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Genome-wide gene expression patterns in dikaryon of the basidiomycete fungus *Pleurotus ostreatus*

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ABSTRACT

Dikarya is a subkingdom of fungi that includes *Ascomycota* and *Basidiomycota*. The gene expression patterns of dikaryon are poorly understood. In this study, we bred a dikaryon DK13 × 3 by mating monokaryons MK13 and MK3, which were from the basidiospores of *Pleurotus ostreatus* TD300. Using RNA-Seq, we obtained the transcriptomes of the three strains. We found that the total transcript numbers in the transcriptomes of the three strains were all more than ten thousand, and the expression profile in DK13 × 3 was more similar to MK13 than MK3. However, the genes involved in macromolecule utilization, cellular material synthesis, stress-resistance and signal transduction were much more up-regulated in the dikaryon than its constituent monokaryons. All possible modes of differential gene expression, when compared to constituent monokaryons, including the presence/absence variation, and additivity/nonadditivity gene expression in the dikaryon may contribute to heterosis. By sequencing the urease gene *poure* sequences and mRNA sequences, we identified the monoallelic expression of the *poure* gene in the dikaryon, and its transcript was from the parental monokaryon MK13. Furthermore, we discovered RNA editing in the *poure* gene mRNA of the three strains. These results suggest that the gene expression patterns in dikaryons should be similar to that of diploids during vegetative growth.

Introduction

Dikaryon is a unique organism in which each compartment of a hypha contains two haploid nuclei, each derived from a different parent. It consists of a subkingdom of fungi *Dikarya*, including *Ascomycota* and *Basidiomycota*. A dikaryon strain is formed by mating two compatible monokaryon strains, resulting in plasmogamy but not karyogamy in the fused compartment. When new hyphae grow, the two nuclei synchronously divide, and each new compartment keeps two nuclei[1]; karyogamy only occurs before the initiation of sexual

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reproduction. This sexual reproduction mode was distinctly different from that in diploids. The interaction between the genetic materials of the two nuclei in dikaryons has not been well characterized. Are the modes of gene action in dikaryons the same as that in diploids during vegetative growth?

The major types of gene expression patterns found in diploids during vegetative growth are mitotic crossover or mitotic recombination,[2,3] DNA methylation and gene silencing by RNAi,[4] monoallelic expression (sex chromosome inactivation, imprinted gene expression, or autosomal random monoallelic expression),[5] RNA-editing,[6] and differential allele expression in hybrids and parents that contributes to heterosis,[7] etc. Mitotic recombination (also named parasexuality in fungi), DNA methylation and gene silencing by RNAi were also found in dikaryons,[8–10] while monoallelic expression and RNA-editing have not been identified in the dikaryon. Although not strictly true for all reported species, in terms of the growth rate, enzyme activity and pathogenicity, diploids have a significant advantage over their parental haploids, which is similar to what is exhibited when dikaryons are compared to their parental monokaryons. It was proposed that the heterosis in diploids resulted from the allele gene differential expression in hybrids and their parents, such as presence/absence variation and additive/non-additive (high- and low-parent dominance, underdominance, and overdominance) gene expression.[11–14] The mechanism of heterosis in dikaryons remains obscure.

An effective approach for exploring the allele gene differential expression in dikaryons is the comparison of soluble protein profiles or isoenzyme patterns between a dikaryon and its constituent monokaryons. The soluble protein profile of *Schizophyllum commune* dikaryon was dramatically different from that of its parental monokaryons, and there are many new bands in the dikaryon[15]; further studies showed that 14 out of 15 isoenzyme patterns changed between the dikaryon and two monokaryons.[16] Similar results were also reported in other basidiomycetes, such as *Coprinus congregatus*[17] and *Coprinopsis cinerea*.[18] Those studies indicated that alleles had different expression patterns in dikaryons and monokaryons. However, subsequent studies found no such difference in higher basidiomycetes and suggested that those reported differences were probably caused by growth conditions and the electrophoresis procedure.[19,20] Since then, many other observations have confirmed such findings. For example, comparing *S. commune* monokaryons and the dikaryon, protein two-dimensional gel electrophoresis showed only 6.6% and 7.7% differences,[21] and the sequence complexities and coding properties of polysomal RNA and total RNA had no detectable difference.[22,23] Nevertheless, using gene expression profiling, the relative differences in the transcription quantity of the 12 laccase genes in the *Pleurotus ostreatus* dikaryon and its two parental monokaryons showed that the dikaryotic superiority in laccase activity was due to non-additive transcriptional increases in two genes.[24] Genome-wide gene expression pattern analysis of dikaryons and their parental monokaryons has not been reported.

Oyster mushroom *P. ostreatus* (Jacq. Fr) Kumm. is a white rot basidiomycete that is an important edible and medical mushroom,[25–27] and it has been studied as a model organism for basidiomycete genetics and genomic studies.[24] In this study, we compared the genome-wide transcriptional profiles among the dikaryon and its two constituent monokaryons of *P. ostreatus* by Solexa-based RNA-Seq with a focus on the transcriptomic profiling difference analysis between the dikaryon and monokaryons, investigation of the mechanisms of the advantages of sexual reproduction, monoallelic expression, and RNA-editing in dikarya.

