A secretome tailored to endure oxidative stress in wood decomposer *Postia placenta*

ECFG15
ROME • ITALY 2020

University of Minnesota

Pacific Northwest
NATIONAL LABORATORY

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1. Background

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Ligninolytic Fungi

White rot

Oxidative enzymes

Peroxidases, laccases

BR fungi usually faster decomposers, yet have fewer lignocellulolytic genes.

How?

Brown rot

Small oxidants (ROS)

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + HO^- + HO^*$$

Fenton Reaction

Soft rot

Peroxidases, laccases less efficient than those used by WR

1. Background

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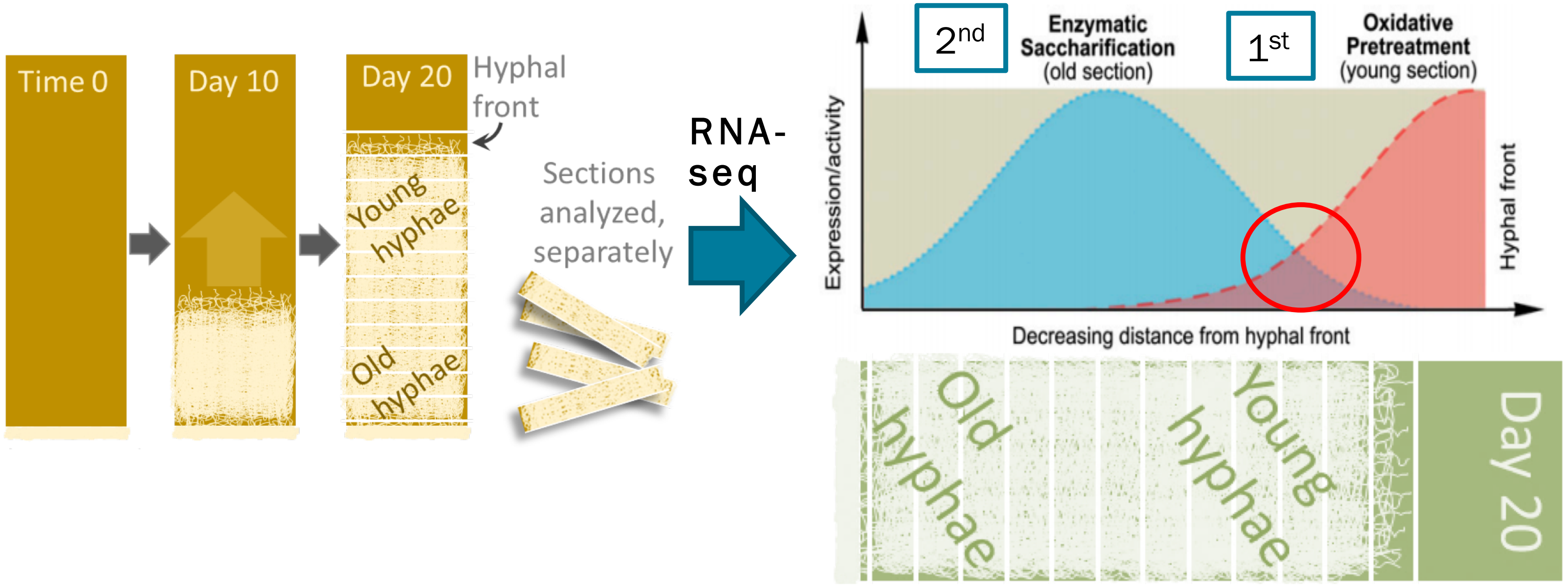
Brown rot decay → Two-step mechanism (1st oxidative, 2nd saccharification)



Hemicellulases, cellulases, pectinases

Zhang et al. (2016) PNAS 113: 10968-10973; Presley et al. (2018) AEM 84 (15)

1. Background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Biodegradation

Oxidative Damage Control during Decay of Wood by Brown Rot Fungus Using Oxygen Radicals

