An Attempt at Determining the Species of Fish Eggs in Sushi through DNA Barcoding:

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Abstract

There are many different fish species whose eggs can be made into caviar. Traditional method of morphology isn't very effective at identifying the species of fish eggs. The objective of our research is to identify the species of fish eggs in sushi by DNA barcoding. DNA barcoding requires extraction of DNA and amplification of certain segment of genes. After DNA sequencing, the sample's DNA and known species' DNA in the database is aligned to identify the species of the sample. After PCR amplifies the CO1 gene in all of our samples, only one sample shown positive PCR result. However, its DNA sequence is far different from our hypothesis, and our group members try to be explain that fact. We suspect that the poor PCR quality could be a result of bad sample quality as well as property of fish eggs.

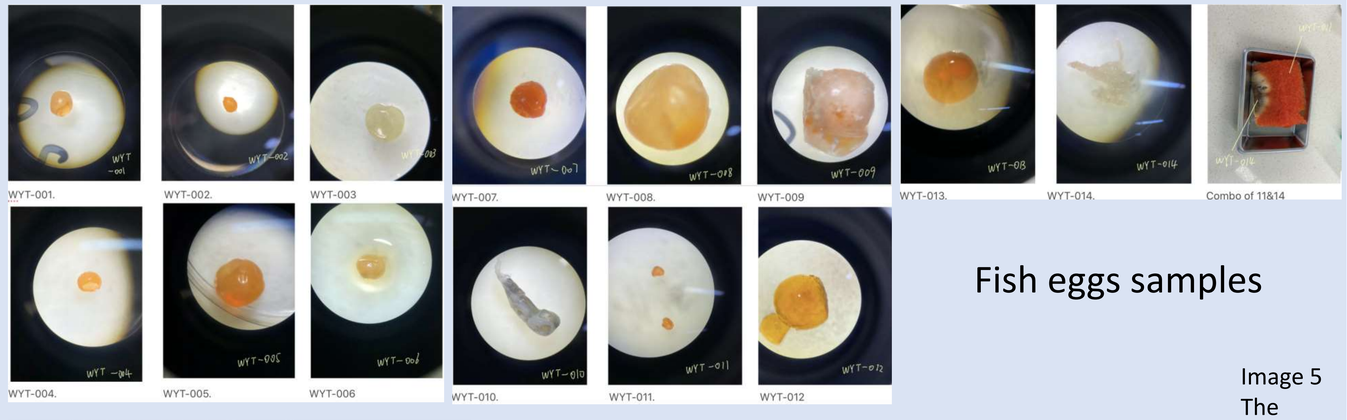
Introduction

Caviar is common food enjoyed by many people. In some sushi stores, customers cannot know which kind of fish eggs are sold from menus. But it is crucial to know what is eaten because of consumer rights, interests and health. In order to identify the fish eggs' species, we used DNA barcoding to determine the kinds of fish eggs provided by sushi stores while it is hard to differentiate fish eggs simply by morphology.

DNA barcoding can determine species by aligning samples' DNA sequence with known species' DNA sequence in the databank. DNA barcoding has lots of advantages over traditional morphology. It can analyze a sample that is an incomplete organism, organism that has different morphology in different life stages, etc. Moreover, DNA Barcoding has great potentials in terms of identifying fish eggs' species. It can be applied to marine biological conservation, anti-smuggling, bio-diversity research, and so on.

Materials & Methods

Our group collected14 samples. Samples No.1- 10 used silica method and No.1 ,2, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14 used rapid method, which turns out to be ineffective. No. 1, 2, 4-10 used Qiagen® DNeasy Blood and Tissue kit to extract DNA. No. 1, 2, 4, 6, 7, 10, 11, 12, 13, 14 used the Chelex method. We also repeated rapid method for Sample No. 4, 6, 10. Also, we used another primer to test No. 3. For all of the samples, we used COI/ Vertebrate (Fish) Cocktail for PCR. For WYT-007, we used Vertebrate (Nonfish) Cocktail. For WYT-003, we used Vertebrate (Nonfish) Cocktail for the second test.



Fish eggs samples

Image 1
The electrophoresis image of results of rapid method and silica method on 7/29

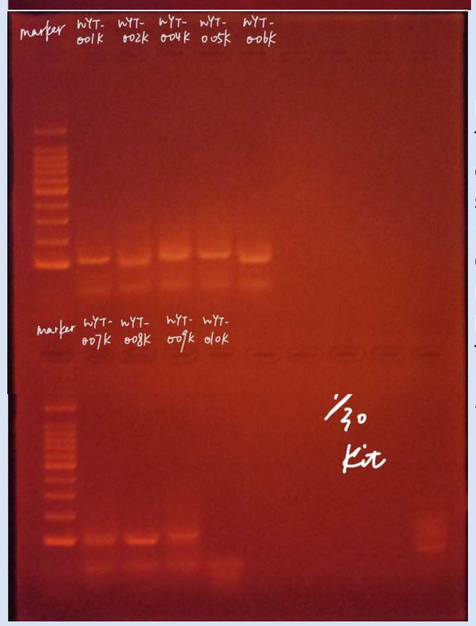


Image 4
The electrophoresis image of results of Qiagen® DNeasy Blood and Tissue kit method on 7/30