Materials and methods

Strains and culture conditions

Monokaryons MK13 and MK3 were from the basidiospores of *P. ostreatus* TD300, which is a commercial cultivation strain in China and was obtained from Zhengzhou Composite Experiment station, China Edible Fungi Research System (Zhengzhou, China). The mycelial growth rate of MK3 was faster than MK13 on potato dextrose agar (PDA) plates (Fig. 1). Dikaryon DK13 × 3 was from MK13 and MK3 through A_1B_1 and A_2B_2 mating, as identified using mating tests.[28] DK13 × 3 grew faster than its constituent monokaryons in PDA and formed normal fruiting bodies with a biological efficiency that was similar to TD300 in cottonseed hull medium (Fig. 2). The three strains were cultured in potato dextrose broth (150 mL in a

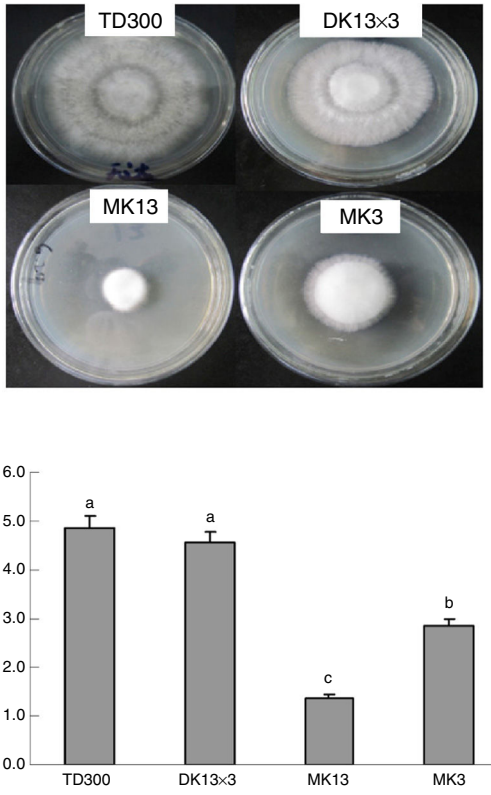

Fig. 1 – Mycelial growth of the monokaryons and reconstituted dikaryon of *Pleurotus ostreatus* on PDA plates. MK13, monokaryon; MK3, monokaryon; DK13 × 3, dikaryon; TD300, dikaryon and the two monokaryons' parent; MGR, mycelial growth rate. Data are given as the means and SE of four replicates. Data with the same lower case letter do not significantly differ from other data at $p < 0.05$.

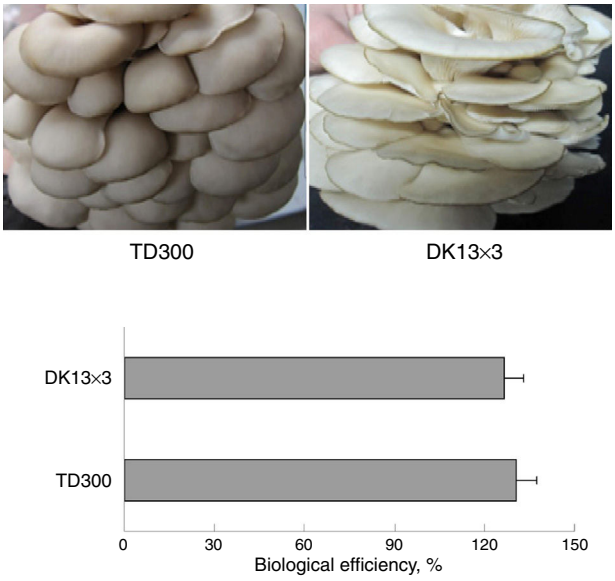

Fig. 2 – Fruiting body morphology and biological efficiency of TD300 and DK13 × 3 in cottonseed hull medium. Biological efficiency indicates the percentage of the fresh weight of harvested 1st and 2nd flush mushrooms over the dry weight of inoculated substrates.

500-mL flask) at 25 °C under 150 rpm shaking; mycelia were harvested in the late exponential phase (10 and 25 days of culturing for dikaryon and monokaryons, respectively) for DNA or total RNA extraction.

RNA extraction, cDNA library construction and RNA-Seq

Mycelia were isolated from culture broth by centrifugation at 5000 × *g* for 10 min; 100 g of fresh mycelia was homogenized in liquid nitrogen; and total RNA was extracted using an RNA pure total RNA fast isolation kit (Bioteke, Beijing, China). The total RNA was used for RT-PCR or enrichment of mRNA (poly(A) + RNA) with a Dynabeads mRNA Purification Kit (Invitrogen, Grand Island, NY), and mRNA was then broken into short fragments. Using these short fragments as templates, first- and second-strand cDNA were synthesized. Sequencing adapters, which also served as sample markers, were ligated to short fragments after purification with a QiaQuick PCR Extraction Kit (Qiagen, Hilden, Germany). Fragments that were 200–700 bp were then separated by agarose gel electrophoresis and selected for PCR amplification as sequencing templates. The three strain libraries were sequenced using Illumina HiSeq™ 2000 by the Beijing Genome Institute (BGI) (Shenzhen, China).

Sequencing reads filtering

Raw reads contained low-quality, adaptor-polluted and high contents of unknown base (N) reads, and these noise reads should be removed before downstream analyses. We used internal software to filter reads. After filtering, the remaining reads were called "Clean Reads" and stored in the FASTQ format.

De novo assembly and sequencing assessment

Contigs were assembled from clean reads using a de novo assembler Trinity[29]; then, non-redundant unigene sets for all three strains were constructed using the EST assembly program TGICL.[30] An all-unigene set was produced from the three contig datasets by further sequence overlap splicing and non-redundancies.

