible to identify females, a system based on the size and sequence of the Internal Transcribed Space-2 of the nuclear ribossomal cistron has been developed (Stouthamer *et al.*, 1999). To identify an individual *Trichogramma,* DNA is extracted and used a polimerase chain reaction (PCR), followed by gel-electrophoresis to determine its size. In some cases an additional restriction digest is needed to confirm identification. An important step in the identification process is the extraction of DNA. Here we study the effect of DNA degradation on durability to extract sufficient DNA from individuals when they are not properly preserved.

MATERIAL AND METHODS

Trichogramma cultures

Two populations: *T. acacioi*, from Botucatu-SP, *T. pretiosum* from ESALQ/USP, Piracicaba-SP, both from Brazil, were used in this study. The populations were reared on *Ephestia kuehniella* eggs and placed inside glass vials of 1,2 x 15,0cm and stored in climate room at 25 ± 2 °C and 70 ± 2 % of relative humidity. After emergence the wasps were killed by freezing them in –80°C. The dead wasps were subsequently placed in glass vials and stored in the climate room. 1, 5, 10, 15 and 20 days after their death the wasps were used for DNA extraction.

DNA extraction and PCR

Five individuals of each population were ground in eppendorf tubes of 0,5 µl. The wasps were ground with 100 µl of 5% Chelex-100 and 4 µl of proteinase K (20mg/ml), incubated at least 6 hours at 56°C followed by 10 minutes at 95°C. The samples used in this work were 0 (control), 1, 5, 10, 15 and 20 days after death with 6 replications. The PCR was done in a total volume of 50 µl. For one reaction, we used 5 µl of DNA template, with 45 µl of the PCR mix (5 µl (10x PCR- buffer, 1µl of dNTP's (each in a 10mM concentration), 1µl of the ITS2 forward primer (5'-TGTGAACTGCAGGACACATG-3') located in the 5.8s region of rDNA, 1 µl of the reverse primer (5'-GTCTTGCCTGCTCTGAG-3'), located in the 28s region of rDNA, 0,14 µl of TAQ polymerase 5 units/µl and 36,86 µl of distilled and autoclaved water). The primers used to amplify the ITS2 region were the same as those used by Stouthamer (1999). The termocycler program used was: 3 minutes at 94°C, followed by 33 cycles of 40 seconds at 94°C, 45 seconds at 55°C and 45 seconds at 72°C, with 5 minutes at 72°C after the last cycle. PCR products were run on a 1% agarose gel stained with ethidium bromide to determine if the PCR had been successful.

RESULTS AND DISCUSSION

DNA bands are present in 100% of the cases to *T. pretiosum* and of 83,33% to *T. acaioi* up to 10 days of death. At 15 days after death only 33 and 50% of DNA bands are visible respectively to *T. pretiosum* and *T. acaioi*. From 20 days only 16,67% of the PCR reactions on *T. pretiosum* were successful. These results allow use to estimate the correct time to send *Trichogramma* samples by mail to other institutions with enough DNA to be extracted if no preservation steps are taken. DNA bands of *T. pretiosum* and *T. acacioi* is presented in figure 1.

According to Bleicher & Parra (1989) the life cycle from egg to adult of *Trichogramma* spp in lab rearing conditions is about 11 days. If the wasps die one day after emergence we have at least 21 days after parasitism to extract DNA with success from the time that the sample is sent to other research institution. This is important information for people that want to send live *Trichogramma* samples for molecular identification. Storing and sending samples in alcohol, is another alternative, but sometimes the DNA can degrade in alcohol if the quality or the concentration of the ethanol is not sufficient (Oliveira, personal obs.). Transport in ethanol is also restricted because of the flammable nature of the ethanol. Storing insects at 4 °C in ethanol 100% or frozen at - 80°C, when is needed, seems to result in the least DNA degradation (Austin & Dillon, 1997, Dillon *et al.*, 1996, Post et al., 1993). Drying material before transport and either sending them under dry conditions or sending them with dessicant enclosed may be a good alternative as well (Post *et al.*, 1993).

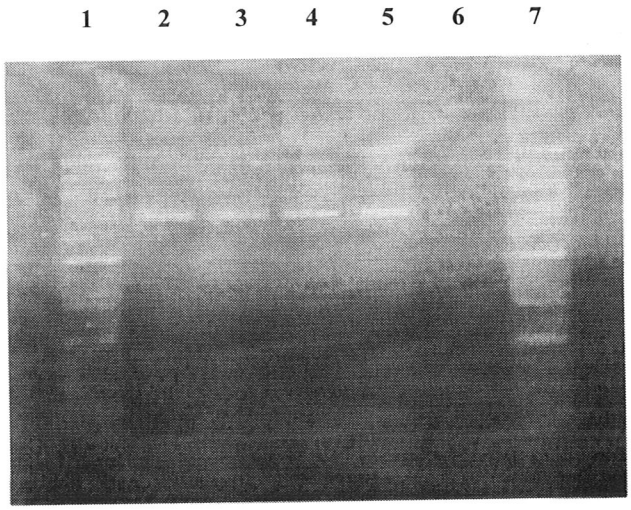

Figure 1.Agarose (1%) gel showing DNA bands of *Trichogramma*. Lane 1 and 7 are low ladder markers.10 days after death: Lane 2 (*T. pretiosum*), lane 3 (*T. acacioi).* 15 days after death: Lane 4 (*T. pretiosum*), lane 5 (*T. acacioi).* Lane 6 = Negative control.

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