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(RESEARCH ARTICLE)

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Genetic discrimination of button mushroom *Agaricus bisporus* by DNA fingerprinting using RAPD marker

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Abstract

The investigation was conducted for DNA fingerprinting and genetic diversity analysis of five button mushroom strains (*Agaricus bisporus-6, Agaricus bisporus-2, Agaricus bisporus-3, Agaricus bisporus-8* and *Agaricus bisporus-5*) using eight decamer RAPD primers (OPA01, OPA02, OPA03, OPA04, OPA07, OPB17, OPB12 and OPA10). A modified CTAB technique was used to isolate DNA. RAPD analysis resolved 152 scorable bands, 89 bands were monomorphic and 63 bands were polymorphic. The primer OPB17 amplified the highest number of band (30) and OPA01 amplified the lowest number of bands (09). The primer OPA04 produced the 8 polymorphic bands. Thus, it showed higher level of polymorphism (80%). Dendrogram based on linkage distance using Unweighted Pair Group Method of Arithmetic Means (UPGMA) indicated segregated of five button mushroom strains into two main clusters. Only *Agaricus bisporus-6* (V1) grouped in cluster 1 (C1) while the major cluster C2 included all other strains, as well as V2 (*Agaricus bisporus-2)*, V3 (*Agaricus bisporus-3*), V4 (*Agaricus bisporus-8*) and V5 (*Agaricus bisporus-5)*. At the linkage distance of 50.5, the major cluster C2 formed sub-cluster C2A (*Agaricus bisporus-3*) and C2B (*Agaricus bisporus-2, A. bisporus-8 & A. bisporus-5*). Overall results from the dendrogram indicated that the V1 (*Agaricus bisporus-6)* button mushroom was shown to be outliers in the dendrogram, that is different from other strains because of its unique genetic makeup and high yields of this starin. The present work revealed that the DNA fingerprinting of five button mushroom strains based on RAPD showed significant different among the strains.

Keywords: Mushroom strains; RAPD primers; Genetic diversity; Linkage distance; PCR.

1. Introduction

The most extensively grown edible mushroom species is *Agaricus bisporus*. Due to its high nutritional content and probiotic qualities, button mushrooms (*Agaricus bisporus*) are advised worldwide to be included in diet plans. [1]. 1.5–6.7% carbohydrates, 1.5–3% protein, 0.3-0.4% lipids, and vitamins are all present in mushrooms [2]. There is a concerning scenario with the population's rapid expansion and the limited supply of nutrients, especially protein. Investigating unconventional protein manufacturing sources is one way to solve the issues. Growing mushrooms appears to be a viable alternative food source to help with this problem. Additionally, this fungus generates certain proteins with possible industrial uses and significant secondary metabolites with therapeutic uses [3]. Mushrooms are an excellent anticancer agent and can help with lung disorders, diabetes, ulcers, and ulcers [4]. Many human diseases can be cured by using mushrooms and their metabolites as immunostimulants, adaptogens, and anticancer agents. According to reports, mushrooms have antioxidant capacity [5], are utilized as a food supplement as well as in the pharmaceutical business, and exhibit anticancer, antibacterial, antiviral, and hematological action. They are also used

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in immunomodulation treatments [4]. One of the most important tools for characterizing mushrooms today is DNA fingerprinting [6]. The creation of RAPD markers has made it possible to generate repeatable, dependable DNA fragments in a large range of mushroom species. Due to a lack of effective markers, research on variation within filamentous fungi has been restricted [7, 8]. It was suggested that RAPD markers could help identify mushroom strains and be useful in protecting elite strains when they were used to discriminate between different cultivars of mushrooms [9, 10]. The present study was conducted with the aim to analyze genetic diversity within five button mushroom strains and determine genetic relationship by DNA fingerprinting using RAPD markers.

2. Materials and methods

2.1. Selection of mushroom strains

Five different button mushroom strains such as (*Agaricus bisporus-6, Agaricus bisporus-2, Agaricus bisporus-3, Agaricus bisporus-8, Agaricus bisporus-5*) were used as materials for DNA extraction.

Table 1 List of mushroom strains along with their common name

SL. No.	Varieties	Common name
1	*Agaricus bisporus-6* (V1)	Button mushroom
2	*Agaricus bisporus-2* (V2)	Button mushroom
3	*Agaricus bisporus-3* (V3)	Button mushroom
4	*Agaricus bisporus-8* (V4)	Button mushroom
5	*Agaricus bisporus-5* (V5)	Button mushroom

2.2. Collection of Sample

Five button mushroom strains were collected from Mushroom Development Institute, Sobhanbag, Savar, Dhaka. It was in test tube. Then to it was culture in our laboratory procedure.

