DNA extraction and amplification of faecal samples from Buller's Shearwater (*Puffinis bulleri*), Fairy Prion (*Pachyptila turtur*), & Fluttering Shearwater (*Puffinus gavia*)

Report

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APPLIED MOLECULAR SOLUTIONS

Executive Summary

The intention of the current research was to test the feasibility of applying DNA-based prey item identification to analysing the diet of three New Zealand sea bird species, using ethanol preserved faecal samples. DNA was extracted from 20 faecal samples collected from Buller's shearwaters (*Puffinis bulleri*), Fluttering Shearwaters (*Puffinus gavial*), and Fairy prions (*Pachyptila turtur*), received from the Northern New Zealand Seabird Trust. PCR amplification was then used to selectively amplify short sections of the 16S gene which can be used for identification. PCR products were run on an agarose gel, as a success/fail test for amplification. DNA was successfully amplified in only three of the twenty samples despite suitably high nucleotide concentrations in the DNA extracts, indicating that the primers used may not be suitable for this research.

Introduction

Doyle & Adams (2018), using various samples from Buller's Shearwaters, found that many of the various samples collected from Buller's Shearwaters (*Puffinis bulleri*) had a large amount of oil in the sample and limited solid material. The limited amounts of solid material in these samples may have resulted in a failure to amplify DNA from these samples. Alternatively, the high oil content may have prevented the Qiagen® DNA Stool extraction method that was initially used from working effectively. After an initial failure to amplify DNA from these samples, DNA was re-extracted from four samples, three from regurgitate and one from faeces, using an isopropanol extraction method. These four isopropanol-extracted samples were then PCR amplified using primers targeting 150-200bp regions of the 16s gene of Chordata and Malacostraca. All four samples failed to amplify using the primers targeting Chordata. Amplification of Malacostraca DNA appeared to be weakly successful, with each sample producing a band in the electrophoresis gel which matched the squid (*Teuthida*) sample used as a positive control, however positive and negative amplification results were visually difficult to differentiate, with the band produced under electrophoresis being of low quality.

The current study continues on from this initial trial. DNA was extracted from faecal samples collected from three different bird species, Buller's shearwaters, Fairy Prions (*Pachyptila turtur*), and Fluttering Shearwaters (*Puffinus gavial*), to test the effectiveness of the approach when using samples with containing greater amounts of solid material.

Method

The ethanol the faecal samples were preserved in presented in a range of colours, from clear to orange. This feature was recorded for each sample. DNA was extracted from a total of twenty faecal samples, sourced from three different bird species, using an isopropanol extraction method. However, nine of these samples had a final product was significantly discoloured, suggesting high levels of contamination. These samples were re-extracted using the Qiagen® DNA Stool kit, which is designed to remove contaminants from faecal samples. Following DNA extraction, estimates of nucleotide concentrations for each sample were made using spectrophotometry.

A 155bp section of the 16S gene in Chordata was PCR amplified using the primers Chord_16S_F_TagA (5'- ATG CGA GAA GAC CCT RTG GAG CT) and Chord_16S_R_Short (5'- CCT NGG TCG CCC CAA C) (Deagle et al., 2009) in 20µL PCR reactions containing of 10µL GoTaq® PCR Master Mix (Promega), 0.8µL the forward primer (10µM), 0.8µl of the reverse primer (10µM), 1.0 µL BSA, 0.2 µL MgCl (25mM), 3µL of DNA template, and 4.2µl of MQ water. A negative control without DNA, in which 3µL of MQ water was substituted for the template DNA, was included in addition to a positive control using 3µL of whitebait (*Galaxis sp.*). A second PCR reaction was carried out using the primers Mala_16S1F (5'-TGA CGA TAA GAC CCT) and Mala_16S2R (5'- CGC TGT TAT CCC TAA AGT AAC T) (Deagle et al., 2005), which target a 200bp section of the 16S gene in Malacostraca. These PCR reactions had the same composition as those using

Chordata primers, and incorporated three positive controls using 3µL each of shrimp (*Decapoda*), squid (*Teuthida*), and muscle (*Bivalvia*) DNA, in addition to a negative control using 3µL of MQ water.

With both reactions, thermal cycling was conducted in a Surecycler 8800 (Agilent Technologies). An initial denaturation period of 15 minutes at 94°C was followed by 33 cycles of denaturation for 20 seconds at 94°C, annealing for 90 seconds at 48.7°C, and extension for 45 seconds at 72°C, followed by a final extension for 2 minutes at 72°C. PCR products were run on a 1% agarose gel and visualised in an UVIDOC HD6 Touch (UVITEC Cambridge), to determine the amplification success of each of the samples.

