OPEN ACCESS Asian Journal of Plant Sciences

ISSN 1682-3974
DOI: 10.3923/ajps.2022.XX.XX

Research Article
Natural Strain Observation of Button Mushroom (*Agaricus bisporus*) from South Africa Using 18S rDNA

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Abstract

Background and Objective: *Agaricus bisporus*, known as button mushroom, is an edible basidiomycete, which is distributed worldwide. The purpose of the study was to determine the native button mushroom growing in the natural land of South Africa. Besides, the white button mushroom is the most cultivated *A. bisporus* in South Africa. **Materials and Methods:** During a survey on the soil and aquatic biology and ecology in South Africa, a natural strain of button mushroom (*Agaricus bisporus*) was recovered in association with a pine tree in Sovenga Hills in Limpopo Province. Then the DNA was extracted to assess the 18S rDNA as a marker. **Results:** The NBLAST showed 99% similarity with an American strain of *A. bisporus* (acc. no. AY787216) and Chinese strain (acc. no. FJ869172). The phylogenetic analysis using the 18S rDNA marker showed that all *A. bisporus* strains could be differentiated from *A. silvaticus*. However, the South African strain of *A. bisporus* stands close to the Chinese strain of *A. bisporus*. The genetic distance indicated the highest differences between the South African and a Chinese (acc. no. JX268572) strain of *A. bisporus*. **Conclusion:** The results suggest that 18S rDNA could be a helpful marker for the natural strain of the South African *A. bisporus*. This is the first report of 18S rDNA for *A. bisporus* in South Africa.

Key words: *Agaricus bisporus*, pine tree, natural area, SSU rDNA, South Africa

Citation: Aminisarteshnizi, M., 2022. Natural strain observation of button mushroom (*Agaricus bisporus*) from South Africa using 18S rDNA. Asian J. Plant Sci., 21: XX-XX.

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The genus *Agaricus* includes the edible and poisonous types of mushrooms. However, *Agaricus bisporus* (J. Lange), a button mushroom, is globally cultivated[1]. Fresh *A. bisporus* contains Vitamins, Thiamine (B1), Riboflavin (B2), Niacin (B3), Pantothenic acid (B5), Vitamin B6, Folate (B9), Vitamin B12, Vitamin C, Vitamin D and minerals such as Iron, Magnesium, Phosphorus, Potassium, Sodium, Zinc. It is the cause of this mushroom becoming very popular and having a significant economic value. In South Africa, the white button mushroom is widely cultivated. McGee[2] reported the economic value of *A. bisporus* was US$ 4.7 billion in 2009. *Agaricus bisporus* produce mainly in North America, Europe, India and China. China is the largest producer of mushrooms globally and produces over 30 million tons (87%)[3]. Due to the daily need for protein by humans, this food source is considered a proper alternative that it is towards food security programs worldwide[4]. Therefore, mushrooms are becoming more popular in various countries, including South Africa.

Morphologically, commercial mushrooms such as *A. bisporus* are similar and producing them with tissue cultures is easy. However, this is challenging to distinguish different cultivars and detect new varieties. Therefore, the accurate identification of cultivars is required[1]. However, the lack of morphological features of mushrooms and the environment can affect their features and their identification is challenging. Molecular markers, such as Single Nucleotide Polymorphism (SNP)[5], sequence characterized amplified region (SCAR)[6,7], Restriction Fragment Length Polymorphism (RFLP)[8], Random Amplified Polymorphic DNA (RAPD)[9,10] and Inter Simple Sequence Repeat (ISSR)[11,12] have been widely used in mushroom identification, phylogenetic analysis and genetic polymorphism analysis[1]. During a survey on the ecology and biology of the organism in the natural lands in South Africa, a natural button mushroom was recovered in association with the pine tree in the Sovenga Hills, Limpopo Province. Therefore, the study aimed to identify and study the phylogenetic position of the *A. bisporus* using 18S rDNA.