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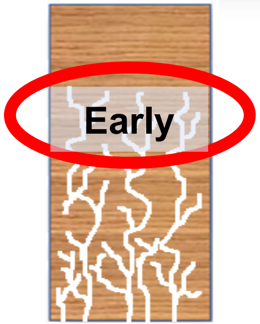
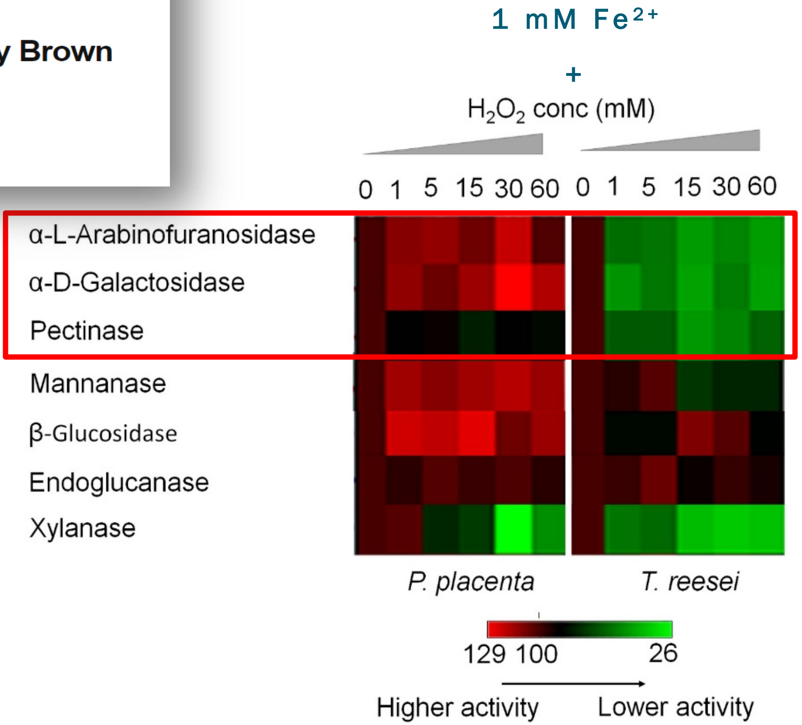