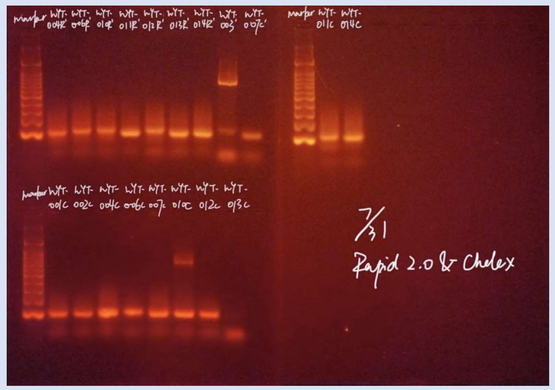


Image 5
The electrophoresis image of results of repeated rapid method and Chelex method on 7/31

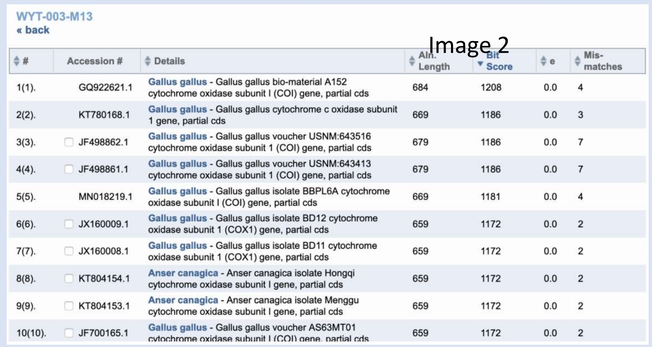


Image 2

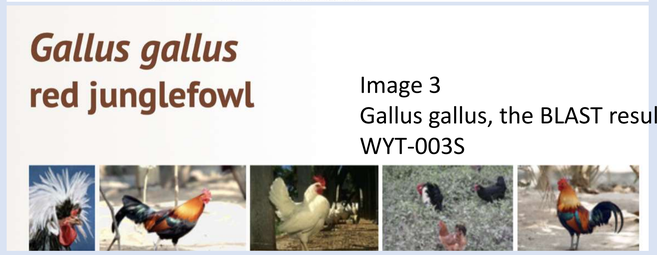


Image 3
Gallus gallus, the BLAST result of WYT-003S

Results

We employed four methods to isolate DNA from samples. First we tried the Rapid and Silica methods. In the first image we can see that only one sample was successful, which was WYT-003S, a fish egg went through Silica method. Next, we used Qiagen® DNeasy Blood and Tissue kit, but disappointingly, none of the samples had a clear band on the image as be seen on image 3. On the same day we analyzed the DNA sequence of WYT-003S on the "DNA subway" website. Its DNA sequence was BLASTed--being aligned with known species' DNA sequence in databank--and turned out to be a rooster as shown in image 2. The top 7 of the BLAST results were all Gallus gallus, which means the sample was very likely to be a rooster. At last, we used Chelex method, which was said to be effective on isolating DNA from fish eggs. The result on the image 5 indicates that WYT-003' and WYT-010C shown positive PCR result and had roughly 300-500 base pairs. We sent them to DNA sequencing, but their DNA concentration was too low and DNA sequencing failed. Our last vanished.

Discussion

During the experiment, we have encountered some difficulty when extracting the DNA of the sample. The results of our PCRs and electrophoresis are not ideal and, despite trying multiple methods, most of our extractions failed. After some discussion, we concluded that there are multiple reasons that could have caused the bad quality of the PCR. Many of our sample is processed, which could affect the quality of DNA. Anothher possible source of error is the storage of the samples, but that is unlikely because we have put all of our samples in ethanol and low temperature to preserve the DNA. One noteworthy thing is that we found out is that some of the sample, especially those of the salmon egg, is very difficult to grind during the rapid or silicon method. The solid substance in those eggs takes a long time to break down under the lysis solution. And for some samples, namely sample 5,8,9,10, had a lot of difficultly extracting the supernatant from the mixture. We suspect that this could be caused by the ethanol having somehow made the substance in the egg solidifies, which greatly increase the difficulty of grinding the sample during the rapid and silicon method, and could result in a bad DNA quality when doing the PCR.

One hypothesis that could explain the difficulty in extracting DNA is the property of fish eggs itselves. Fish eggs are the egg cells of fish, and the amount of DNA in a fish egg will be miniscule. This caused the extraction of DNA from fish egg to be very difficult, especially for larger fish eggs. One way that we have found that can reduce this difficulty is by firstly extracting the nucleus, where the genetic material is stored, out of the fish egg and than do the extraction, sadly we do not have the equipment to do such procedures.

Reference

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