Investigation of the evolutionary relationship between edible and wild mushroom species in Suzhou through DNA Barcoding

Alessa Dai; Nora Tang; Kenneth Yan; Saber Su
Cold Spring Harbor Asia DNA Learning Center

冷泉港亚洲DNA学习中心
Cold Spring Harbor Asia DNA Learning Center

INTRODUCTION

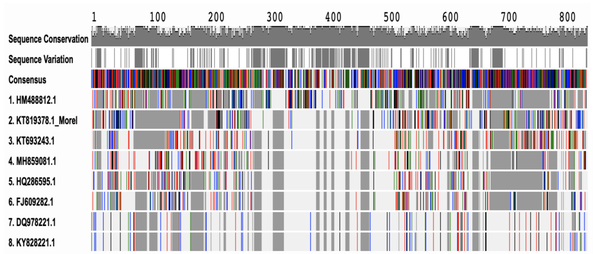
Mushrooms are commonly seen in both natural and developed environment, playing important roles in ecosystem. However, huge amount of various edible mushrooms is often thought to be the same species because of their similar characteristics. So, they are often labelled incorrectly by merchants and bought by consumers. And this can be a problem that consumers cannot get the nutrition quality equal to their cost. Therefore, we want to clearly identify their species and evolutionary relationship in molecular level. According to previous studies, ITS barcoding regions can help to identify different species. We isolated their DNA through Qiagen DNeasy Plant Kit, sequencing them with the help of GENEWIZ company and produce their DNA barcodes on DNA Subway. In our investigation, we tried to discuss two questions: 1) the feasibility of using the commercial kit for DNA extraction of fresh fungi sample, and the utility of ITS primers in PCR. 2) the identification of the common edible mushrooms in Suzhou market and their inter-species relationship.

Materials & Methods

Ten of the mushrooms were bought in the local market in Suzhou, and the other two samples were collected around the DNALC. Tissues were removed from each sample to enable the sufficient lysis. Qiagen© DNeasy Plant Kit enabled us to work out the DNA extraction rapidly and productively.PCR was used to make the DNA sequences of our samples exponentially amplified. Specially, ITS primers were used to initiate the reaction. However, we did not get any result shown in our gel analysis. Therefore, we turned to the taxonomic method. We identified them and got the DNA sequences of them on GenBank. Subsequently, we input these data to DNA Subway to get MUSCLE and finally got the DNA barcode of seven samples.Phylogenetic trees using Maximum Likelihood method allowed us to study about the evolutionary relationship of our samples.

Results & Graphs

After the first gel running of our experiment , we were not able to get the positive results from the samples that we collected. Later we think that the problems may arise during the process of DNA isolation. Then we tried silica method to isolate the DNA, but there was still almost nothing on the gel. As a result, we considered that the primers we used during the PCR (universal ITS1 &ITS4 primer) may not be suitable enough to test our samples. Since the ITS region does not work well in some highly speciose genera, such as Penicillium, Aspergillus, Cladosporium, and Fusarium as these taxa have narrow barcode gaps in their ITS regions. Moreover, the highly mutated regions in the ITS region could be hard for us to cope with. That was why we tried another way, searching the sequence of our samples from database in Genbank then do the next processes. This is our DNA barcode graph, from the graph we could not judge how much these amples are similar to each other, so we calculate the percentage of similarities for ever 2 samples. We found that none of these data was higher than 95 %, which meant that partition of species was clear, and then we turned it into the phylogenetic tree. The evolutionary relationship was showed in the following phylogenetic tree.



DNA barcode

	C	1	2	3	4	5	6	7	8
C	-	60.22	80.24	74.71	80.54	80.88	82.77	91.51	87.76
1	60.22	-	44.95	47.22	49.02	48.39	50.57	52.58	52.04
2	80.24	44.95	-	58.45	59.47	62.66	66.17	61.77	55.40
3	74.71	47.22	58.45	-	70.09	72.25	72.25	69.61	68.14
4	80.54	49.02	59.47	70.09	-	72.43	76.40	74.42	71.87
5	80.88	48.39	62.66	72.25	72.43	-	77.23	76.27	73.73
6	82.77	50.57	66.17	72.25	76.40	77.23	-	78.23	75.04
7	91.51	52.58	61.77	69.61	74.42	76.27	78.23	-	95.61
8	87.76	52.04	55.40	68.14	71.87	73.73	75.04	95.61	-

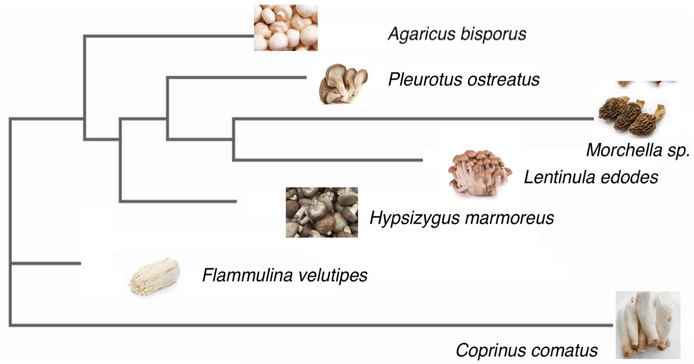
Percentage of similarity for every 2 samples

ZTT-001 ZTT-002 ZTT-003 ZTT-004 ZTT-005 ZTT-006

ZTT-007 ZTT-008 ZTT-008 ZTT-009 ZTT-010 ZTT-011 ZTT-012

Conclusion

Even though the ITS barcoding regions are vital in our experiments, we still fail to amplify them correctly. There is a Chinese proverb that ‘‘the beginning of wisdom is to call things by their proper name”. Although we know the proper Latin binomial names of those mushroom, It is still essential for us to identify them into the molecular and species level. We cannot only rely on the appearance and basic taxonomy to identify them. We need a better method—DNA barcoding to do this, and that this study could serve as a platform to build an ITS database of fungal sequences used in mushroom food industry.



Phylogenetic tree

Besides that, the primers we used during the PCR (universal ITS primer) may not be suitable enough to test our samples. Since the ITS region does not work well in some highly specific genre, such as Penicillium, Aspergillus, Cladosporium, and Fusarium as these taxa have narrow barcode gaps in their ITS regions. Moreover, the highly mutated regions in the ITS region could be hard for us to cope with. The knowledge obtained via ITS fungal barcoding can thus be used as a partial solution for product certification. According to the results, we found that many of these mushrooms with similar differences are actually the same species, and have a very close evolutionary relationship. Our future research may testify some of the mushrooms seals in the markets are actually the same species, but their names are labeled with different names and prices.

References

[1]Huzefa A. Raja, Timothy R. Baker, Jason G. Little,etc.DNA barcoding for identification of consumer-relevant mushrooms: A partial solution for product certification?[J].Food Chemistry,2017

[2] Raja, H. A., Baker, T. R., Little, J. G., & Oberlies, N. H. (2017). DNA barcoding for identification of consumer-relevant mushrooms: A partial solution for product certification?. Food chemistry, 214, 383-392.

[3] Orosz, E., Van de Wiele, N., Emri, T., Zhou, M., Robert, V., de Vries, R. P., & Pócsi, I. (2018). Fungal Stress Database (FSD)—a repository of fungal stress physiological data. Database, 2018.

[4] Vu, D., Groenewald, M., De Vries, M., Gehrmann, T., Stielow, B., Eberhardt, U., ... & Boekhout, T. (2019). Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. Studies in mycology, 92, 135-154.

Acknowledgements

We thank CSHA and Dr.P for providing experimental guidance and proper equipment. Also we thank the supermarket for providing us the source of edible mushroom.