2. Objective- Understanding why they are tolerant

Research questions

1. Is tolerance of ROS unique to brown rot fungi (vs white rot)?

2. What's the basis of the ROS tolerance?

White rot

Trametes versicolor

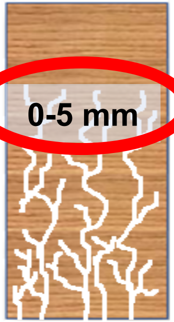
Soft rot

T. reesei

Brown rot

P. placenta

3. Experimental set-up



Early decay section

Protein extraction

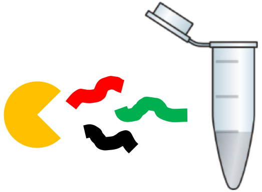
$H_2O_2 + Fe^{2+}$

1, 5, 15, 30, 60 mM

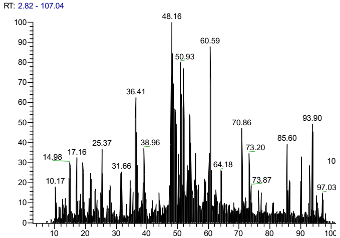
1 mM

Oxidative treatment

Residual enzyme activity



Protein precipitation and digestion



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C-18 LC and proteomics

4. Results

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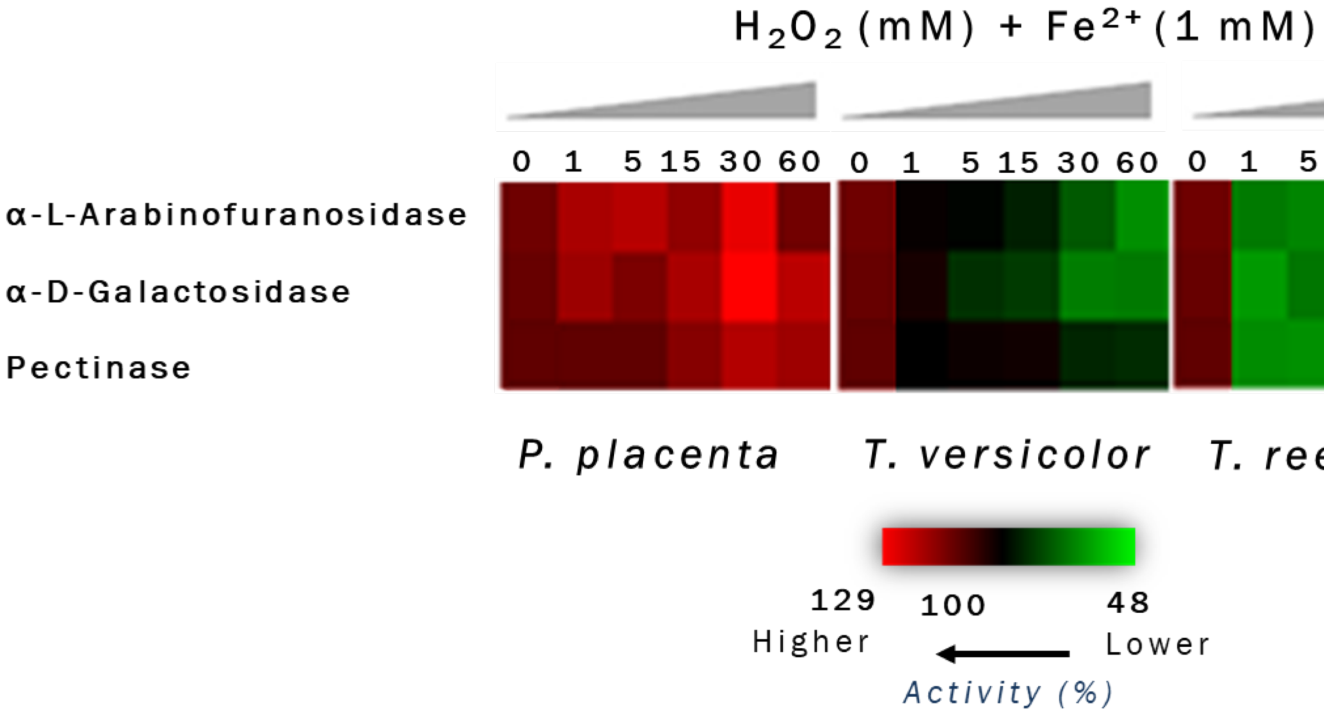
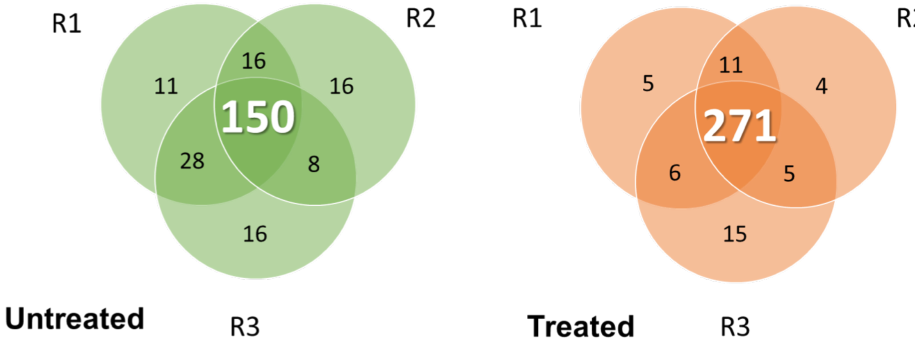
Residual activity after ROS exposure



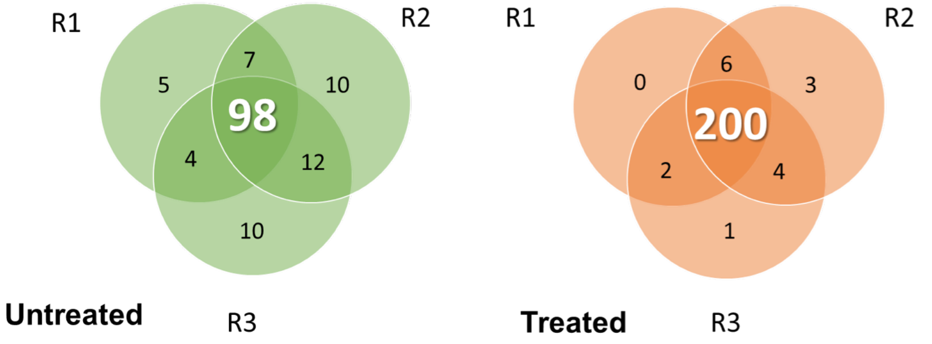
4. Results

Oxidation dynamics I (oxidized protein count)

