January 2014, Vol. 8, No. 1, pp. 27-34
Journal of Life Sciences, ISSN 1934-7391, USA

Molecular Discrimination of Wild Philippine Paddy Straw Mushroom (*Volvariella volvacea*)

Evaristo A. Abella[1], Geoff D. Robson[2] and Richard F. Preziosi[2]

1. Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Muñoz, Nueva Ecija 3120, Philippines

2. Faculty of Life Sciences, The University of Manchester, United Kingdom, Oxford Rd., M13 9PL, UK

Received: October 09, 2013 / Accepted: December 05, 2013 / Published: January 30, 2014.

Abstract: Genetic diversity study of wild Philippine paddy straw mushroom (*Volvariella volvacea*) was conducted to establish a germplasm collection accessible to volvariella producers in the Philippines. Forty one wild strains were collected from different geographical areas in Northern and Central Luzon region. Strains were differentiated using random amplified polymorphic DNA (RAPD). A single 10-based primer was used to generate randomly amplified polymorphic DNA (RAPD) in *V. volvacea* and differences were noted in band size (bp) ranging from 1,800 bp to 550 bp. Principal component analysis (PCA) of the RAPD data revealed 4 groups from wild strains. One strain showed RAPD pattern with band appearance at 1,750, 950 and 750 bp; 3 strains at 1,800 and 750 bp; 8 strains at 1,500 and 550; and the most abundant group with 29 strains at 750 bp. With observed lack of heterogeneity among strains, it is recommended that more collections from the wild should be undertaken for more diverse germplasm collection. Moreover, it is suggested that RAPD can be used to delineate strains of *V. volvacea* with potential importance on genetic diversity conservation and breeding.

Key words: *Volvariella volvacea*, genetic diversity, random amplified polymorphic DNA (RAPD).

1. Introduction

Volvariella volvacea, is a leaf-litter decomposing edible mushroom, commonly found growing saprophytically on decomposing piles of rice straw, banana leaves, coffee hull and sugarcane bagasse during the onset of the rainy season [1]. Growers in the villages of the Philippines usually cultivate this mushroom on bundled rehydrated rice straw or stubbles or banana leaves [2].

Three major problems usually encountered by paddy straw mushroom growers in the Philippines are the lack of practical technological expertise, availability and accessibility to spawn, and continuous availability of cultures. The first two problems have been properly addressed through our research efforts and regular training and extension programme being undertaken at the Centre for Tropical Mushroom Research and Development in the Philippines. However, sustainability and availability of viable cultures and its accessibility to farmers remains a major problem in sustaining *V. volvacea* production in the countryside. A need to continuously search and screen strains from the wild in order to ensure the availability of promising strains to growers is of paramount importance, since its still from the wild which is the best source of viable strains. Determining the genetic diversity among strains is of significance in order to delineate variation and serve as a basis for strain improvement [3]. Accessibility of promising strains to growers would ultimately lead to a more sustained year round production.

The conventional method of identifying and characterising strains of *V. volvacea* is based on the morphological and cultural characteristics of the

Corresponding author: Evaristo A. Abella, Ph.D., associate professor, research field: biodiversity. E-mail: resty.abella@gmail.com.

isolates. These characteristics include the size, colour, weight, shape of the pileus and stipes, optimum temperature, pH and relative humidity for mycelial growth and fruiting. Although edible mushrooms like *V. volvacea* are conventionally characterised by the specific morphological characteristics of the fruiting bodies [4], it is impractical to always fruit different strains in order to characterise and identify them. This procedure is quite tedious and difficult when knowledge on the fruiting initiation mechanism is inadequate. To ease this problem, the use of available molecular methods to delineate the strains is recommended. With the use of this method, different strains can be characterised at a molecular level in a highly reproducible manner. Since its discovery, it has been used intensively in a wide range of applications [5].

