DNA Barcoding---Reliable Test of Fern Phylogenies

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Abstract

DNA barcoding is widely used to identify and classify organisms. The author focuses on using DNA barcoding to identify common ornamental ferns found at a Flower-and-Fish market and concluded that this method is reliable and effective for determining fern phylogenies.

Introduction

Ferns, which belong to the phylum *Pteridophyta*, are an ancient group of plants whose ancestor first appeared more than 4 hundred million years ago, and they prove to be very valuable plants to humans. Being able to identify the correct fern genus or species that could serve a specific function would be the first step in exploiting the potential of the plants. The traditional morphology methods work on separating large groups of ferns, but it is more difficult to make finer distinctions without special knowledge and experience, so an easy and efficient method that does not require too much professional skill is needed.

DNA barcoding is a new technique developed for identifying different organisms efficiently with a standardized, sufficiently varied, easily amplified and relatively short DNA sequence that exists in all e.g plant chloroplast DNA. DNA barcoding is a simpler test for nonspecialists to do than the traditional morphology approach, which requires expertise and years of experience. Another advantage of DNA barcoding is that it only needs a tiny mass of tissue from the original organism to gain reliable results. Traditional morphology requires at least a whole organism to get the best results. Besides that, DNA barcoding safely avoids the problems of genetic and phenotype variation among the same species and evolution convergence between different species. For these reasons, DNA barcoding is used widely to help determine the phylogenetic relationships between organisms around the world.

In this paper, I focused on using DNA barcoding to identify the fern samples using other ferns with known DNA sequence from the databank, and thus proved that DNA barcoding is indeed a sufficiently accurate method to separate different fern samples collected.

Materials & Methods

The ferns were found in a Flower-and-Fish market, and newly grown leaves were collected, photographed and documented. The samples were labeled and numbered XTT-001, XTT-002, XTT-003 to XTT-015 and so on with the only exception of XTT-013a and XTT-013b, which were two leaf samples that have been mislaid and put into the same plastic bag.

I used the silica DNA extraction method to isolate DNA from all the samples and selected ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit genes (rbcL) suitable for all green leafy plants, then I employed polymerase chain reaction (PCR) to amplify the desired length of DNA sequence, and gel electrophoresis has been done to determine which of the DNA samples could be sent for sequencing. A QIAGEN DNeasy Plant Mini Kit (50) has been used on all failed samples.

Data and Analysis

Fig.1 Gel result for XTT-001 to XTT-008 (silica)

Fig.2 Gel result for XTT-009 to XTT-015 (silica)

Fig.3 Gel result for failed samples (kit)

Fig.4 Left to right: XTT-001, XTT-002, XTT-003, XTT-004

Fig.5 Left to right: XTT-005, XTT-006, XTT-007, XTT-008

Fig.6 Left to right: XTT-009, XTT-010, XTT-011, XTT-012

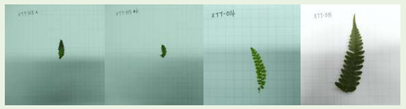

Fig.7 Left to right: XTT-013a, XTT-013b, XTT-014, XTT-015

Fig.4-7: All 16 fern samples were fresh leaves collected from healthy plants and were labeled and documented. Notice that XTT-013a and XTT-013b look very similar, but later results showed that they are actually from two rather distantly related fern genera.

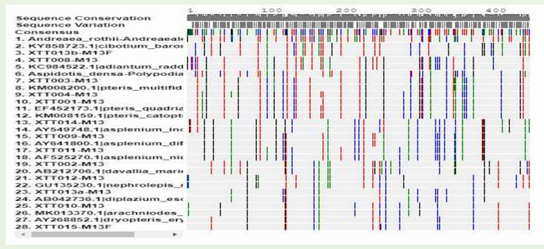

Fig.8 MUSCLE result shown in Alignment Viewer

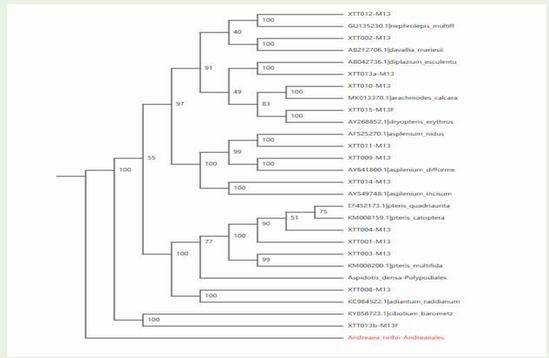

Fig.9 NJ tree. The one marked red is a moss and is used as an out group to construct the tree. The samples cover about

