**Bisulfite sequencing analysis**

Raw reads from bisulfite treatment were checked for quality using FastQC (www.bioinformatics.babraham.ac.uk/projects/fastqc/) and aligned to the reference genome (PC15 v2.0) using the BS-Seeker2 pipeline[1]. DNA methylation content was estimated at each cytosine level as a relative value of methylated cytosines (5mC) to unconverted (5mC) and converted (C→T) cytosines after bisulfite modification. Bisulfite non-conversion rate of 0.27% was estimated from an unmethylated lambda DNA spike-in, according to previous studies[2]. Default parameters of the whole-genome bisulfite alignment pipeline were unchanged, except for using Bowtie2[3] as aligner (local alignment) and allowing unique alignments. For the following analyses, we only considered cytosines covered by at least four reads. Pairwise comparison to detect differentially methylated regions (DMR) was performed using the SMART software[4], setting 200 bp as the minimum window size, $p$-value < 0.05 for DMR calling.

**MSRE-qPCR analysis for gene-specific DNA methylation**

The methylation levels were analyzed at specific genic regions according to the MSRE-qPCR method previously described[5]. We performed the enzymatic digestion of a region (methylated in PC9 genes and unmethylated in PC15 genes) with MSRE (methylation-sensitive restriction enzymes). In parallel, mock digestions were performed in the same PC9 and PC15 regions by replacing the enzymes with water. A total of 1 ug genomic DNA was digested with 40 U of AccII and HpaII enzymes overnight at 37°. After incubation, digested samples were ethanol precipitated and dissolved in 50 µl water. The "digested" and "mock" samples were subjected to real-time PCR amplification, selecting gene regions containing at least three recognition sites for the enzymes used. Primer design was performed by using Primer3 software[6] and the list of oligos used in this study are available in Supplementary Table S8. Reactions were carried out in a CFX96 thermal cycler (Bio-Rad Laboratories, S.A.) and amplification products were monitored at each cycle of PCR using SYBR green fluorescent dye. Each assay was performed in duplicate in 96-well plates and PCR and reactions were run according to the following cycling conditions: DNA templates were denatured at 95 °C for 10 min, followed by 40 cycles of amplification (95 °C for 30 sec, 54 °C for 1 min, and 72 °C for 1 min) and final extension at 72 °C for 7 min. The methylation level for any amplified region was estimated using a relative quantitative approach based on the following equation;

$$\text{Methylation level} = 2^{-\Delta Ct}$$

where ΔCt = average Ct value from the "digested reaction" minus the average values from the "mock reaction" for each sample (PC9 and PC15).

**mRNA-seq analysis**

RNA-seq data were filtered for quality using FastQC and trimmed with BBDuk from the BBMap package (https://sourceforge.net/projects/bbmap/) to remove sequences of adapters and low quality reads. The resulting reads were aligned to the reference genome assembly using STAR v2.3.1[7] restricted to single hit mapping using the following parameters: --outReadsUnmapped Fastx --outFilterMismatchNoverLmax 0.04 --outFilterMultimapNmax 1. Expression levels were quantified using python script rpkmforgenes.py (www.sandberg.cmb.ki.se/media/data/rnaseq/rpkmforgenes.py) to calculate values of fragments per kilobase of transcript per million mapped reads (FPKM).

**sRNA-seq analysis**

Small RNA (sRNA) raw reads were first processed to trim the adapter sequence and filter by size (17 to 30 nt) using BBDuck software. Reads mapping to a custom database of ribosomal RNA (rRNA) and transfer RNA (tRNA) were also removed. The remaining sRNA reads were mapped to the reference genome using Butter 0.3.2, a variant of Bowtie optimized for small RNA included in the ShortStack package[8]. This program uses iterative read assignment, and reads with multiple possible alignments were mapped to a single best location. The program was run using the parameters: --mismatches 1 --bam2wig combined. After mapping, sRNA reads were overlapped to features listed in TE and gene annotation datasets. Scripts used for sRNA analyses are available at https://github.com/RaulCas/smallRNApipeline.

**Differential gene expression analysis**

Differentially expressed gene (DEG) analyses were performed using the EdgeR Bioconductor package[9] using three biological replicates per sample, and considering a $p$-value < 0.05 and log2 (foldchange) > 3 as cutoffs for statistical significance. A heat map plot combined with hierarchical clustering was constructed with all DEG. Clusters of co-expressed genes were trimmed using the define_clusters_by_cutting_tree.pl tool of the TRINITY package[10] using 55 as the cutoff value. Hierarchical clustering was constructed using hclust R function, with "complete" method and Euclidean distance, all parameters integrated in the TrinityRNAseq pipeline[10]. Gene ontology (GO) enrichment analysis of DEGs was performed using GOATOOLS software[11].

**Whole genome alignment**

The draft genome assembly of *P. ostreatus* PC9 was aligned to the reference PC15 v2.0 genome to determine the overall nucleotide sequence identity and determine the unique regions of both genomes. PC9_V1.0 (35.6 Mb) is assembled in 572 scaffolds accounting for a total of 476 gaps covering 9.72 % of the assembly length. Unmasked assemblies were obtained from the MycoCosm browser (www.genome.jgi.doe.gov) and aligned using the NUCmer script from the MUMmer package 3.0[12]. NUCmer was selected to align highly conserved genome assemblies and delta-filter parameter applied to assign the optimal placement avoiding alignments to each repeat location.

**General manipulation of sequencing data**

Conversion between data formats and manipulation of BS-seq, RNAseq, and sRNAseq datasets was carried out with BEDTools[13], SAMtools[14] and custom Python scripts. All the results generated after mapping to genomic features (methylation levels, RNAseq counts, and sRNAseq counts) are reported in Supplementary Tables S1.

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