Biodiversity is a key resource for cultivated edible mushroom improvement. To assess the biodiversity within species and to obtain useful genetic information for future breeding, it is necessary to use neutral markers as well as genetic markers [6]. Application of molecular genetic methods has allowed studies of the pattern of genetic variation in natural populations of fungi [7]. These recently applied methods allow polymorphisms of the nucleic acid and protein molecules providing additional insight into systematic relationships often useful for initial selection of lines [8]. Basic tools used to study genetic variations within and between populations are called genetic markers. Markers allow one to determine what alleles are present in populations, and are therefore very useful for studying a wide variety of questions in ecology and evolution [9]. These methods provide additional insight into systematic relationships often useful for initial selection of lines [8].

In this study, random amplified polymorphic DNA (RAPD) was used to differentiate strains of *V. volvacea*. In RAPD, randomly generated primers, 10 bases in length, are used for PCR with the entire genome of the study organism serving as a template. If a sequence complementary to the primer(s) occurs on both strands of DNA, then this fragment will be amplified and appear as band on the gel when the products of the PCR reaction are electrophoretically separated [7]. This PCR-based method avoids the need for restriction enzymes, blotting, probing or cloning and produces fragment differences similar to RFLP analysis. This analysis has been particularly popular for studies of population genetics and mapping of fungi [7].

2. Materials and Methods

2.1 Sources of Wild V. volvacea

Wild strains of *V. volvacea* were collected from different geographical areas in Central and Northern Luzon, Philippines. Collection was conducted during the rainy season when they are expected to produce fruit bodies. Samples were collected from different geographical locations in central and northern Luzon, Philippines (Fig. 1). Immediately after collection, sterile tissue of the fruit body was aseptically inoculated in a flat bottle with PDA and was incubated until its mycelia had fully ramified the culture medium. Cultures were revived and used as sources for molecular characterisation.

2.2 Revival of Cultures

Once the strains were collected, secondary mycelium were inoculated aseptically on to PDA in flat bottles and were incubated for seven days at room temperature (28-30 °C). This was to determine if the strains were viable. Revived cultures were labelled according to its source and stored.

2.3 Sample Preparation and DNA Extraction

Forty one wild strains were used in this study. Cultures were grown in 50 mL potato dextrose broth (FOrMedium LTD., Norwich, UK) in 250 mL conical flasks shaken at 200 rpm at 35 °C for 14 days. Flasks were inoculated using a 10 mm mycelial disc from a 7 day old PDA plated culture. Mycelium was harvested onto sterile muslin, washed with sterile water, excess