Results

The spectrophotometry readings for nucleotide concentration were highest for those samples extracted using the isopropanol method, with an average nucleotide concentration of 96.4ng/µL. The average nucleotide concentration of the samples extracted using the Qiagen® DNA Stool kit was 15.68ng/µL (Table 1).

Electrophoresis examination of the PCR products showed that the 3 of the 20 samples were successfully amplified using the Chordata primers (Table 1). All 3 of the samples which were successfully amplified were extracted using the isopropanol method. The samples amplified using the Malacostraca primers produced large amounts of primer dimers with no amplification seen in any of the samples.

In the current trial, shrimp, squid, and muscle samples were sourced for use in the PCR reactions targeting malacostraca, whereas only squid was used in the initial work. This change allowed for improved differentiation by providing a stronger visual example of the desired result. This small improvement to the electrophoresis allowed for the determination of the negative results to be made with greater confidence. However, this brought into question of the validity of the results from the first trial in which the four samples extracted by isopropanol appeared to have amplified. Consequently, these samples were re-amplified and found to be negative.

Table 1: Summary of DNA extractions and amplification success using different primers.

Sample	Source species	Colour of ethanol	Extraction method used	DNA concentration ng/uL	Chordata amplification	Malacostraca amplification
TA02	Fluttering Shearwater	Orange	Isopropanol	43.2	+	-
TA05	Fluttering Shearwater	Clear	Isopropanol	0.1	+	-
TA06	Fluttering Shearwater	Orange	Isopropanol	55.5	-	-
MW01	Fluttering Shearwater	Light yellow	Isopropanol	10.5	-	-
MW04	Fluttering Shearwater	Dark orange	Isopropanol	88	-	-
MW06	Fluttering Shearwater	Light orange	Isopropanol	94.7	-	-
MW08	Fluttering Shearwater	Light orange	Blood & Tissue kit	4.8	-	-
MW10	Fluttering Shearwater	Yellow	Isopropanol	68.9	-	-
TR1	Fairy Prion	Yellow	Blood & Tissue kit	0.7	-	-
TR2	Fairy Prion	Orange	Isopropanol	319.6	-	-
TR3	Fairy Prion	Orange	Isopropanol	42.9	-	-
TR4	Fairy Prion	Orange	Blood & Tissue kit	0.7	-	-
TR5	Fairy Prion	Orange	Blood & Tissue kit	-0.1	-	-
TR10	Fairy Prion	Orange	Isopropanol	328	+	-
TR12	Fairy Prion	Clear	Isopropanol	9.1	-	-
TR6	Buller's Shearwater	Yellow-orange	Blood & Tissue kit	96.1	-	-
TR7	Buller's Shearwater	Yellow	Blood & Tissue kit	4.1	-	-
TR8	Buller's Shearwater	Yellow	Blood & Tissue kit	1.5	-	-
TR9	Buller's Shearwater	Yellow	Blood & Tissue kit	2.1	-	-
TR11	Buller's Shearwater	Yellow	Blood & Tissue kit	31.2	-	-

Discussion

The colour of the ethanol used to preserve the faecal samples did not appear to correlate with amplification success, however sequence analysis could potentially show a relationship between the colour of the ethanol and the species being consumed. The estimated nucleotide concentration of the samples varied between the two extraction methods tested, with the samples extracted using the isopropanol method having an average concentration 53.7ng/µL higher than those extracted using the Qiagen® DNA Stool kit. However, the average concentration of the samples extracted using the Qiagen® Stool kit remained sufficiently high to allow for amplification by PCR.

The successful amplification of Chordata DNA indicates the presence of amplifiable DNA in the samples collected from the fluttering shear water and the fairy prion. However, with amplification only being seen in three samples of the twenty samples it is possible that chordates were not a significant component of the diet of the birds sampled. There is the possibility that some of the DNA being amplified originated from the birds themselves, as opposed to their food items.

A recent study carried out by Unitec researchers (unpublished), found the malacostraca primers to be considerably more successful when used on gut contents as opposed to faecal samples, suggesting that the DNA from this taxon is readily broken down in the digestive tract, leaving little to be detected in faecal material. This supports the literature, which suggests that there is a strong bias towards the detection of organisms which are slow to digest, while soft bodied prey may be fully digested and remain undetected (Barrett et al., 2007; Casper et al., 2007; Dunshea, 2009). This trend suggests that some food items in the sea birds' diet may go undetected in faecal samples even were they are common components of the diet.

Both the current work and the initial pilot for this study had similar difficulties with achieving amplification with both the Chordata and Malacostraca primers (Doyle & Adams, 2018). This, in additional to the high rate of failure in the current work, indicates that different primer options need to be investigated for use with faecal samples of New Zealand sea birds. This avenue is now being explored.

References

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