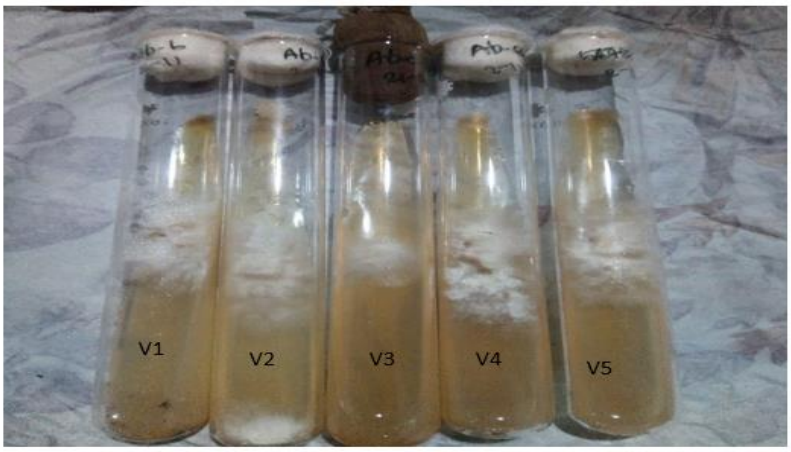

Figure 1 Different strains of mycelium of *Agaricus bisporus*

2.3. Principle for PCR application

Five randomly selected individuals from five distinct strains were used as a sub sample, and eight random sequence primers (Operon Technologies, Inc., Alameda, California, USA) were checked on them to determine their appropriateness for properly scored DNA sequence amplification. For each DNA sample, PCR reactions were conducted in a 10µl reaction mixture that contained 6 µl of the Master Mix, 2 µl of genomic DNA, and 2 µl of RAPD primer from the 10µM working solution. PCR amplification was carried out in an oil-free thermal cycler (Genius, Techne, Cambridge Limited) using the initial denaturation PCR profile, which was set for 94ºC for one minute. There were then 35 cycles of 30 seconds at 94ºC for denaturation, one minute for annealing at 37ºC, and two minutes for elongation or extension at

72ºC. A last step was introduced after the last cycle, which allowed all amplified fragments to fully extend, and it lasted for 7 minutes at 72ºC. Following the conclusion of the cycling session, the responses were maintained at 10ºC. Each sample's PCR results were verified using a 1.5% agarose gel with 3 µl nucleic acid dye and 35 ml 1X TAE buffer run at 120 V for 1.5 hours. After adding 2 µl of loading dye to the PCR products, the wells were loaded. Additionally, a 100 bp DNA ladder serving as a molecular weight marker was placed onto both sides of the gel. Using a trans illuminator and ultra violet light, RAPD bands were detected. Images were captured with a digital camera.

2.4. Primer used

Eight primers of random sequence (Operon Technologies, Inc., Alameda, California, USA) were screened on a sub sample of five randomly chosen individuals from five different strains to evaluate their suitability for amplification of the DNA sequences, which could be scored accurately (Table 2).

Table 2 Parameters of the Operon random primers used in the present study for screening

Primer Code	Sequence (5/-3/)
OPA01	CAG GCC CTT C
OPA02	TGC CGA GCT G
OPA03	AGT CAG CCA C
OPA04	AAT CGG GCT G
OPA07	GAA ACG GGT G
OPB17	AGG GAA CGA C
OPB12	CCT TGA CGC A
OPA10	GTG ATC GCA G

3. Results and discussion

3.1. Cultivation of button mushroom strain

Two culture media were used for the growth of mycelium. One was PDA media and another was PDB media.

Figure 2 Mycelium grown on PDA media

Figure 3 Mycelium grown on PDB media

3.2. DNA isolation, qualification and quantification

Total genomic DNA was recovered from mushrooms by using a modified CTAB techniques of Aljanabi et al. [11], the mini-prep approach taken from Hossain et al. [12]. As a result, the DNA sample was assessed quantitatively using a spectrophotometer and qualitatively using agarose gel electrophoresis to visually estimate if the sample had more molecular weight or if there had been significant shearing, degradation, or contamination.

3.3. RAPD data analysis

The Thermal Cycler (Genius, Techne) and 1.5% agarose gel electrophoresis were used to generate 152 bands with sizes ranging from 180 bp to 1500 bp, utilizing eight primers and the five button mushroom strains. By comparing the migration of each amplified fragment to that of known-size molecular weight marker fragments (a 100 bp DNA ladder), the size of the amplification products was calculated. The five button mushroom strains had amplified bands ranging in size from 180 bp to 1500 bp. Out of the 152 bands, 89 were monomorphic bands and 63 bands were polymorphic bands. The primer OPA04 produced the 8 polymorphic bands. Thus, it showed higher level of polymorphism (80%). The highest number of bands (6.00) per variety was amplified from the primer OPB17 (Table 3).