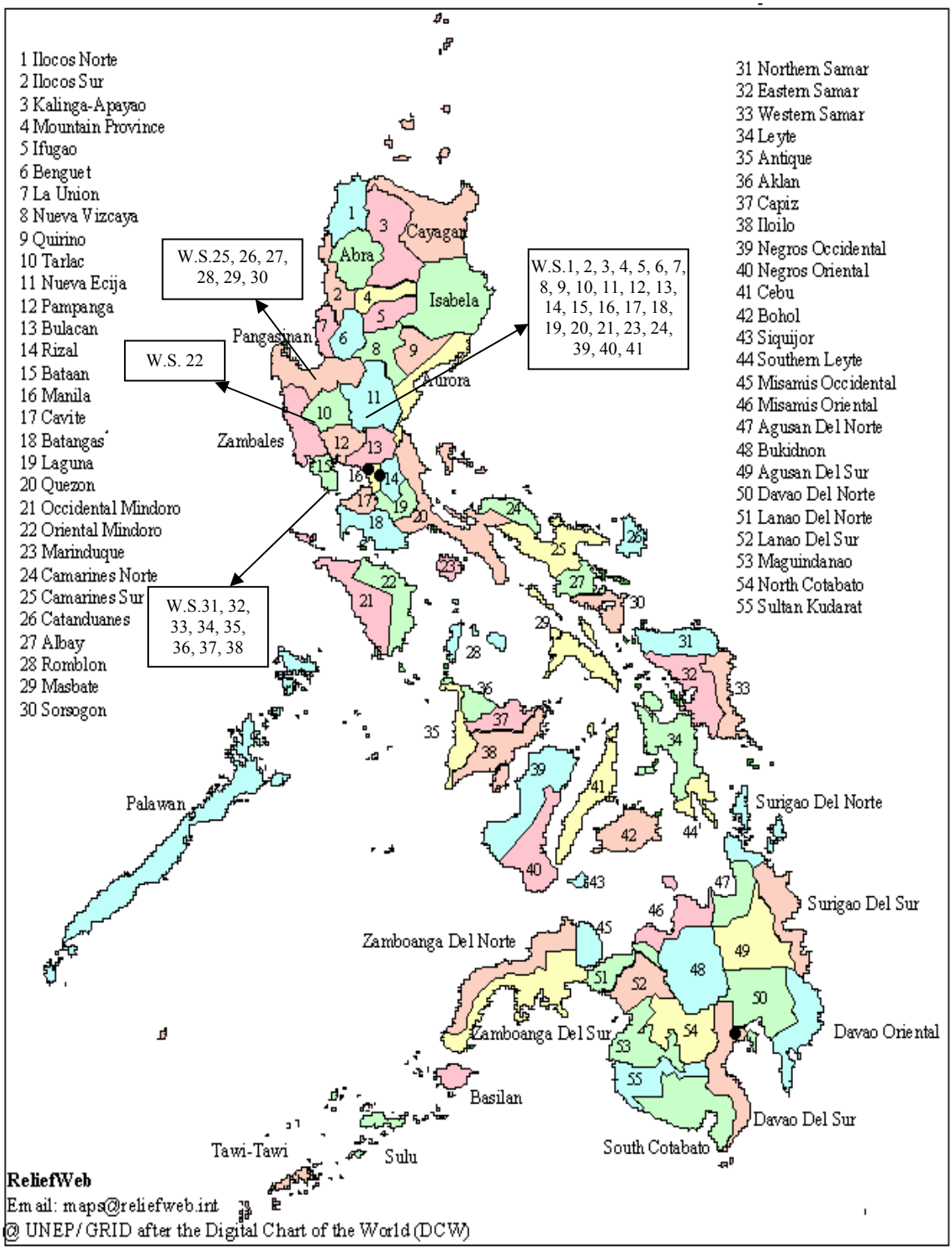

Fig. 1 Location map of origins of the collected commercial and wild *V. volvacea* strains.
Locations of origins of wild *V. volvariella* strains were determined using the global positioning system (GPS).

liquid removed by blotting and ground in a mortar and pestle in liquid nitrogen until a fine powder was obtained. DNA was extracted using a DNeasy Plant Mini Kit (Qiagen Ltd., Crawley, United Kingdom) according to the manufacturer's instructions. DNA was stored at -20 °C until required.