MATERIALS AND METHODS

Study area: The sample was collected in the natural land in Sovenga Hills (GPS coordinates: S 23°53'41.763''E, 29°44'21.292''), in Limpopo Province, South Africa, under the pine tree. This study was carried out in December, 2020.

Molecular analysis: DNA extraction was done using the Chelex method[13]. A piece of *A. bisporus* was picked up with a sterilized needle and then transferred to a 1.5 mL Eppendorf tube containing 30 µL double distilled water. The mushroom pieces in the tube were crushed with the fine needle and vortexed. Fifty microliters of 5% Chelex® 50 and 5 µL of proteinase K were added to the tube containing crushed mushroom. These separate microcentrifuge tubes with the mushroom lysate were kept at 56°C for 2 hrs and then incubated at 95°C for 10 min to deactivate the proteinase K and finally spin for 3 min at 16000 rpm[14]. The supernatant was then extracted from each of the tubes and stored at -20°C. Following this step, the forward and reverse primers, 18s (5'-TTGATTACGTCCCTGCCCTTT–3') and 26s (5'-TTTCACTCGCCGTTACTAAGG–3'), was used to amplify the ITS rDNA. PCR was conducted with 5 µL of the DNA template, 12.5 µL of 2X PCR Master Mix Red (Promega, USA), 1 µL of each primer (10 pmol μL^{-1}) and nuclease-free water for a final volume of 25 µL. The amplification was processed using a Thermo Fisher Scientific Thermal Cycler (USA), with the following program: Initial denaturation for 3 min at 94°C, 40 cycles of denaturation for 45 sec at 94°C, 53°C annealing temperatures for ITS rDNA, extension for 45 sec to 1 min at 72°C and finally an extension step of 5 min at 72°C followed by a temperature on hold at 4°C. After DNA amplification, 5 µL of PCR product was loaded on a 1.5% agarose gel in TBE buffer (40 mM Tris, 40 mM boric acid and one mM EDTA) to observe the DNA. The bands were stained with RedGel and pictured under a UV illuminator. The PCR product was stored at -20°C. Finally, the PCR product was purified for sequencing by Inqaba Biotech Company (South Africa, Pretoria). The phylogenetic analysis was done using maximum likelihood implemented in Mega-X software[15]. The 18S rDNA sequence for *A. bisporus* from South Africa was deposited in the GenBank under the accession number MZ087879.

RESULTS AND DISCUSSION

Molecular characterization: An 860 bp for 18S rDNA was obtained from a strain of *A. bisporus* from South Africa. The sequence was analyzed and compared with other 18S rDNA sequences of *A. bisporus* available from GenBank. The NBLAST for 18S rDNA revealed a 99% similarity with the American strain of *A. bisporus* (acc. no. AY787216) and Chinese strain (acc. no. FJ869172). The genetic distance estimation ranged from 0.011-0.633. The pairwise genetic distance for the strains of *A. bisporus* revealed the highest distance (0.633) between the South African and a Chinese (acc. no. JX268572) strain of *A. bisporus* in Table 1. In contrast, the lowest genetic distance (0.011) was observed between the South African and the Chinese (acc. no. FJ869172) and American (acc. no. AY787216)