Table 3 Band scoring of five button mushroom

Primer Code	Size ranges (bp)	Total No. of bands scored	Number of monomorphic bands	Number of polymorphic bands	Polymorphism (%)	No. of band per variety
OPA01	490-1400	09	02	07	77.78%	1.80
OPA02	180-1020	25	17	08	32%	5.00
OPA03	210-1000	19	13	06	31%	3.80
OPA04	300-1000	10	02	08	80%	2.00
OPA07	650-1500	11	06	05	45.45%	2.20
OPB17	230-1200	30	19	11	36.67%	6.00
OPB12	210-1500	22	16	06	27.27%	4.00
OPA10	230-1500	26	14	12	46.15%	5.20
Total		152	89	63		
Average		19	11.13	7.88		

The eight primers' combined data were used to calculate the pair-wise comparisons of linkage distances between varieties using the computer program "Origin Pro." These comparisons yielded values ranging from 30.16 to 60.00. The highest linkage distance (60.00) was found in *Agaricus bisporus*-6 (V1) vs. *Agaricus bisporus*-3 (V3) variety pair (Figure 4).

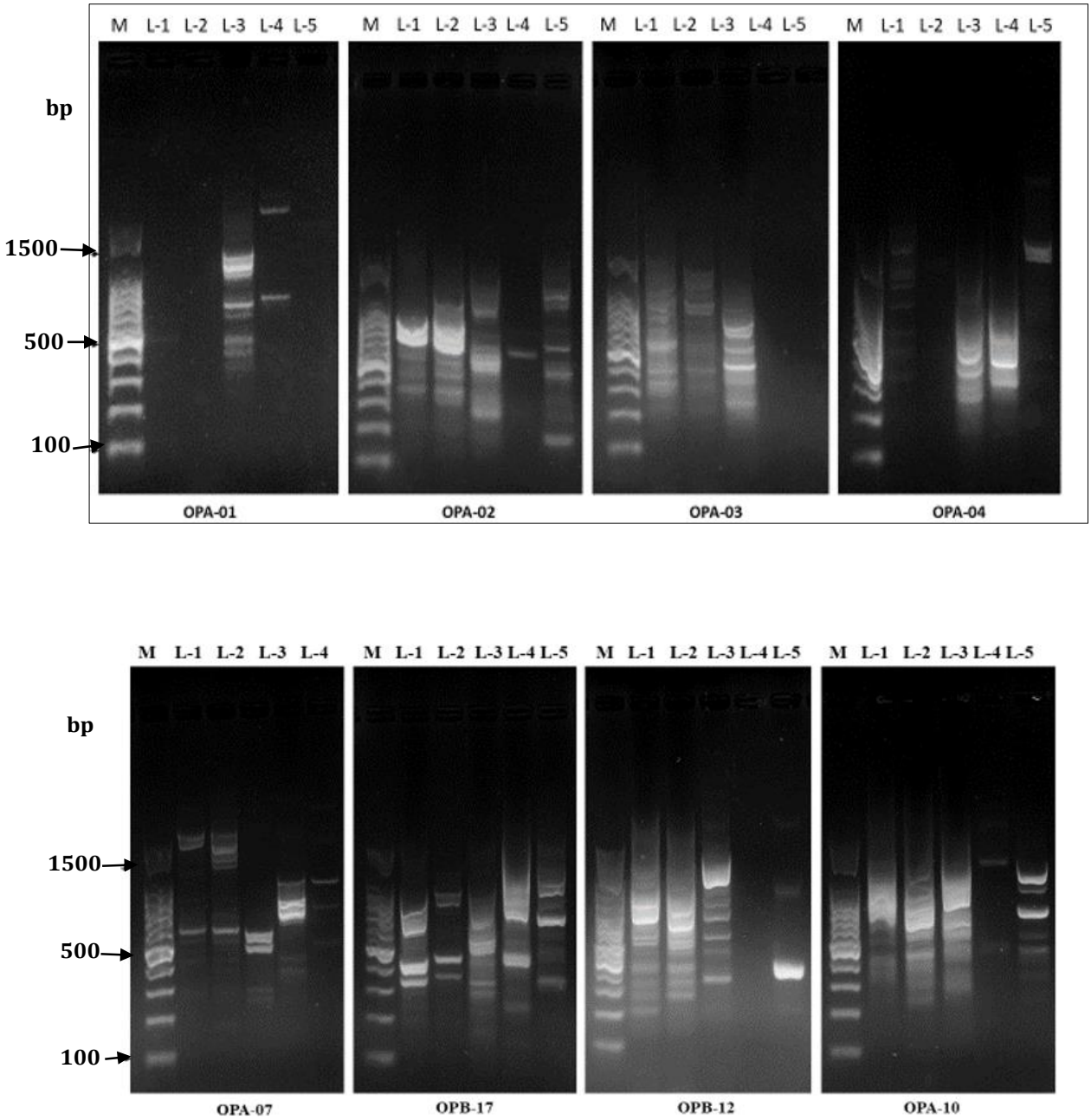

Figure 4 DNA fingerprinting of five button mushroom varieties based on RAPD primers OPA01, OPA02, OPA03, OPA04, OPA07, OPB17, OPB12 & OPB10 through 1.5% agarose gel. Lane M: Molecular weight marker (100bp DNA ladder); Lane 1: *Agaricus bisphorus*-6 Lane 2: *Agaricus bisphorus*-2, Lane 3: *Agaricus bisphorus*-3, Lane 4: *Agaricus bisphorus*-8, Lane 5: *Agaricus bisphorus*-5.