2.4 RAPD Method

Ten commercial random oligonucleotide primers (Invitrogen, UK) were screened for optimum number of RAPD fragments with sample strains (Primer S12 [CCT TGA CGC A], Primer6 [GAA ACA GCG G], Primer8 [GGA GCC CAC], Primer14 [GCC GTC TAC G], Primer17 [GGC ATC GGC C], Primer21 [GTG AGC GTC], PrimerOPL1 [GGC ATG ACC T], PrimerOPS5 [TTT GGG T], PrimerS1 [GTT TCG CTC C] and PrimerS10 [CTG CTG GGA C]). PrimerOPL1 was chosen for RAPD analysis on the basis that it produced the most number of DNA fragments visualised on gel electrophoresis. Each RAPD reaction was carried out in a final volume of 50 μL volume containing 0.50 μM primer OPL1 (GGC ATG ACC T), 2 mM $MgCl_2$, 200 μM of each of the 4 dNTPs, and 1.25 units of *Taq* DNA polymerase (Roche Diagnostics Ltd., Lewes, United Kingdom) and at least 10 ng of template DNA. Amplification was performed with initial denaturation at 92 °C for 5 min followed by 40 cycles at 94 °C for 1 min, annealing at 37 °C for 1 min, and 72 °C for 1.5 min, and final extension at 72 °C for 10 min. Amplified products were electrophoresed at 80 V through a 1.5% (w/v) flat bed agarose gel (Appligene, UK). Marker fragments of known molecular weight (1 kb ladder, Gibco BRL) were used to determine PCR product sizes by comparison with the marker. The running buffer used was 1 × TPE from a 10 × TPE stock [108 g Trizma base, 15 mL Orthophosphoric acid (BDH, Analar) and 40 mL 0.5 M EDTA in a total volume of 1 L]. A fifth volume of DNA loading buffer [0.25% (w/v) bromophenol blue, 0.25% (w/v) xylene cyanol, 15% (v/v) Ficoll] was added to the PCR product prior to loading into the wells. The gel was stained with Ethidium bromide (20 μL of a 10 $mg \cdot mL^{-1}$ solution diluted in 500 mL 1× TPE buffer) for 30 min to visualize the DNA using a UV transilluminator. Gel photographs were taken using an UVl Tec gel imaging system.

2.5 Analysis

Principal component analysis (PCA) was determined using the Paleontological Statistics Software Package for Education and Data analysis [10].

3. Results and Discussion

V. volvacea strains were subjected to molecular characterisation using the Random amplified polymorphic DNA (RAPD) method. Differences were determined by the presence and absence of bands of specific base pairs (bp). Hyperladder I was used as the molecular marker for comparison. It was noted that bands were observed approximately from 1,800 to 550 bp. The DNA profile of wild strains revealed by RAPD is presented in Figs. 2-4. Strains 1-12; 17-27; 28-30 and 39-41 were found to be closely related with one band appearing after RAPD at 750 bp. Strains 13-15 showed bands at 1,800 and 750 bp, whereas strain 16 was unique with bands observed at 1,750, 950 and 750 bp. Strains 31-38 on the other hand, yielded bands at 1,500 and 550 bp. Principal component analysis (PCA) (Fig. 5) resolves four strain groups. Moreover, relating the collection sites (Fig. 1) with the RAPD patterns yielded (Figs. 2-4), strains coming from the same geographic location tend to be more closely related.

There are a number of reasons or factors that may cause differences in morphological characteristics of strains. It is likely that differences are the result of interaction between the environment, the nutrients available in the media or growing substrate, and the inherent characteristics of the strain and capability to utilise these nutrients. Determining whether the differences are environmentally or genetically influenced is difficult when using the conventional way of characterising mushrooms. It is in this context that molecular method was applied to delineate the wild strains of *V. volvacea* at molecular level.

Molecular evaluation of the strains suggests that there are four strains groups from forty one collected