Genome mapping and gene expression analysis

Clean reads were mapped to the reference genome sequence of *Pleurotus ostreatus* PC15 (http://genome.jgi-psf.org/PleosPC15_2/PleosPC15_2.home.html) using Bowtie2[31]; then, the gene expression level was calculated using RSEM.[32]

Differential unigene expression analysis

The unigene expression levels were calculated using the Reads per kb per Million reads (RPKM) method.[33] Under the null hypothesis of equal expression between two samples, the following test gives the *p*-values for identifying differentially expressed genes (DEGs) between two samples.[34]

$$P(y|x) = \left(\frac{N2}{N1}\right)^{y} \frac{(x+y)!}{x!y!(1+(N2/N1))^{(x+y+1)}}$$

*N*1 is the total number of clean tags in MK3 or MK13; *N*2 is the number in DK13 × 3; *x* is the number of the clean tags of the target gene in MK3 or MK13, and *y* is the number in DK13 × 3. $p \leq 0.001$ and $|\log2\text{Ratio}| \geq 1$ were used as the threshold to filter DEGs.

The DEGs expressed in all three strains were used to estimate the mid-parent expression value (MPV). The MPV was calculated by averaging the expression level of the parental monokaryons, assuming an (MK3:MK13) ratio of RNA abundance in the nucleus of Dikaryon DK13 × 3 of 1:1, as described elsewhere.[35]

Cloning and sequencing of the urease gene

To validate the gene expression profiles obtained by RNA-seq, urease gene *poure* of the monokaryons and dikaryon was cloned, amplified, and sequenced. Cloning was performed by colony direct PCR[36] using primers POU1 (GCATTTTGATTGGCAGGGT) and POU2 (AGTGATTACGGCAGGGCG) at PCR conditions of 94 °C for 30 s, 51 °C for 40 s, and 72 °C for 3 min, which were repeated 31 times. mRNAs were amplified using RT-PCR with primers POU3 (TTACCGAGGGAAGAAGCGAA) and POU4 (GGTGGTGACAGAAACGGGAGTA), and PCR conditions were set at 94 °C for 30 s, 52 °C for 40 s, and 72 °C for 2 min, which was repeated 31 times. The PCR products of DNA and mRNA were purified and were then cloned into the pGEM-T Vector (Promega, Madison, WI, USA). The vectors were transformed into *E. coli* DH5α, and five transformants were randomly selected and sequenced by the Beijing Genome Institute (BGI) (Shenzhen, China).

Table 1 – Throughput and quality of RNA-Seq of the dikaryon and its constituent monokaryons of *Pleurotus ostreatus*.

Strain	Total raw reads (Mb)	Total clean reads (Mb)	Total clean bases (Gb)	Clean reads Q20 (%)	Clean reads ratio (%)	Total mapping ratio (%)	Uniquely mapping ratio[a] (%)
MK13	20.27	20.27	1.82	97.19	100.00	64.72	59.17
MK3	20.81	20.81	1.87	97.10	100.00	57.28	52.56
DK13 × 3	20.50	20.50	1.84	97.24	100.00	59.33	54.59

[a] Unique mapping: reads that map to only one location of the reference, called unique mapping.

Results

Quality assessment of RNA-seq datasets and mapping of the reference genome

Table 1 lists the statistics of the reads. The RNA-seq reads were of high quality; almost all mRNA fragments were sequenced, and 97% of the reads had a Phred quality score greater than 20. We mapped clean reads to the reference genome sequence of *Pleurotus ostreatus* PC15 (http://genome.jgi-psf.org/PleosPC15_2/PleosPC15_2.home.html) using HISAT.[37] On average, 60.44% of reads are mapped, and the uniformity of the mapping result for each sample suggests that the samples are comparable. The GenBank accession number for the RNA-seq datasets of the three strains is BioProject Accession: PRJNA326297.

Gene expression analysis

After genome mapping, we used StringTie[38] to reconstruct transcripts, and with genome annotation information, we can identify novel transcripts in our samples using cuffcompare, a tool of cufflinks.[39] In total, we identified 4261 novel transcripts. Then, we merged novel coding transcripts with the reference transcript to obtain a complete reference, mapped clean reads using Bowtie2,[40] and calculated the gene expression level for each sample with RSEM.[41] Thereupon, the total mapping ratios of the clean reads in the transcriptomes of the three strains were increased. Total transcript numbers were all more than ten thousand (Table 2).

We then calculated the read coverage and read distribution on each detected transcript. The Pearson correlation between the transcriptomes of the three strains was obtained. The Pearson correlations of the dikaryon DK13 × 3 to its constituent monokaryons, MK13 and MK3, were 0.8523 and 0.8100, respectively, while the Pearson correlation between the two monokaryons was 0.8124, indicating that the expression profile in DK13 × 3 was more similar to MK13 than MK3 (Fig. 3).

