

Effects of secondary metabolism on sexual development and wood decay in agaricomycetes

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Research aims

Agaricomycetes are characterized by two main criteria. One is dramatic morphological differentiation, fruiting development, or mushroom formation. Mushrooms are relatively large multicellular structures that agaricomycetes form during sexual reproduction. The second is wood decay, especially white rot. White-rot fungi play an important role in the global carbon cycle since they biodegrade almost all wood lignin found in nature. Molecular genetic studies on the regulation of morphogenesis and secondary metabolism in ascomycetes, and physiological and biochemical studies on wood decay, indicate a correlation between mushroom formation, wood decay, and the secondary metabolism found in white-rot fungi. In this study, we investigated this hypothesis through an extensive molecular genetics approach using the white-rot fungus *Pleurotus ostreatus*.

Methods

In this study, *P. ostreatus* strains 20b and 20b/1 (a *ku80* disruptant from wild-type strain PC9) was used for gene targeting experiments. Examination of the effects of gene disruption on fruiting development and the ability of white rot was carried out according to the procedure described in the previous study¹⁾. Quantification of wood lignin-containing sawdust media was performed according to the Klason method. *P. ostreatus* strains were grown on sawdust media for 13 days, after which total RNA was extracted from mycelial cells of each to perform comparative RNAseq analyses.

Results

Effects of the targeted disruption of *veA* and *laeA* genes on the ligninolytic activity in *P. ostreatus*

We disrupted *P. ostreatus* genes homologous to ascomycete genes *veA* and *laeA*, which were reported to affect secondary metabolism and morphogenesis in various ascomycetes. *LaeA* and *veA* disruptions did not cause significant defects in either sexual

development or ligninolytic activity (Table 1), although extracellular manganese peroxidase (MnP) activity was diminished in the *veA* disruptant when it was grown on GP (glucose-peptone) liquid medium.

Effects of targeted disruption of genes involved in sexual development on the ligninolytic activity of *P. ostreatus*

We disrupted *P. ostreatus* genes homologous to agaricomycete *Coprinopsis cinerea* genes *snf5*, *ubc2*, and *rmt1* (Table 1), which were reported to be essential for sexual development. The results showed that *ubc2* is essential for dikaryosis in *P. ostreatus* as well as *C. cinerea*, while *snf5* does not affect dikaryosis but is essential for fruiting development in *P. ostreatus*. Unlike the case of *C. cinerea*, *rmt1* disruption did not cause any defects in either fruiting initiation or hyphal growth rate in response to carbon sources in *P. ostreatus*. Targeted disruption of *ubc2* caused defects in wood lignin biodegradation, while disruption of *rmt1* did not. We could not examine the effects of *snf5* disruption on the ability to biodegrade wood lignin because the hyphal growth rate of *snf5* disruptants was much lower than that of the wild-type strain.

Effects of mutations that cause defects in ligninolytic activity on sexual development in *P. ostreatus*

We also examined the effect of disrupting genes that impede wood lignin biodegradation on fruiting development in *P. ostreatus*. *Pex1* and *chd1* disruption, which cause significant defects in ligninolytic activity, were also shown to be essential for fruiting initiation, unlike *wtr1* disruption, which was shown to cause slight defects in the ligninolytic activity but no effect on fruiting initiation.

Recently, we identified some new genes likely responsible for mutant phenotype defects in wood lignin biodegradation using the methodology demonstrated in the previous study²). Specifically, two genes encoding putative transcription factors were identified. One is involved in the regulation of secondary metabolism and morphogenesis in *Aspergilli*. Another two genes were identified that encode putative proteins involved in nucleosome assembly, which is one of the epigenetic regulatory mechanisms involved in the regulation of secondary metabolisms in ascomycetes. We are now confirming that these genes are indeed responsible for the mutant phenotypes. Furthermore, a dominant mutation that causes defects in both lignin biodegradation and fruiting initiation was also identified, along with the gene responsible for this mutation.

Gene lists that are significantly inactivated by *snf5* and *ubc2* disruption overlap with those inactivated by *pex1*, *chd1*, and *wtr1* disruptions

We investigated the effects of the gene disruptions performed in this study on gene expression via RNAseq. Many of the genes that were significantly inactivated by *snf5* and *ubc2* disruption, which caused defects in sexual development, were also inactivated by *wtr1*, *chd1*, and *pex1*.

Conclusion

This research shows that some gene disruptions that cause defects in wood lignin biodegradation also cause defects in fruiting development. Comparative RNAseq analyses also suggest a correlation between the regulation mechanisms of sexual development and the ligninolytic system at transcription level, although it is unclear whether this is a direct effect or not. Reverse genetics data acquired in this study did not support the hypothesis that the malfunction of regulation mechanisms in *P. ostreatus* homologous to the regulation of secondary metabolism in ascomycetes affect sexual development and/or ligninolytic activity in *P. ostreatus*. However, the extensive forward genetics study identified four genes (four mutants) that are likely responsible for the mutant phenotype and defects in ligninolytic activity. These genes encode putative transcription factors homologous to those involved in secondary metabolism and morphogenesis in Aspergilli. The putative proteins are involved in nucleosome positioning, which may be involved in epigenetic regulation. Based on these results, we must investigate whether the regulatory mechanisms of secondary metabolism investigated in ascomycetes are present in agaricomycetes. and the molecular mechanisms that affect both lignin biodegradation and fruiting development.

References

- 1) Shioya, T. *et al.* (2013) The *Coprinopsis cinerea* septin Cc.Cdc3 is involved in stipe cell elongation. *Fungal Genet. Biol.* **58-59**: 80–90.
- 2) Nakazawa, T. *et al.* (2017) Identification of two mutations that cause defects in the ligninolytic system through an efficient forward genetics in the white-rot agaricomycete *Pleurotus ostreatus*. *Environ. Microbiol.* **19**: 261–272.

Table 1. Effects of gene disruptions on the ligninolytic activity and sexual development of *P. ostreatus*.

Disrupted gene	Effect on the ligninolytic activity	Effect on sexual development
<i>pex1</i>	significant decrease	defects in fruiting initiation
<i>wtr1</i>	slight decrease	no effect
<i>chd1</i>	significant decrease	defects in fruiting initiation
<i>veA</i>	no effect	no effect
<i>laeA</i>	no effect	no effect
<i>snf5</i>	no effect ¹	defects in fruiting initiation
<i>ubc2</i>	decrease	defects in dikaryosis
<i>rmt1</i>	no effect	no effect
<i>pcc1</i>	no effect	defects in dikaryosis

¹ Effects of *snf5* disruption on gene expression pattern are very similar those of *pex1*, *wtr1* and *chd1*.