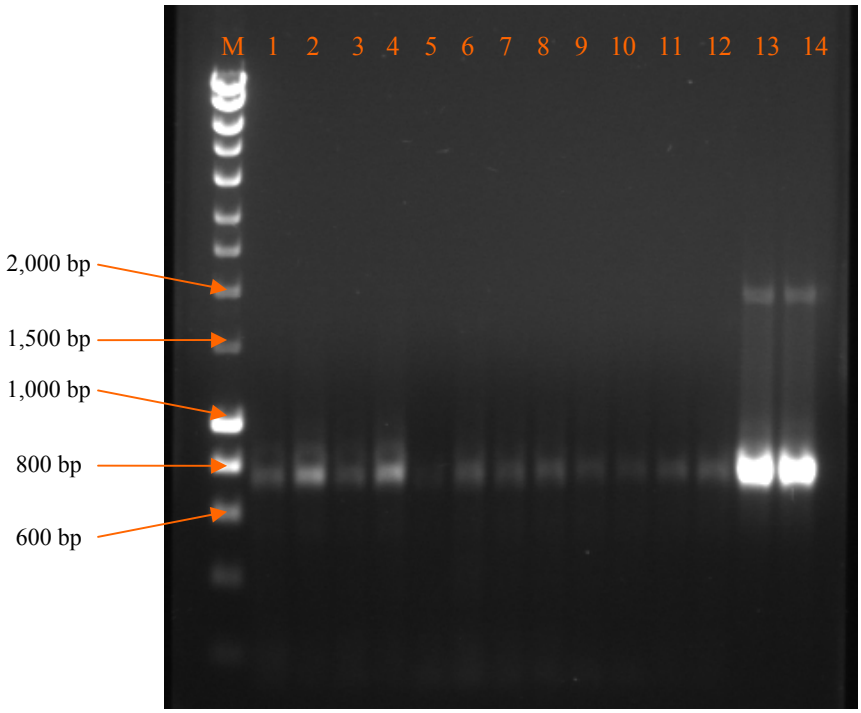

Fig. 2 RAPD patterns of wild *V. volvacea* strains (1-14).

Hyperladder I was used as molecular marker (M) as basis for determining the presence and absence of bands at specific base pairs (bp).

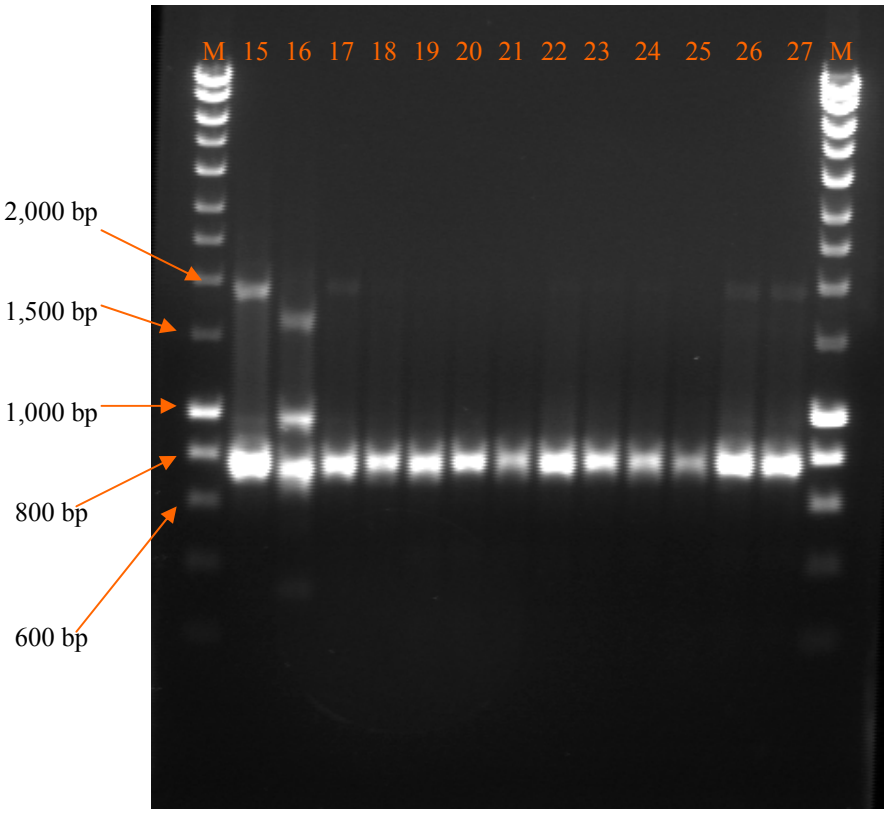

Fig. 3 RAPD patterns of wild *V. volvacea* strains (15-27).

Hyperladder was used as molecular marker (M) as basis for determining the presence and absence of bands at specific base pairs (bp).