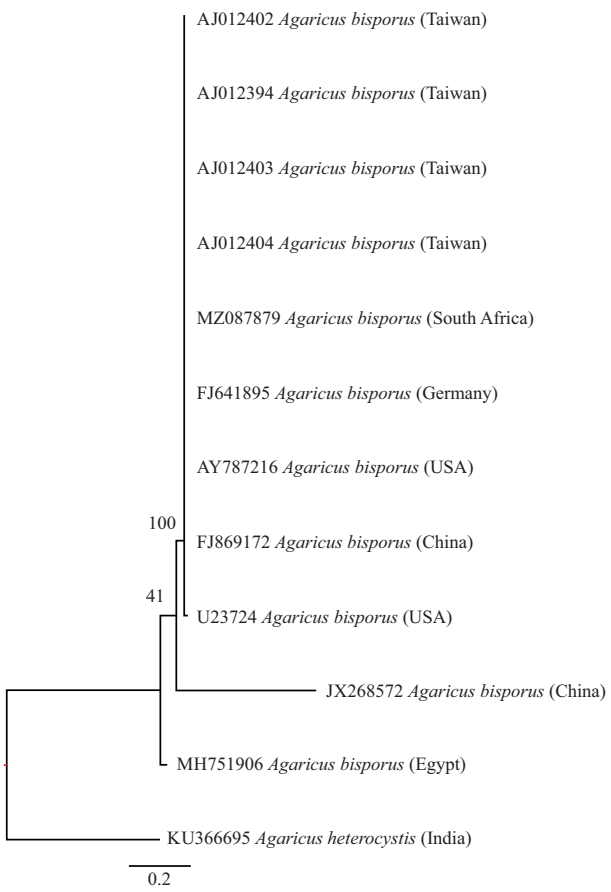

Fig. 1: Maximum likelihood tree for 18S rDNA of the button strain of *A. bisporus* recovered from South Africa under the pine tree in Sovenga Hills

Table 1: Estimation of genetic pairwise distance for *A. bisporus* strains using 18S rDNA (the upper side is a standard error)

Accession number	Locality	1	2	3	4	5	6	7	8	9	10	11
MZ087879	South Africa		0.013	0.013	0.207	17.116	0.013	0.013	0.013	0.013	0.013	0.018
FJ641895	Germany	0.011		0.000	0.202	5.872	0.000	0.000	0.000	0.000	0.000	0.011
FJ869172	China	0.011	0.000		0.202	5.872	0.000	0.000	0.000	0.000	0.000	0.011
MH751906	Egypt	0.121	0.108	0.108		5.244	0.202	0.202	0.202	0.202	0.202	17.473
JX268572	China	0.633	0.602	0.602	0.740		5.872	5.872	5.872	5.872	5.872	6.518
AY787216	USA	0.011	0.000	0.000	0.108	0.602		0.000	0.000	0.000	0.000	0.011
AJ012404	Taiwan	0.011	0.000	0.000	0.108	0.602	0.000		0.000	0.000	0.000	0.011
AJ012403	Taiwan	0.011	0.000	0.000	0.108	0.602	0.000	0.000		0.000	0.000	0.011
AJ012402	Taiwan	0.011	0.000	0.000	0.108	0.602	0.000	0.000	0.000		0.000	0.011
AJ012394	Taiwan	0.011	0.000	0.000	0.108	0.602	0.000	0.000	0.000	0.000		0.011
U23724	USA	0.021	0.011	0.011	0.121	0.631	0.011	0.011	0.011	0.011	0.011	

strain of *A. bisporus* (Table 1). In addition, the South African *A. bisporus*, showed a low genetic distance (0.011) with the Taiwanese strains (acc. no. AJ012402, AJ012403, AJ012404) of *A. bisporus*.

Phylogeny of *A. bisporus*, as derived from the corresponding tree in Fig.1, reveals a clear relationship with *A. bisporus* strains. Additionally, the *A. bisporus* strains were placed separately from the outgroup *A. heterocystis* (acc. no. KU366695). The phylogenetic analysis revealed that the South African strains placed close to the Taiwanese (acc. no. AJ012402, AJ012403, AJ012404) and American (acc. no. AY787216) strains of the same species with 100 bootstrap value. Besides, the Egyptian strains (acc. no. MH751906) were grouped separately from other *A. bisporus.* This is in agreement with the previous record by Wang *et al.*[1] using IGS rDNA.

The 18S rDNA tree placed the present South African *A. bisporus* close to the same strain from China (acc. no. FJ869172), USA (acc. no. AY787216) and Taiwan (acc. no. AJ012404) with 100 bootstrap values (Fig.1).