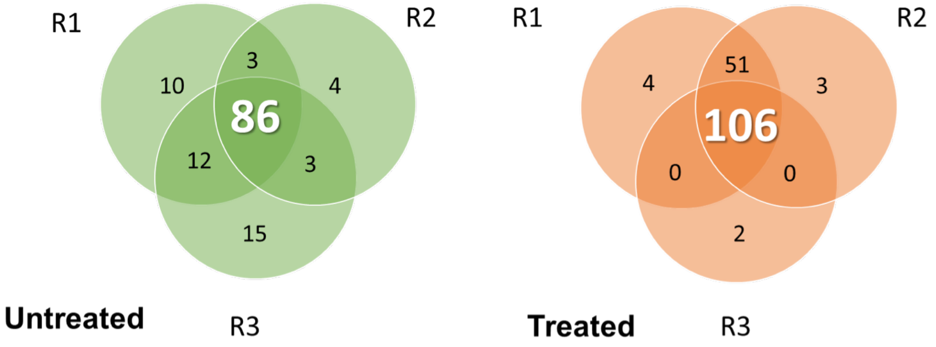
T. reesei

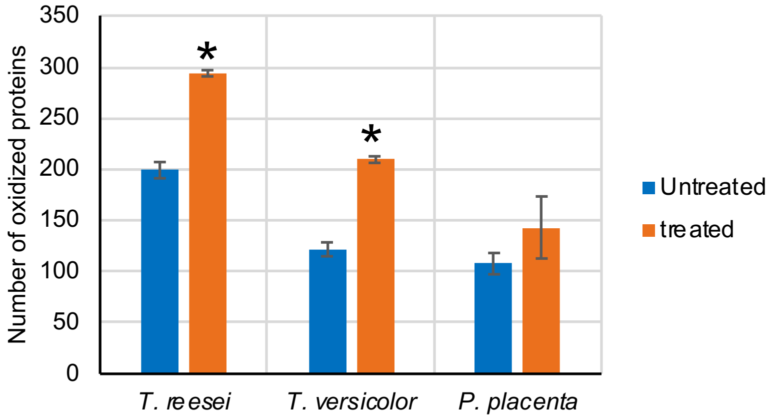


T. versicolor



P. placenta

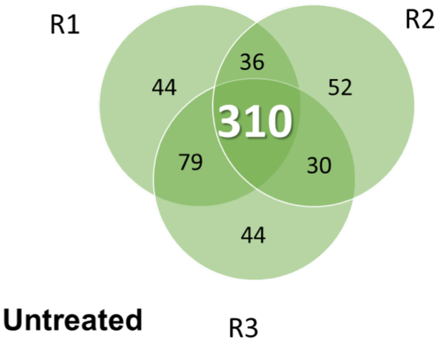


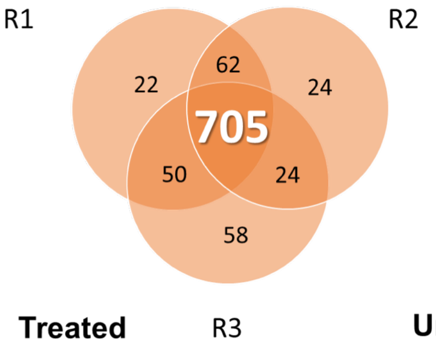


4. Results

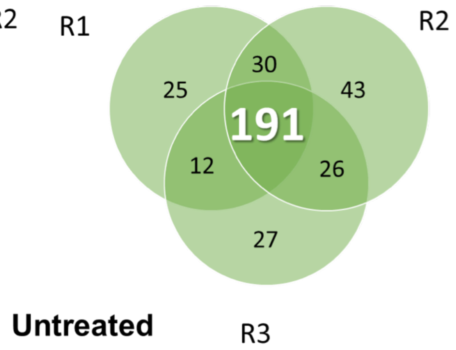
Oxidation dynamics I (oxidized peptide count)

