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Original Article

Amplification and sequence analysis of '*nad*1' gene from edible mushroom *Lentinula edodes*

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Abstract

Edible mushrooms are being used as food supplement worldwide for its nutritional attributes. Crop improvement through molecular breeding is adopted in mushroom for different significant targets as well. Thus the molecular genetic analysis is prerequisite and had been carried out for last few decades. *Lentinula edodes* is a popular shiitake mushroom having medicinal properties and high volume of food value and consume in mainly Asian countries. Genetic studies of *L. edodes* already been done due to its popularity in breeding programme too. Here, the NADH complex subunit is chosen first time in mushroom to standardize its amplification conditions and sequence analysis. Through the *nad*1 gene is a conserved region, we tried to give an account that it can be used as genetic marker in various studies in future. However, we have successfully standardized the PCR conditions and showed the nucleotide similarity of *L. edodes* with its mitochondrial genome. The amplified gene showed 378 bp in size and 99% similarity in this study.

Keywords: *Edible mushroom, Lentinula edodes, 'nad1' gene, BLAST*

Introduction

Macro fungus *i.e*., mushrooms are used as food supplement due to its nutritional attributes specially for rich in protein content. Commonly edible mushrooms are oyster *Pleurotus spp*., button *Agaricus spp.* paddy straw *Volveriella spp., S*hiitake *Lentinus spp.* milky *Calocybe spp. etc* having high amount of lysine; minerals like sodium, calcium, potassium and phosphorus; vitamins like B, C, D and K and very little amount of fat. These are recommended as ideal food for heart and diabetic patients too. *Shiitake* mushroom *Lentinula edodes* is a popular edible mushroom native to East Asia which is commercially cultivated and consumed in many Asian countries. It is also considered as medicinal mushroom in some forms of traditional medicine. The fleshy and nutrient rich *L. edodes* contains proteins (18%), potassium, niacin, calcium, magnesium, phosphorus and vitamin B. This mushroom is credited with lowering serum cholesterol levels by 12% through eritadenine. *Shiitake* healing properties are also reflected in its anti-viral strengths. It is said that once metabolised, the glucan based compound therein is able to fight

the influenza virus, bacterial infection, and other infectious elements like cancerous cells.

Genetic analysis of edible mushrooms have been carried out earlier through morphology (Kevei and Peberdy, 1984; Yanagi *et al*, 1988; Park *et al*, 1991; Sonnenberg *et al*, 1991) and molecular DNA markers like RAPD, SSR, ISSR (Chakraborty and Sikdar, 2008; Zhang *et al*, 2012; Mallick and Sikdar, 2014); RFLP of rRNA-ITS genes (Jorgenson and Cluster, 1988; Cullings *et al*, 1996; Vogler and Bruns, 1998; Mallick and Sikdar, 2016) etc. DNA markers also used to study about population ecology, hybrid polymorphism, strain identification (Challen *et al*, 2003; Callac *et al*, 2003; Mallick and Sikdar, 2015, 2016) etc in mushroom crop. In the past *L*. *edodes* genome is also characterized through RAPD, ISSR and RFLP of rRNA-ITS genes (Zhang and Molina, 1995; Mallick and Sikdar, 2014, 2016). In mushroom the '*nad*' (NADH dehydrogenase subunit) genes are not used before to detect the polymorphism or genetic diversity due to having the concept of conserved sequence. The mitochondrial genome of several mushroom species have been identified where the NADH subunits complex *i.e*., *nad1, nad2, nad3, nad4, nad4L, nad5, nad6* etc. have been sequenced (Albert and Sellem 2002). In plant system the *nad* gene is often used to study about the phylogenetic relationship among demonstrated and wild species (Sanjur *et al*, 2002).

However, in this study we refereed the '*nad*1*'* gene from mushroom mitochondrial genome and successfully amplified, sequenced. Actually, in mushroom this particular single gene is not amplified before for genetic analysis. In this case, we tried to amplify that gene from genomic DNA and performed BLAST at NCBI genbank for sequence matching.

Materials and Methods

Mycelial culture and DNA extraction:

L. edodes culture was obtained from National Research Centre for Mushroom, Solan, Himachal Pradesh, India. Routine maintenance of the strain was carried out in PDA (Potato Dextrose Agar, pH 6.2) medium at 24±1°C. For DNA isolation culture was grown in liquid MYG (10 g/l malt extract, 4 g/l yeast extract and 10 g/l glucose, pH 6.2) medium for two weeks at 24±1°C.