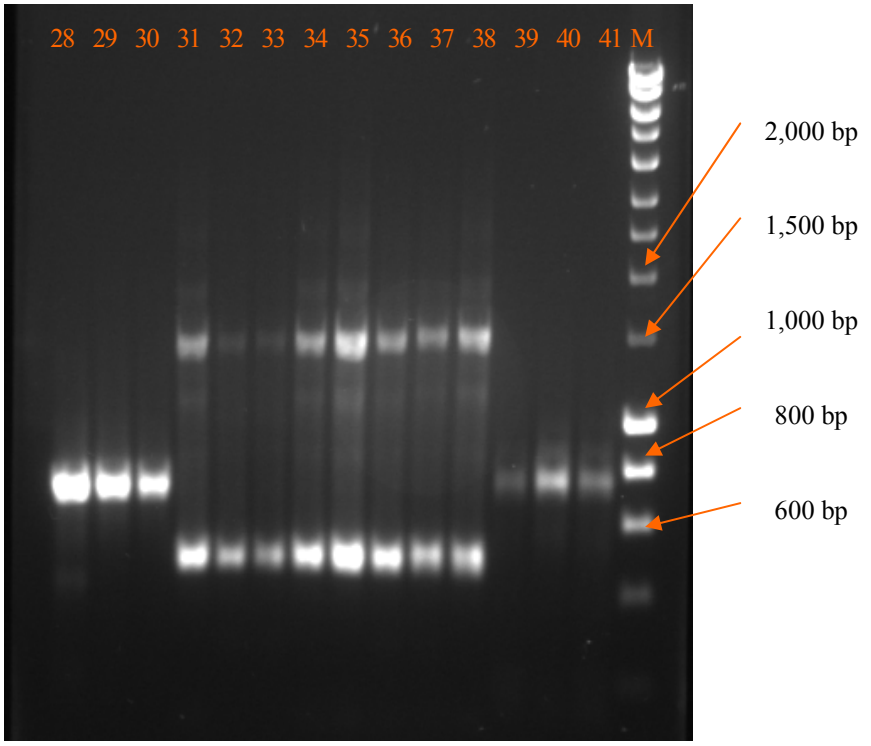

Fig. 4 RAPD patterns of wild *V. volvacea* strains (28-41).

Hyperladder I was used as molecular marker (M) as basis for determining the presence and absence of bands at specific base pairs (bp).

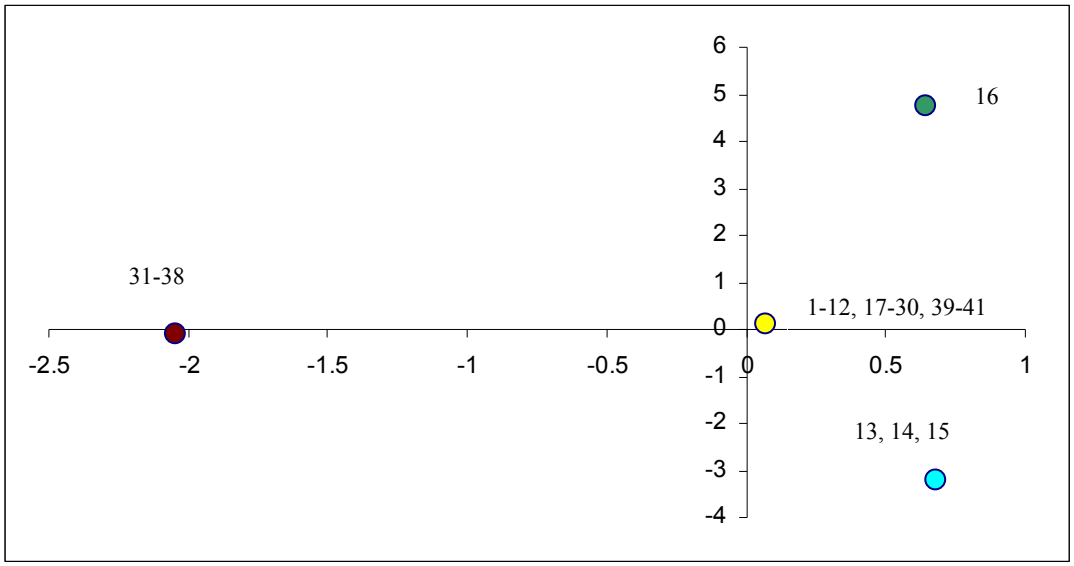

Fig. 5 Principal component analysis of wild *V. volvacea* strains' RAPD pattern. 4 strain groups were delineated as represented by the different colours.