The small subunit ribosomal DNA (SSU) is useful for the examination of close relationships in fungus[16]. Recently, domestic breeding has been analyzed for its divergences in the nucleotide sequences of IGS1[17]. Their result indicated that *A. bisporus* is a monophyletic group, which is in agreement with the present study. Kwon *et al.*[18] showed that the IGS1 rDNA region in *A. bisporus* is not inheritable with entirely homologous sequences. It seems that it is a quickly shifting region in the process of breeding. Nevertheless, using IGS regions has been suggested for discriminatory analysis among intra-specific individuals of mushroom species[19]. DNA barcoding is an approach to rapidly identify species using short and standard genetic markers[8]. The mitochondrial cytochrome oxidase I gene (COI) has been proposed as the universal barcode locus[20], but its utility for barcoding in mushrooms has not been established. Determining and realizing the best DNA barcode markers for identifying mushrooms is essential[4]. Dentinger *et al.*[21] compared COI and ITS as DNA barcode markers for mushrooms their result indicated a similar performance of COI and ITS as a barcode. The 18S rDNA phylogenetic tree indicated that genetic variation exists among the sequenced mushrooms from South Africa and those deposited in the gene bank (https://www.ncbi.nlm.nih.gov/). However, 18S rDNA is a useful marker for the phylogenetic and molecular identification of the natural button mushroom. The result indicated that the chelex was also a suitable method for edible mushroom DNA extraction. This is the first report of *A. bisporus* using 18S rDNA from South Africa.

CONCLUSION

In molecular studies, we need to know the different methods for extracting DNA. Also, increase the credibility of work by using different primers. This study, used 18S rDNA for *A. bisporus* in South Africa for the first time. Current results suggest that 18S rDNA could be a helpful marker for the natural strain of the South African *A. bisporus*. On the other hand, the result indicated that the Chelex method was a good option for extracting DNA in *A. bisporus* which is cheap and affordable.

SIGNIFICANCE STATEMENT

This study was discovered for identification of the *A. bisporus*, using 18S rDNA was useful that can be beneficial for molecular analysis of this mushroom. Furthermore, this study will help the researchers uncover the critical areas of molecular research that many researchers could not explore. Thus, a new theory on identifying button mushrooms may be arrived at.

ACKNOWLEDGMENT

I acknowledge the Aquaculture and Genetic Lab of the Faculty of Health for using the laboratory for processing the specimen.

REFERENCES

1. Wang, L.N., W. Gao, Q.Y. Wang, J.B. Qu, J.X. Zhang and C.Y. Huang, 2019. Identification of commercial cultivars of *Agaricus bisporus* in China using genome-wide microsatellite markers. J. Integr. Agric., 18: 580-589.
2. McGee, C.F., 2018. Microbial ecology of the *Agaricus bisporus* mushroom cropping process. Appl. Microbiol. Biotechnol., 102: 1075-1083.
3. Kabel, M.A., E. Jurak, M.R. Mäkelä and R.P. de Vries, 2017. Occurrence and function of enzymes for lignocellulose degradation in commercial *Agaricus bisporus* cultivation. Appl. Microbiol. Biotechnol., 101: 4363-4369.
4. Lebel, T., 2013. Two new species of sequestrate *Agaricus* (section *Minores*) from Australia. Mycol. Prog., 12: 699-707.
5. Xu, J., H. Guo and Z.L. Yang, 2007. Single nucleotide polymorphisms in the ectomycorrhizal mushroom *Tricholoma matsutake*. Microbiology, 153: 2002-2012.
6. Su, H., L. Wang, L. Liu, X. Chi and Y. Zhang, 2008. Use of inter-simple sequence repeat markers to develop strain-specific SCAR markers for *flammulina velutipes*. J. Appl. Genet., 49: 233-235.
7. Liu, J.Y., Z.H. Ying, F. Liu, X.R. Liu and B.G. Xie, 2012. Evaluation of the use of scar markers for screening genetic diversity of *Lentinula edodes* strains. Curr. Microbiol., 64: 317-325.
8. Pawlik, A., G. Janusz, J. Koszerny, W. Małek and J. Rogalski, 2012. Genetic diversity of the edible mushroom *Pleurotus* sp. by amplified fragment length polymorphism. Curr. Microbiol., 65: 438-445.
9. Ro, H.S., S.S. Kim, J.S. Ryu, C.O. Jeon, T.S. Lee and H.S. Lee, 2007. Comparative studies on the diversity of the edible mushroom *Pleurotus eryngii*: ITS sequence analysis, RAPD fingerprinting, and physiological characteristics. Mycol. Res., 111: 710-715.
10. Sharma, S., P.K. Khanna and S. Kapoor, 2014. Molecular characterization of shiitake medicinal mushroom, *Lentinus edodes* strains (higher basidiomycetes) using RAPD and ITS sequencing. Int. J. Med. Mushrooms, 16: 169-177.
11. Du, P., B.K. Cui and Y.C. Dai, 2011. High genetic diversity in wild culinary-medicinal wood ear mushroom, *Auricularia polytricha* (Mont.) Sacc., in tropical China revealed by ISSR analysis. Int. J. Med. Mushrooms, 13: 289-298.
12. Nazrul, M.I. and B. YinBing, 2011. Differentiation of homokaryons and heterokaryons of *Agaricus bisporus* with inter-simple sequence repeat markers. Microbiol. Res., 166: 226-236.