Gene expression difference between the three strains

The total RPKMs of the unigenes in MK13, MK3 and DK13 × 3 were 559494, 550716, and 586583. The total RPKMs of the unigenes in DK13 × 3 were 4.8% and 6.5% higher than those in MK13 and MK3 ($p < 0.05$) (Fig. 4). Among the unigenes between DK13 × 3 and MK13 or MK3, the common unigenes of the three strains were 27.6%, the common unigenes for DK13 × 3 and MK13 were 10.8%, and the common unigenes for DK13 × 3 and MK3 were 11.3%. The special unigenes in DK13 × 3, MK13

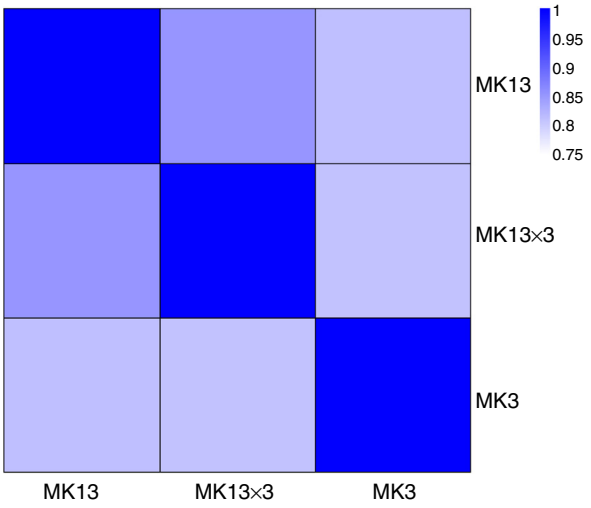

Fig. 3 – Heatmap of Pearson correlations between the dikaryon and its constituent monokaryons of *Pleurotus ostreatus*.

and MK3 were 13.5%, 17.6%, and 15.5%, respectively. Up to 38% of unigenes in DK13 × 3 were derived from its parental monokaryons (Fig. 5), indicating that the gene expression pattern of present/absent variation occurred among the three strains, and more than one-third of the DEGs in the dikaryon were monoallelic expression genes.

Using $p \leq 0.001$ and $|\log2Ratio| \geq 1$ as the standard to screen the differentially expressed genes (DEGs) between DK13 × 3 and MK13 or MK3, compared to MK13, the number of genes whose expression levels were up-regulated in DK13 × 3 was 11323; 7953 were up-regulated more than 3-fold, and 114 were up-regulated more than 15-fold. Additionally, 8421 genes were down-regulated; 2573 were down-regulated more than 3-fold, while none were down-regulated more than 15-fold (Fig. 6A). Compared to MK3, the number of genes whose expression was up-regulated in DK13 × 3 was 11578; 7787 were up-regulated more than 3-fold, and 116 were up-regulated more than 15-fold. Furthermore, 7425 genes were down-regulated; 2176 were down-regulated more than 3-fold, and 1 was down-regulated more than 15-fold (Fig. 6B). The results suggest that the number of up-regulated genes in the dikaryon was much higher than that of down-regulated genes, especially compared to the constituent monokaryons.

The genes in the dikaryon that were 15-fold up- or down-regulated compared with the monokaryons were examined with an NCBI online BLASTP homology analyzer. Additionally, 28 and 21 up-regulated genes were found to have related

Table 2 – Summary of gene expression in the dikaryon and its constituent monokaryons of *Pleurotus ostreatus*.

Strain	Total mapping ratio (%)	Uniquely mapping ratio (%)	Total gene number	Known gene number	Novel gene number	Total transcript number	Known transcript number	Novel transcript number
MK13	66.91	41.60	9559	9467	92	11,134	7667	3467
MK3	66.94	42.81	9380	9293	87	10,883	7497	3386
DK13 × 3	65.45	42.40	9659	9565	94	11,319	7827	3492

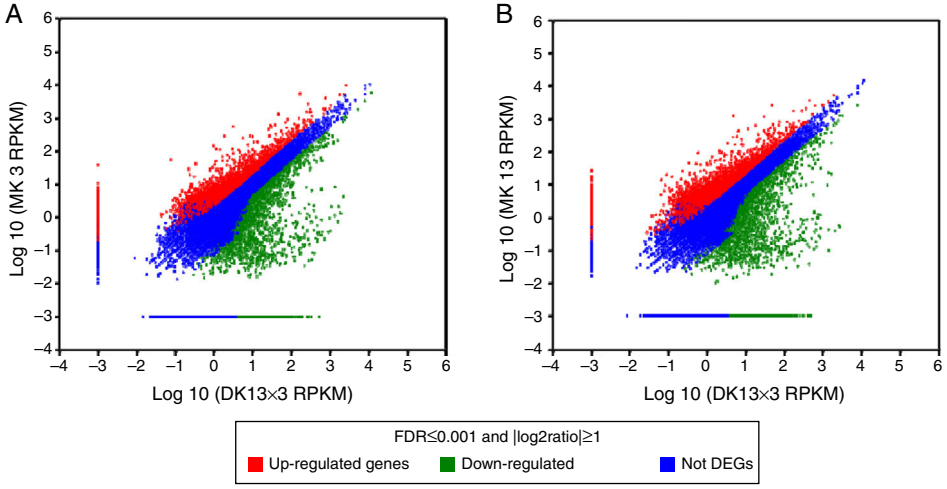

Fig. 4 – Comparison of the unigene expression levels between MK3 or MK13 and DK13 × 3. Up-regulated genes, down-regulated genes, and NOT DEGs were determined using a threshold of $p \leq 0.001$ and |log2Ratio| ≥ 1. A, MK3 vs DK13 × 3; B, MK13 vs DK13 × 3; NOT DEGs, Unigenes were not obviously changed upon MK3 or MK13 to DK13 × 3.