Cluster analysis of five button mushroom strains based on RAPD data using Unweighted pair group method arithmetic mean (UPGMA) based on Nei's (1978) genetic distance. Genetic relationships among the button mushroom Agaricus bisporus strains at the average distance of 50.7 showed two major clusters. V1 (*Agaricus bisporus*-6) shows only in major cluster C1 while the major cluster C2 included all other strains as well as V2 (*Agaricus bisporus*-2), V3 (*Agaricus bisporus*-3), V4 (*Agaricus bisporus*-8) and V5 (*Agaricus bisporus*-5) (Figure 5).

Table 4 Summary of linkage distance (based on Origin Pro) values for different cultivar pairs of button mushroom

	V1	V2	V3	V4	V5
V1	0				
V2	50.74	0			
V3	60.00	50.92	0		
V4	50.48	50.00	50.29	0	
V5	50.10	40.58	50.10	30.16	0

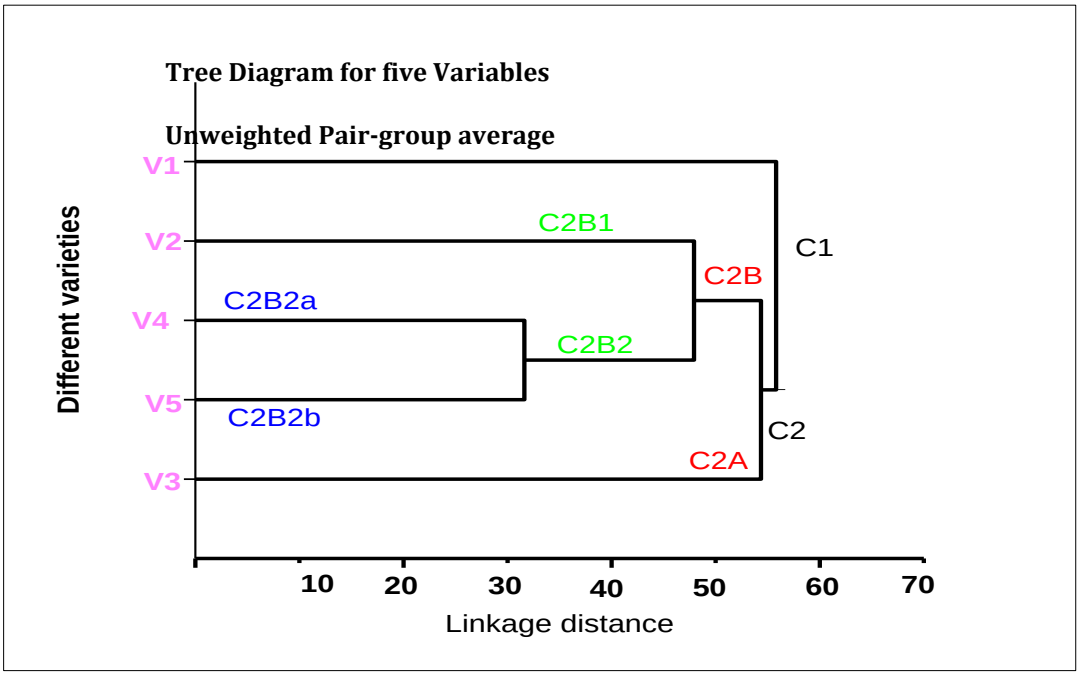

Figure 5 Cluster analysis by Unweighted Pair Group Method of Arithmetic Means (UPGMA) of five button mushroom strains based on eight RAPD markers.

4. Conclusion

According to the investigation's findings, adequate amounts of high-quality DNA can be isolated utilizing basic tools and without the need for a tissue homogenizer. DNA was isolated in order to undertake PCR amplification and RAPD DNA fingerprinting. The results of this research also showed that, based on their genetic relationships, the eight RAPD primers may distinguish between different types of mushrooms. The results of this study showed that there aren't many papers on the use of DNA markers for variation estimate; as a result, this work should serve as a standard for future research.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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