13. Shokoohi, E., 2021. First report of *Bitylenchus ventrosignatus* (Tobar Jiménez, 1969) Siddiqi, 1986 associated with wild grass in Botswana. J. Nematol., Vol. 53. 10.21307/jofnem-2021-037.
14. Shokoohi, E. and J. Abolafia, 2021. Redescription of a predatory and cannibalistic nematode, *Butlerius butleri* Goodey, 1929 (Rhabditida: Diplogastridae), from South Africa, including its first SEM study. Nematology, 23: 969-986.
15. Kumar, S., G. Stecher, M. Li, C. Knyaz and K. Tamura, 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol., 35: 1547-1549.
16. Kwon, H.W., M.A. Choi, D.W. Kim, Y.L. Oh, M.W. Hyun, W.S. Kong and S.H. Kim, 2016. Ribosomal intergenic spacer 1 based characterization of button mushroom (*Agaricus bisporus*) strains. Mycobiology, 44: 314-318.
17. Banos, S., G. Lentendu, A. Kopf, T. Wubet, F.O. Glöckner and M. Reich, 2018. A comprehensive fungi-specific 18S rRNA gene sequence primer toolkit suited for diverse research issues and sequencing platforms. BMC Microbiol., Vol. 18. 10.1186/s12866-018-1331-4.
18. Kwon, H.W., M.A. Choi, Y.H. Yun, Y.L. Oh, W.S. Kong and S.H. Kim, 2015. Genetic and biochemical characterization of monokaryotic progeny strains of button mushroom (*Agaricus bisporus*). Mycobiology, 43: 81-86.
19. Saito, T., N. Tanaka and T. Shinozawa, 2002. Characterization of subrepeat regions within rDNA intergenic spacers of the edible basidiomycete *Lentinula edodes*. Biosci. Biotechnol. Biochem., 66: 2125-2133.
20. Férandon, C., S. Moukha, P. Callac, J.P. Benedetto, M. Castroviejo and G. Barroso, 2010. The *Agaricus bisporus cox1* gene: The longest mitochondrial gene and the largest reservoir of mitochondrial group I introns. PLoS One, Vol. 5. 10.1371/journal.pone.0014048.
21. Dentinger, B.T.M., M.Y. Didukh and J.M. Moncalvo, 2011. Comparing COI and ITS as DNA barcode markers for mushrooms and allies (*Agaricomycotina*). PLoS One, Vol. 6. 10.1371/journal.pone.0025081.