wild strains. Looking at the spatial distribution of wild strains, they seemed to be more closely related when they were collected from within geographical area and tend to be different otherwise. There are few strains though, having same RAPD pattern across geographical locations. This parallels the findings of a study, when commercial strains of *Volvariella* from Malaysia, Philippines and Thailand was differentiated using RFLP. Results would suggest that no differences were noted among the commercial strains,

though they came from different geographical locations [11]. It was further cited that the observed highly similar DNA fingerprints reflect the lack of heterogeneity in commercial *V. volvacea* strains. In a study on the development of molecular and biochemical markers for selecting a potential high yielding strain of paddy straw mushroom (*V. volvavea*) reported that RAPD analysis of *V. volvacea* collected strains with common origin showed 85% to 95% similarity [12]. Collected strains of *Lentinula edodes* from different areas analayzed with RAPD revealed that strains showed similar RAPD patterns except for 2 strains [13]. High genetic homogeneity among cultivated strains of shiitake (*L. edodes*) in mainland China was also noted when they were analyzed using RAPD. It was recommended that collection from the wild should be encouraged since this mushroom is depending on a limited gene pool [14].

Volvariella volvacea like *Agaricus bisporus* is a self fertile species that does not require mating of two compatible species for fruiting to occur and is known as homothallism. Two common types of homothallism are found among self-fertile species, primary homothallism in which a homothallic mycelium is established from a single meiotic nucleus that has the potential to progress through dikaryons to completion of the sexual cycle, and secondary homothallism, in which a fertile dikaryotic mycelium is established from a basidiospore carrying two meiotic nuclei of different mating types. Secondary homothallism occurs in *Agaricus bisporus* while primary homothallism is common to *Volvariella* [15]. Unlike most basidiomycetes, mycelium produced by a uninucleate basidiospore of *V. volvacea* can complete the life-cycle producing a fruit body in which meiosis occurs. Since basidiospores are haploid and uninucleate, only one haploid set of chromosomes can occur in such a selfed (homothallic) fruit body and its progeny are expected to be identical to each other and to the parent [16]. Results on progeny analysis on genetical studies on the sexuality pattern of *V. volvacea* reveal that 90% of the monosporous isolates of mutants retained their marker's phenotype from parental phenotype. This proves that *V. volvacea* is a primary homothallic species [17]. This would probably explain why wild strains collected from within geographical areas tend to be more closely related.

4. Conclusions

With only four genetically different strain groups delineated from wild strains the diversity can be considered low. The lack of heterogeneity among wild population could be attributed to the homothallic nature of the organism. Since *V. volvacea* is considered to be primary homothallic, the chance of producing genetically different strains is low. It is essential that more collection efforts from the wild should be carried out to conserve variants within population and to have a more diverse germplasm collection. *Volvariella volvacea* production industry in the Philippines will primarily depend on crop improvement and available modern technology for higher production and income. Technology would always be available to growers; however, crop improvement of *V. volvacea* depends on the genetic resources availability. Genetic resources can be available only through genetic conservation and this is possible through exploring the wild and continuously collecting wild strains. This will facilitate the availability of strains for commercial and research and subsequently reduce the genetic vulnerability.

The natural genetic diversity and the present available technologies can be used in increasing the productivity of small scale mushroom farms [15].

Acknowledgments

Thanks and appreciation is given to the International Fellowship Program (IFP)-Ford Foundation for funding this research and giving the author the opportunity to study at the University of Manchester, United Kingdom.