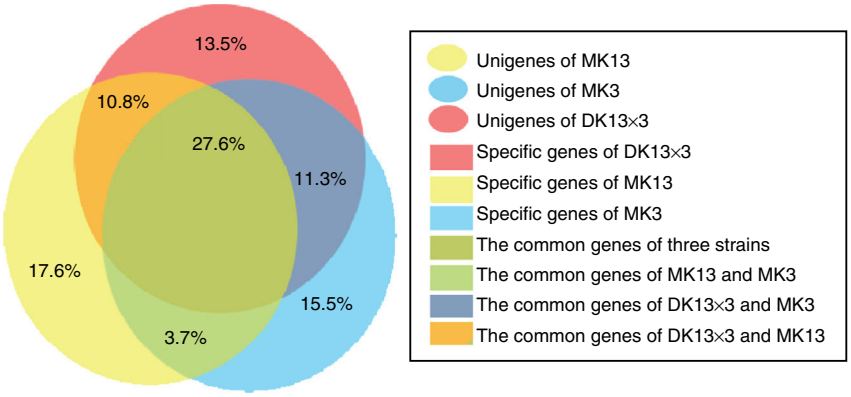

Fig. 5 – Distribution diagram of DEGs between MK3 or MK13 and DK13 × 3. DEGs were screened by a threshold of $p \leq 0.001$ and |log2Ratio| ≥ 1.

functions to annotated genes; no such genes were found for down-regulated genes. The up-regulated genes were primarily involved in macromolecule utilization, cellular material synthesis, stress resistance and signal transduction, etc. (Tables 3 and 4). These findings have provided evidence for the growth advantage that the dikaryon has over the constituent monokaryons.

Among the common DEGs of the three strains, when the DK13 × 3 levels were compared to MPV additive model values, approximately 63.0% (878/2027) of transcripts were identified to be engaged in non-additive gene expression (threshold of greater than two-fold higher/lower). A small plurality of genes, 36.8%, had lower expression levels in DK13 × 3 than expected, while 26.2% were higher and potentially upregulated (Fig. 7).

For example, we obtained the transcription profiling from the RNA-seq of the 17 laccase genes in the three strains. The gene action modes of the 17 laccase genes could be divided into the following three patterns: genes expressed in both parental monokaryons but not in the dikaryon; genes expressed in one parental monokaryon and dikaryon but not in another parental monokaryon; and genes expressed in parental monokaryons and the dikaryon. However, the total RPKMs of these laccase genes in DK13 × 3 did not present significant differences compared to the parental monokaryons (Table 5).

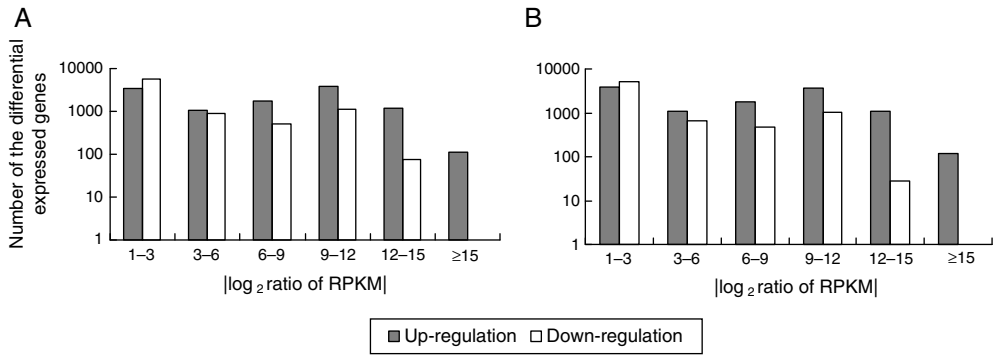

Fig. 6 – Differentially expressed genes in dikaryon DK13 × 3 compared to parental monokaryons MK13 (A) or MK3 (B). RPKM, reads per kb per million reads.

Table 3 – Function annotation of differentially expressed genes in dikaryon DK13 × 3 compared to its parental monokaryon MK13.

Gene ID	Log_2 ratio	Up/down	Homologous protein	NCBI ID	E-value
Unigene24705	18.9046	Up	Mitochondrion protein	XP_567165.1	3E−37
Unigene8016	18.2889	Up	Tetraspanin Tsp2 family	XP_001885708.1	1E−14
Unigene17666	17.5877	Up	Alcohol dehydrogenase superfamily protein	XP_001833941.1	6E−59
Unigene24669	17.4953	Up	NADH kinase	XP_001830329.2	1E−152
Unigene6939	17.286	Up	Glucosamine 6-phosphate N-acetyltransferase	XP_001834733.1	6E−63
Unigene3965	17.2631	Up	Cystathionine beta-synthase (beta-thionase)	XP_754772.1	3E−24
Unigene4053	17.126	Up	Calcium:hydrogen antiporter	XP_002911846.1	4E−71
Unigene12949	16.6756	Up	Large surface exposed glycoprotein PsrP	CBW35224.1	6E−28
Unigene22941	16.5483	Up	Histone-like type 2	XP_001831684.1	3E−36
Unigene24800	16.5407	Up	MCMA	XP_001835736.2	1E−111
Unigene12396	16.2922	Up	OmpA family protein	YP_001236439.1	2E−10
Unigene24789	16.252	Up	123R	NP_149586.1	3E−09
Unigene12755	16.18	Up	Membrane fraction protein	XP_001837650.1	1E−122
Unigene24727	16.1761	Up	Endopeptidase	XP_001837196.1	1E−179
Unigene24787	16.0545	Up	KLTH0E05940p	XP_002553740.1	5E−06
Unigene22994	15.9919	Up	Calcium/calmodulin-dependent protein kinase	BAF75875.1	1E−168
Unigene24636	15.7398	Up	Proteasome subunit alpha type 4	XP_001830819.2	1E−131
Unigene24738	15.7175	Up	Type VI secretion system Vgr family protein	YP_001812335.1	3E−14
Unigene17540	15.5547	Up	Alpha/beta hydrolase fold protein	YP_002430731.1	6E−24
Unigene2861	15.5003	Up	Mucin-like protein 1	XP_001835597.2	3E−08
Unigene9632	15.4441	Up	Ribosomal protein P2	XP_001831572.2	2E−41
Unigene17732	15.3692	Up	NADH-ubiquinone oxidoreductase 21 kDa subunit	XP_001835740.1	2E−63
Unigene18012	15.3683	Up	Endo-1,3(4)-beta-glucanase	XP_001828985.1	1E−144
Unigene12721	15.2793	Up	NADH-ubiquinone oxidoreductase 51 kDa subunit	XP_001840875.1	0
Unigene24653	15.189	Up	Ubiquitin-conjugating enzyme 16	EFP75491.1	7E−47
Unigene1749	15.1234	Up	Carboxy-cis,cis-muconate cyclase	XP_002850491.1	6E−10
Unigene21455	15.1112	Up	Mitochondrial ribosomal small subunit	XP_001840218.2	1E−60
Unigene23368	15.064	Up	Glycoside hydrolase family 16 protein	XP_001875740.1	1E−129