Results

The 16 samples were divided into two batches of 8 and went through the silica method separately, and a kit was used to repeat the whole process on all failed samples from the previous two groups. As can be seen on the results of gel electrophoresis, 14 out of 16 samples gave a positive PCR result (bright, clear bands on the top of each column), indicating that the desired length of DNA of these samples has been amplified successfully during PCR. The gels also gave evidence of functional polymerase, as in all of the columns (including those of failed samples) there is a second-to-bottom band whose number of base pairs shows that a product called primer dimer has been made by the polymerase. With a primer that can locate and bind with the DNA strands in almost ninety percent of all samples and a functional polymerase, the absence of a bright band in samples XTT-006 and XTT-007 shows that the primer did not settle down on the chloroplast DNA of these two samples and so no DNA was amplified during PCR.

After sequencing results came back (13 pairs of forward and reverse DNA sequences), I uploaded them to DNA Subway and went through the procedure to determine sequence relationships. One thing to note is that for samples XTT-013b and XTT-015, only the forward sequences were obtained, so for these two samples no consensus of two stands could be made, and the following BLAST has to be conducted with the information on the forward sequence only. The flaws of the reverse sequences could be seen visually in the Sequence Viewer. They are much shorter, and they contain many more "N"s than the other sequences, which indicate every misalignment detected. These features of the two failed sequences seem to show that very little of the target DNA segment was amplified and so DNA concentration in the PCR product was too low to sequence.

Discussion

The purpose of using DNA barcoding to sequence these fern samples in the first place is to identify them and see whether this method is accurate enough to separate the samples. This can be done by first analyzing the NJ and ML trees to get the phylogenetic relationships of each sample with the BLAST results I have selected, and then look up for photographs of the closest BLAST results taken by field researchers on a online plant database. Comparing the images I find with the results of the trees would help me determine the accuracy of DNA barcoding in distinguishing the ferns.

The NJ tree, or neighbor joining tree, gives information about the confidence level that individual samples are related to the BLAST results. The numbers on the horizontal branches range from 1 to 100, and larger the number, the more confidence one can place in the relationships shown by a particular branch. The relationships shown are not the precise species to which the samples belong, but rather the possible genus, family or order shared between the samples and BLAST results. One point of interest is that 6 samples out of 12 could be narrowed down to what genera they belong to. In the middle of the tree, there are two prevailing genera, namely *Asplenium* and *Pteris*. As the two branches at the sides of the cluster of branches that contain XTT-011, XTT-009 and XTT-014 both belong to the genus *Asplenium*, it is almost definite that these three samples all belong to that same genus. This also applies to the branch that contains all the *Pteris* ferns. The BLAST results on both sides of this branch belong to the genus *Pteris*, indicating that the samples XTT-004, XTT-001 and XTT-003 all belong to that same genus.

It is not so definite with the other samples, and the phylogenetic relationships of these samples with the ones discussed in the last paragraph may be traced further up the tree to the family or order level. It is unlikely that XTT-012 and XTT-002, located on the top of the tree, share the same genus, since the BLAST results that have the highest confidence level with each of the two samples come from different genera. XTT-013a, XTT-010 and XTT-015 belong to different genera for the same reason. XTT-008 shares a common ancestor with ferns from the genus *Pteris*, but its closest BLAST result comes from a different genus, so it might be of the same family with the *Pteris* ferns. XTT-013b evolved from an ancestor that deviated from the main branch much earlier than that of all other samples, so it can be considered to be the most distant to all the other samples, and its BLAST result, *Cibotium barometz*, is its closest possible relative.

I paired the samples with their closest BLAST results and searched for their photographs on an online plant database. Generally, all the photographs of the BLAST results fit my samples with small deviations in color and size. It is explainable, since gardeners must have artificially selected the ferns that have a particular color or size to make them more appealing to customers, so it is quite natural that the fern samples are not exactly the same with their wild counterparts.

So much for the analysis of the phylogenetic trees. As I was extracting DNA according to the silica method protocol, one procedure that may affect the whole outcome of the experiment proved to be very problematic. The original protocol stated that the tiny mass of leaves were to be grinded up with a small plastic pestle in lysis solution until there was no visible matter left, and there was no allusion to repeating this step. This is evidently a flaw in the procedure, since grinding up the matter with hand does not mean that all the cells have been sufficiently broken up. These two aspects suggested that some other method that would break up the cells more thoroughly was needed. I recommend using ultrasound to break up the cells because it ensures better rupture of cell walls in a short time.

References

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Acknowledgements

Thanks to John Mark Olson and Xinyue Wang for teaching me the techniques of DNA barcoding,
Thanks to cooperative shopkeepers of Luzhi Flower-and-Bird Market for letting me take bits off their potted ferns.