PCR condition and amplification of nad1 gene:

The genomic DNA from *L. edodes* was isolated from actively growing mycelia in liquid MYG medium using modified *CTAB* method (Dellaporta *et al*., 1983). The '*nad*1' gene is amplified using PCR performed in a total volume of 25µl reaction mixture, containing 10ng template DNA, 20µM of each primer (*nad1 exon* B Forward: GCATTACGATCTGCAGCTCA and *nad1 exon* C Reverse: GGAGCTCGATTAGTTTCTGC), 10X Taq buffer (with KCl), 25mM $MgCl_2$, 2mM dNTPs mixture and 5U/l of Taq DNA polymerase. The negative control (without template DNA) was also made in this reaction. Additional ingredient like BSA was added 0.1µl/25µl rxn. and *βME* 0.05µl/25µl rxn as an inducers. The PCR was conducted in a DNA thermal cycler (Applied Bio-systems 2027) by preliminary denaturation of DNA at 94°C for 4 minutes consisting of 30 cycles; DNA template denaturation at 92°C for 45 sec, primer annealing at 58°C for 45 sec, initial extension at 72°C for 2 min 30 sec, followed by a final extension at 72°C for 10 min.

Sequencing of nad1 gene and blast analysis:

PCR products were purified by sodium acetate precipitation. The purified PCR products were sequenced using Big Dye Terminator v 3.1 method and nucleotide bases were read by an automated sequencer (Applied Biosystems). Sequencing PCR was conducted in a normal PCR tube of 10µl total reaction volume containing RR mix 1µl, 5X sequencing buffer 2µl, primers (pmol/µl) forward - 1µl, reverse - 1µl, PCR product (50 ng). The PCR amplification reactions were conducted in a DNA thermal cycler (Applied Bio-systems 2027) by preliminary denaturation at 96°C for 1 min consisting of 25 cycles; template denaturation at 96°C for 10 sec, primer annealing at 37°C for 5 sec, extension at 60°C for 4 min. The sequenced data was then used and performed blast at NCBI Genbank database for similarity matching.

Results

Amplification and sequence of nad1 gene:

The *L. edodes* cultures were successfully maintained in both PDA and MYG medium for different purposes (Fig.1). The primer pair successfully amplified a 378bp sized fragment and visualized in agarose gel (Fig.2). The additional PCR inducers were standardized after tested of several combinations. The primer annealing temperature was also standardized. The amplified PCR product was purified, sequenced and the sequenced data showed 378 bp in length (Table1). The sequence was performed in blast at ncbi genbank database and it showed similarity with a part of *Lentinula edodes* mitochondrial DNA, complete sequence (AB697988.1) with 99% similarity (Table2).

Table1: Nucleotide sequence of *L. edodes nad*1 gene.

5'TACATGATGCTGCTCAGCCATGACAATTCGGTTTCCAAGATAGTGCTGCTCCAGGAT
TTACAGGTCTAGTAACACTTCATAATACTATTGGTTTCTACTTAGTTTTAATTAGTTTCTCAGTATTT
TGAGTTCTTTTCTCTATAATTTATTACTACAATAATAATAGAAATCCTATAGCTTATAAATACTTAAC
TCACGGTTCAATCATAGAACTTATCTGAACAATCACTCCTGCATTAATATTAATTGCTATCGCTTTT
CCTTCATTTAAATTATTATATTTAATGGATGAAGTTCTATGACCAACATTAACTATTAAAGTAGTAG
GCCACCAATGATATTGGTCTTATGAATACAGTGATTTTATAACAGAAAGTG3'

Table2: Blast matching of *L.edodes nad*1 gene.

Lentinula edodes mitochondrial DNA, complete sequence, strain: akiyamaA567_pro_pm_17; Sequence ID: AB697988.1; Length: 121394; Number of Matches: 1

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Query  1      TACATGATGCTGCTCAGCCATGACAATTCGGTTTCCAAGATAGTGCTGCTCCAGGATTTA  60
              || ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  11491  TAAATGATGCTGCTCAGCCATGACAATTCGGTTTCCAAGATAGTGCTGCTCCAGGATTTA  11550

Query  61     CAGGTCTAGTAACACTTCATAATACTATTGGTTTCTACTTAGTTTTAATTAGTTTCTCAG  120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  11551  CAGGTCTAGTAACACTTCATAATACTATTGGTTTCTACTTAGTTTTAATTAGTTTCTCAG  11610

Query  121    TATTTTGAGTTCTTTTCTCTATAATTTATTACTACAATAATAATAGAAATCCTATAGCTT  180
              |||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||
Sbjct  11611  TATTTTGAGTTCTTTTCTCTATAATTTATTACTACAATAATAATAAAAATCCTATAGCTT  11670

Query  181    ATAAATACTTAACTCACGGTTCAATCATAGAACTTATCTGAACAATCACTCCTGCATTAA  240
              |||||||||||||||||||||||||||||||||||||||||||||||||| |||||||
Sbjct  11671  ATAAATACTTAACTCACGGTTCAATCATAGAACTTATCTGAACAATCACTCCAGCATTAA  11730