poure monoallelic expression in the dikaryon

The *poure* gene of the two monokaryons and mRNA of the two monokaryons and karyon were cloned and sequenced by PCR and RT-PCR. The *poure* gene sequences of MK13 (GenBank access number: KF312589.1) were 97% and 97% identical to those of *P. ostreatus* PC15 v2.0, PC9 v1.0, (http://genome.jgi-psf.org/PleosPC15_2/PleosPC15_2.home.html; http://genome.jgi-psf.org/PleosPC9_1/PleosPC9_1.home.html); those for MK3 (GenBank access number: KF312590.1) were 96% and 95% identical. The different bases between the *poure* gene CDS of MK13 and MK3 were 93 (Table 6). The *poure* mRNA sequences of MK13, MK3 and DK13 × 3 were all 100% identical to the RNA-seq results. However, the mRNA sequences and gene CDS of *poure* differed by 4 bases in MK13 and 12 in MK3. In MK13, the differences were two Ts to Cs and two Gs to As. In MK3, the differences were one C changing to G, four Cs to Ts, four As to Gs, and three Gs to As (Table 7). This revealed that *P. ostreatus* simultaneously occurred in numerous RNA editing types. Furthermore, the *poure* mRNA sequences of DK13 × 3 were more identical to that of MK13 than MK3, with only two different bases and one predicted amino acid to MK13, while there were 89 different bases compared to MK3. As with MK13, the mRNA sequence and gene CDS of *Poure* in DK13 × 3 involved 4 bases, one T to C, one C to T, and two Gs to As (Tables 6 and 7). Urease catalyzed the hydrolysis of urea into carbon dioxide and ammonia. Urease was the first enzyme to be crystallized from jack beans, and it was the first protein whose enzymatic properties were demonstrated by Sumner in 1926.[42] Ureases have been

Table 4 – Functional annotation of differentially expressed genes in dikaryon DK13 × 3 compared to its parental MK3 monokaryon.

Gene ID	Log_2 ratio	Up/down	Homologous protein	NCBI ID	E-value
Unigene22495	17.9421	Up	Glycoside hydrolase family 30 protein	XP_001883860.1	7E−13
Unigene33702	17.4268	Up	YOP1	XP_001828571.1	8E−71
Unigene20364	16.6204	Up	Cystathionine beta-synthase (beta-thionase)	XP_754772.1	2E−24
Unigene33752	16.5541	Up	Aspartate amino-transferase	XP_001874806.1	1E−72
Unigene17552	16.5366	Up	Aldo-keto reductase	XP_001838896.2	2E−87
Unigene7733	16.4478	Up	Oligopeptide transporter	XP_001883373.1	0
Unigene12919	16.1909	Up	Symbiosis-related protein	ADD66798.1	6E−10
Unigene33888	16.1005	Up	40S ribosomal protein S12	XP_002475522.1	4E−71
Unigene33770	15.9518	Up	RNA-binding region RNP-1	YP_001022993.1	8E−10
Unigene8068	15.9284	Up	YALI0C17391p	XP_501942.2	4E−07
Unigene15993	15.6892	Up	Nucleoside-diphosphate-sugar epimerase family protein	XP_748586.1	3E−13
Unigene14963	15.6826	Up	Receptor expression-enhancing protein 4	XP_001837879.2	1E−103
Unigene29875	15.6645	Up	TKL/TKL-ccin protein kinase	XP_001838297.2	3E−20
Unigene14774	15.6242	Up	Short-chain dehydrogenase/reductase SDR	XP_001828376.2	1E−108
Unigene15814	15.5711	Up	Glycoside hydrolase family 16 protein	XP_003028746.1	4E−91
Unigene5851	15.3849	Up	Chitinase	BAA36223.1	9E−08
Unigene14448	15.2642	Up	Guanine nucleotide-binding protein alpha-4 subunit	XP_001884704.1	6E−21
Unigene3750	15.2109	Up	Tetraspanin Tsp2 family	XP_001881334.1	4E−11
Unigene16536	15.1569	Up	Mitochondrial protein	XP_001828236.1	4E−92
Unigene17706	15.1209	Up	Aldo-keto reductase	XP_001835654.1	1E−125
Unigene22890	15.0774	Up	Carboxyesterase	XP_002473270.1	3E−50

Table 5 – Laccase gene expression profile in *Pleurotus ostreatus* dikaryon DK13 × 3 and its parental monokaryons MK13 and MK3.