References

[1] R.G. Reyes, E.A. Abella, F. Eguchi, M. Higaki, State of the art of mushroom production activities in the province of Nueva Ecija, Central Luzon region, Philippines. Mushroom science and Biotechnology 4 (1997) 3-8.

[2] R.G. Reyes, E.A. Abella, F. Eguchi, T. Iijima, M. Higaki, T.H. Quimio, Growing Paddy Straw Mushrooms, Mushroom Growers' Handbook, Mushworld, 2004.

[3] J. Labarere, U. Menini, Characterization, Conservation, Evaluation and Utilization of Mushroom Genetic Resources for Food and Agriculture, Bordeaux (France), Global Network on Mushrooms, Under the Aegis of F.A.O., 2000, pp. 17-64.

[4] S.T. Chang, J.A. Buswel, S.W. Chiu, Mushroom Biology and Mushroom Products, The Chinese University Press, Hong Kong, 1993, pp. 73-82.

[5] A.J. Jeffrey, V. Wilsen, S.L. Thein, Hyper variable mini -satellite regions in human DNA, Nature 314 (1985) 67-73.

[6] P.G. Miles, S.T. Chang, Mushroom biology: Concise basics and current developments, World Scientific, 1997.

[7] J. Labarere, U. Menini, Characterization, Conservation, Evaluation and Utilization of Mushroom Genetic Resources for Food and Agriculture, Bordeaux (France), Global Network on Mushrooms, under the Aegis of F.A.O., 2000, pp. 65-70.

[8] J. Labarere, U. Menini, Characterization, Conservation, Evaluation and Utilization of Mushroom Genetic Resources for Food and Agriculture. Boreaux (France), Global Network on Mushrooms, under the Aegis of F.A.O., 2000, pp. 91-96.

[9] J.K. Conner, D.L. Hartl, A Primer of Ecological Genetics, Sunderland, Massachusetts, Sinauer Associates Inc., 2004.

[10] Ø. Hammer, D.A.T. Harper, P.D. Ryan, PAST: Paleontological statistics software package for education and data analysis, Palaeontologia Electronica 4 (1) (2001) 9. http://palaeoelectronica.org/2001_1/past/issue1_01.h.

[11] S.W. Chiu, M.J. Chen, S.T. Chang, Differentiating homothallic *Volvariella* mushrooms by Rflps and Ap-Pcr, Mycological Research 99 (1995) 333-336.

[12] G.P. Ahlawat, O.P.S. Kamal, B.L. Dhar, Development of molecular and biochemical markers for selecting a potential high yielding strain of paddy straw mushroom (*Volvariella volvacea*), Journal of Plant Biochemistry and Biotechnology 17 (2008) 57-63.

[13] M. Sunagawa, H. Neda, K. Miyazaki, Application of random amplified polymorphic DNA (RAPD) markers II, Mokuzai Gakkaishi 41 (1995) 949-951.

[14] S.W. Chiu, A.M. Ma, F.C. Lin, D. Moore, Genetic homogeneity of cultivated strains of shiitake (*Lentinula edodes*) used in China as revealed by the polymerase chain reaction, Mycological Research 100 (1996) 1393-1399.

[15] E. Labarere, U. Menini, Characterization, Conservation, Evaluation and Utilization of Mushroom Genetic Resources for Food and Agriculture, Bordeaux, France, Global Network on Mushrooms, under the Aegis of F.A.O., 2000, pp. 193-199.

[16] S.W.A. Chiu, D. Moore, Segregation of genotypically diverse progeny from self-fertilized haploids of the Chinese straw mushroom, *Volvariella volvacea*, Mycological Research 103 (1999) 1335-1345.

[17] Mather, Genetical Studies on the Sexuality Pattern of *Volvariella volvacea*, Genetical studies on the sexuality pattern of *Volvariella volvacea*, Balkema, Rotterdam, 1991.