Query  241    TATTAATTGCTATCGCTTTTCCTTCATTTAAATTATTATATTTAATGGATGAAGTTCTAT  300
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  11731  TATTAATTGCTATCGCTTTTCCTTCATTTAAATTATTATATTTAATGGATGAAGTTCTAT  11790

Query  301    GACCAACATTAACTATTAAAGTAGTAGGCCACCAATGATATTGGTCTTATGAATACAGTG  360
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  11791  CACCAACATTAACTATTAAAGTAGTAGGCCACCAATGATATTGGTCTTATGAATACAGTG  11850

Query  361    ATTTTATAACAGAAAGTG  378
              ||||||||||||||||||
Sbjct  11851  ATTTTATAACAGAAAGTG  11868
```

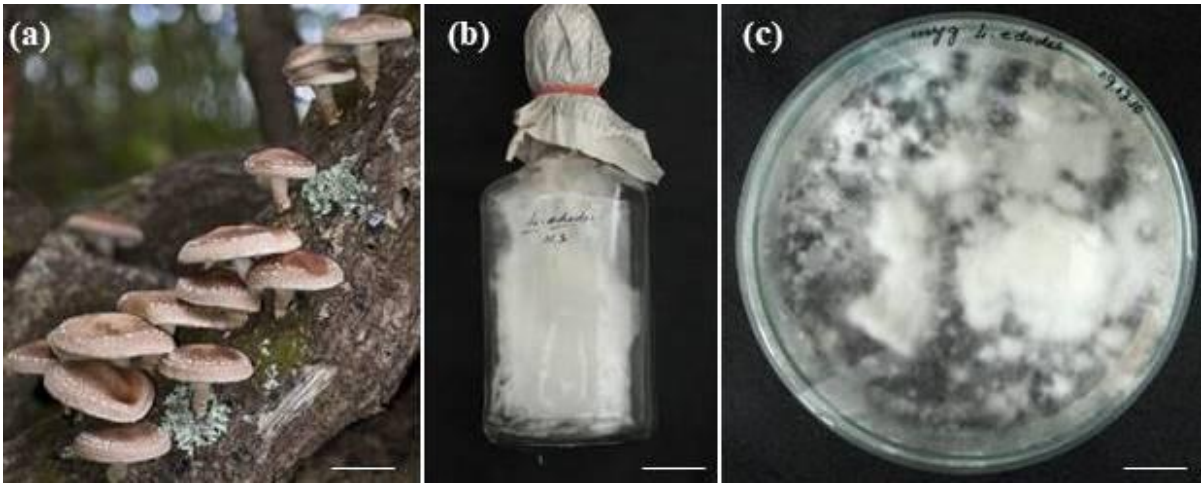

Fig.1: (a) Naturally grown *L. edodes* on tree logs, (b) Vegetative PDA culture, (c) Liquid MYG culture. Bar size: (a) 7cm; (b) 2.5cm and (c) 1.2cm.

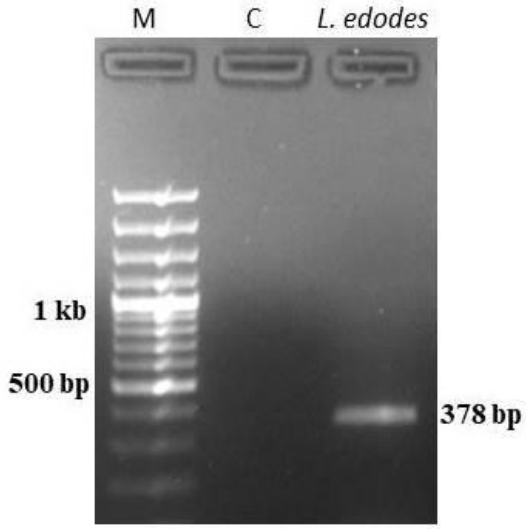

Fig.2: Amplified *nad*1 gene of *L. edodes* in agarose gel

Discussion

The *nad*1 gene is a conserved mitochondrial DNA sequence of NADH subunit complex present in the mitochondrial genome. The responsive DNA sequence in the nuclear genome of such *nad* genes are being studying mainly in plants physiology programme. In mushroom, the single *nad* gene from nuclear genome is not amplified yet except the sequencing to total mitochondrial genome. Here, we have successfully amplified the gene from a popular edible mushroom strain *Lentinula edodes* for the first time. Mainly we tried to amplify the specific gene of interest from mushroom through PCR standardization. However, the purified and sequenced data showed similarities with *L. edodes* complete mitochondrial genome. The sequence present in the mitochondrial genome is also present in the nuclear genome with maximum similarities.

Conclusion

From the present study now we can use this *nad1* gene marker for future uses in like strain identification, characterizations, hybrid polymorphism detection etc.

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