Unigene ID	Nr-annotation	RPKM			Gene differential expression patterns[a]
		MK3	MK13	DK13 × 3	
16937	phenol oxidase	1.74	0.32	0.00	Group 1
36987	laccase 3	0.18	0.19	0.00	
17686	diphenol oxidase	0.00	7.97	7.18	Group 2
17819	phenol oxidase	0.00	3.46	1.92	
32024	phenol oxidase	0.00	1.67	0.42	
33168	laccase	0.00	2.60	0.48	
24223	laccase	3.24	0.00	0.79	
9579	phenol oxidase	3.72	0.00	3.04	
10675	laccase	3.64	2.14	3.23	Group 3
13269	laccase	3.93	2.17	3.44	
17104	phenol oxidase	0.79	2.71	2.19	
21195	laccase 2	1.72	1.96	1.78	
21872	laccase 4	1.78	1.32	0.84	
25117	laccase 3	2.27	0.42	0.85	
31192	poxa3b	0.97	1.08	1.19	
33608	phenol oxidase	0.59	2.13	0.61	
3517	phenol oxidase 1	1.33	1.09	0.28	
Total		25.9	31.23	28.24	

[a] Group 1, genes expressed in both parental monokaryons but not in the dikaryon; Group 2, genes expressed in one parental monokaryon and the dikaryon but not in another parental monokaryon; Group 3, genes expressed in parental monokaryons and the dikaryon.

found in numerous bacteria, fungi, algae, plants and some invertebrates, and they have been found to help microorganisms and plants use endogenous and exogenous urea as a nitrogen source. The ammonia produced is subsequently utilized to synthesize proteins.[43] Ureases of bacteria, fungi and higher plants are highly conserved.[44] In higher plants and fungi, the enzyme is encoded by a single gene.[45,46] Thus, our results showed that the *poure* transcript of DK13 × 3 was from the MK13 *poure* gene and that RNA editing also occurred (Table 6).

Discussion

Our results showed that the global gene expression profile of dikaryon was distinct from its constituent monokaryons, and there was an expression difference in nearly two-thirds of the genes. This change was also confirmed by RT-PCR cloning and sequencing of the *poure* mRNA of the three strains. These results are not in agreement with previous reports,[22,23] which is probably due to the different gene expression

Table 6 – Sequence alignment of the *poure* gene CDS between the two monokaryons of *Pleurotus ostreatus*.

CDS[a]	The position of mismatched bases from the 5′ end of the *Poure* CDS															
	10	30	81	160	177	189	222	264	285	306	325	331	363	365	376	523
MK13	T	G	C	C	C	G	C	C	T	A	A	T	C	A	G	T
MK3	G	A	G	T	T	A	T	T	C	C	C	C	T	C	A	C
	540	558	567	576	586	600	639	642	654	691	741	763	779	795	879	903
MK13	C	C	G	C	T	G	A	A	T	T	A	A	G	G	G	T
MK3	T	T	A	T	C	A	G	G	A	C	G	G	A	A	C	C
	924	966	975	978	1005	1061	1074	1083	1170	1200	1209	1236	1290	1311	1326	1335
MK13	A	C	G	C	T	G	A	C	T	A	C	C	C	A	C	C
MK3	G	T	A	T	C	A	T	T	C	T	T	T	T	G	T	T
	1383	1521	1523	1527	1536	1587	1605	1628	1641	1680	1689	1707	1709	1713	1764	1767
MK13	G	G	T	C	A	T	C	A	C	T	T	C	C	G	T	T
MK3	T	A	A	T	C	G	A	T	T	C	G	A	T	A	C	C
	1769	1782	1788	1808	1848	1857	1876	1917	1992	2061	2067	2070	2076	2079	2158	2208
MK13	C	C	C	A	A	C	A	C	C	C	T	C	T	G	G	C
MK3	T	T	T	G	G	T	G	T	A	T	C	T	C	A	A	T
	2224	2229	2268	2317	2325	2364	2409	2450	2451	2469	2475	2478	2480			
MK13	T	C	A	G	G	C	A	A	C	A	C	T	G			
MK3	C	T	T	A	T	T	G	G	T	G	T	C	A			

[a] The accession numbers in GeneBank of the *poure* gene CDS of *Pleurotus ostreatus* MK13 and MK3 are KF312589.1 and KF312590.1.

Table 7 – Sequence alignment of the *poure* gene CDS, mRNA and predicted AAs between the three strains of *P. ostreatus*.

Strain		The position of mismatched bases from the 5′ end of the *poure* CDS in MK13 or MK3 and contact mismatched AA residues												
		529	1005	1301	1383	2158								
MK13	CDS	T	T	C	G	G								
	AA	Phe				Glu								
MK13	mRNA	C	C	C	A	A								
	AA	Leu				Lys								
DK13 × 3	mRNA	T	C	T	A	A								
	AA	Phe				Lys								
		16	365	523	570	691	779	924	1061	1628	1876	2224	2450	
MK3	CDS	C	C	C	A	C	A	G	A	A	G	C	G	
	AA		Ala				Glu		Glu		Val	Leu	Gly	
MK3	mRNA	G	T	T	G	T	G	A	G	G	A	T	A	
	AA		Phe				Gly		Gly		Ile	Phe	Asp	

profiling approaches. The high throughput RNA-seq was certainly more thorough and comprehensive than traditional DNA hybridization.[47]

Based on the gene transcriptional quantity, heterosis in diploids was considered to result from differential gene expression, including the following five gene expression patterns: (i) genes expressed in both parents but not in hybrids, (ii) genes expressed in one parent and hybrid but not in another parent, (iii) genes expressed in one parent but not in another parent or hybrid, (iv) genes expressed only in a hybrid but not in both parents, and (v) genes expressed in both parents and the hybrid. The first four patterns are the presence/absence variations (PAV)[48]; the fifth could be divided into additive and non-additive gene expression patterns for which hybrids showed a transcript level equal to or deviating from the mid-parent value (average of the two parents).[49–51] In this study, the mycelial growth rate of *P. ostreatus* dikaryon DK13 × 3 was significantly higher than that of the two parental monokaryons, indicating the advantage of sexual reproduction or heterosis in the dikaryon. The total gene expression quantity in the dikaryon was 4.8% and 6.5% higher than its constituent monokaryons, and all possible modes of differential gene expression that were present in the dikaryon when compared to its constituent monokaryons, including presence/absence variation and additive/non-additive gene expression, may be contributing to heterosis. This was confirmed in previous studies.[24]

Monoallelic expression genes have been found in a number of organisms, including humans, rodents, corn, and yeast.[52] They are on the X chromosome in female placental mammals or on autosomes,[5] and the selection of the expressed allele may depend on the parental origin or be random.[53] However, this phenomenon has not been reported in the dikaryon. Those DEGs in the dikaryon can be divided into four groups. The main group was simultaneously expressed in both of the monokaryons. The other two smaller groups were expressed in only one of two monokaryons. The fourth group was expressed in the dikaryon alone. DEGs in the dikaryon

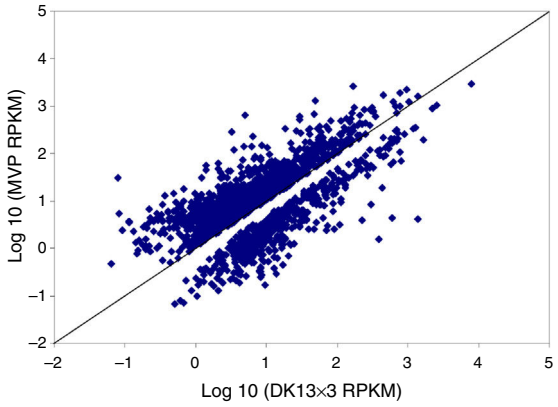

Fig. 7 – Scatter plots showing the expression levels of the differentially expressed genes in dikaryon DK13 × 3 vs. mid-parent expression value model estimates. RPKM, reads per kb per million reads and MPV, mid-parent expression values.

only expressing MK3 or MK13 might be regarded as monoallelic expression genes, as evidenced by RT-PCR cloning and sequencing results. For example, the *poure* transcript in the dikaryon was from the MK13 nucleus gene but not MK3. More than 10% of the monoallelic expression genes in the dikaryon were from each parental monokaryon. However, we could not determine whether they demonstrated autosomal random monoallelic expression, sex chromosome inactivation, or imprinted gene expression. In fungi, the chromosome containing mating genes may be deemed as the sex chromosome. In mice and humans, more than 10% of the genes have autosomal random monoallelic expression.[54,55] The isozyme bands that are only present in the *S. commune* dikaryon were demonstrated to depend on the expression of mating genes A and B.[16] Accordingly, the relationship between the fourth group and the mating genes merits further study.

RNA-editing by base deamination has been reported in plant mitochondria and plastids (C-to-U editing)[56] and mammals (A-to-I editing)[57]; U-to-C and guanosine (G)-to-A changes, which are probably by trans-amination, are also reported in mammals.[58,59] No similar cases have been found in higher fungi. In this study, our results showed that numerous types of RNA editing existed in the *poure* mRNA in *P. ostreatus*, including C-T, A-G, and C-G base substitution.

Taken together, our results suggest that the gene expression patterns in dikaryons should be similar to diploid. Finally, we strongly propose that the fungal dikaryon is a perfect experimental model for studying sex evolution and monoallelic expression due to its unique biology. The two parental monokaryons can independently live with asexual reproduction. It was proposed that the monokaryons were the temporary stage of dikaryons and had less combative ability than dikaryons,[60] but several species models have demonstrated that monokaryons have a similar or more combative phenotype compared to dikaryons.[61,62] Therefore, it was suggested that monokaryons with greater adaptive genetic potential may improve the combative ability to dikaryons.[63] In dikaryons, the two monokaryon nuclei do not fuse to karyogamy, and the two chromosomal sets only occasionally recombine during vegetative growth[63]; therefore, it is easy to determine the origins of alleles in a dikaryon. Although there is no paternal and maternal distinction in the mating of two compatible monokaryons, as with other sexual reproduction, the mitochondrion in almost all dikaryons is from only one monokaryon.[64] The example donor can be regarded as the female parent.

Conflicts of interest

The authors declare no conflicts of interest.

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