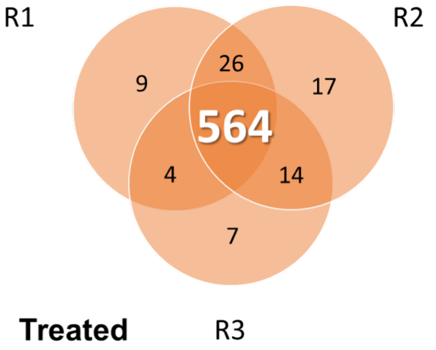
T. reesei



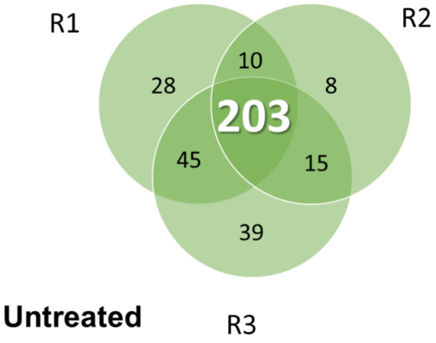


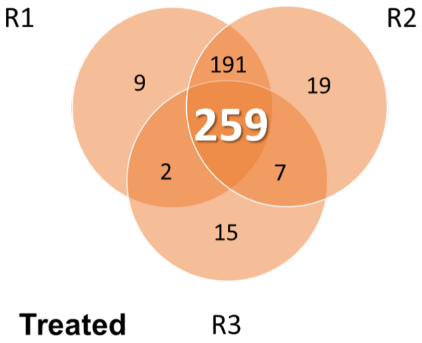
T. versicolor

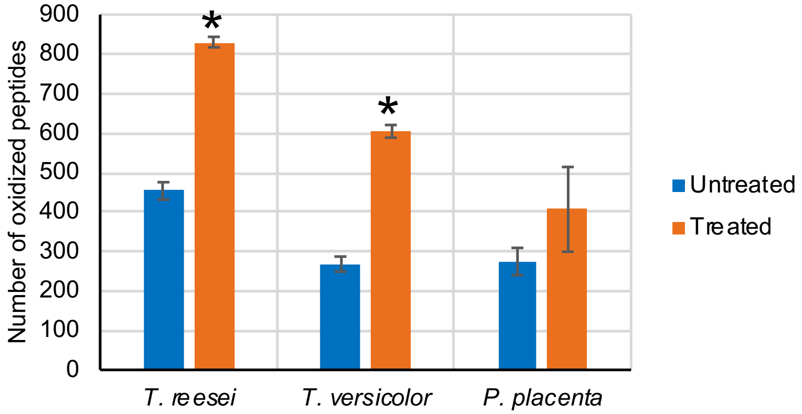




P. placenta

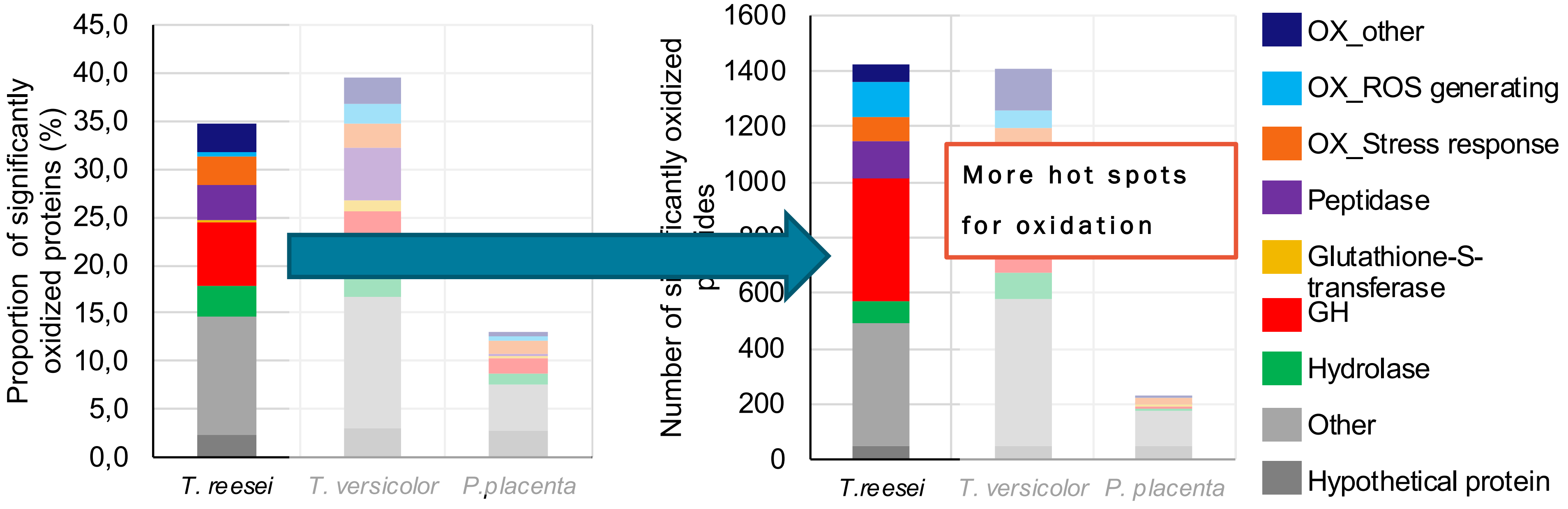






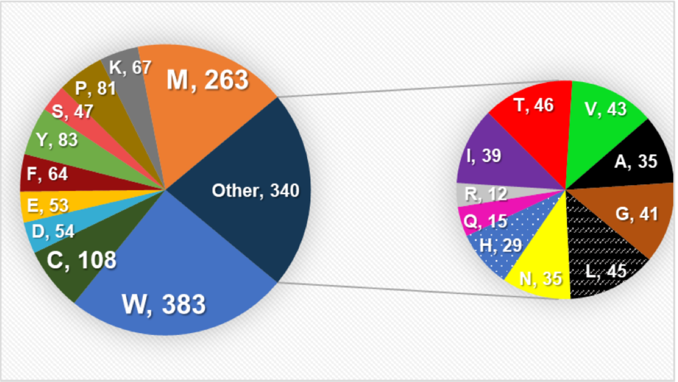
4. Results

Oxidation dynamics II (Significant oxidation events)



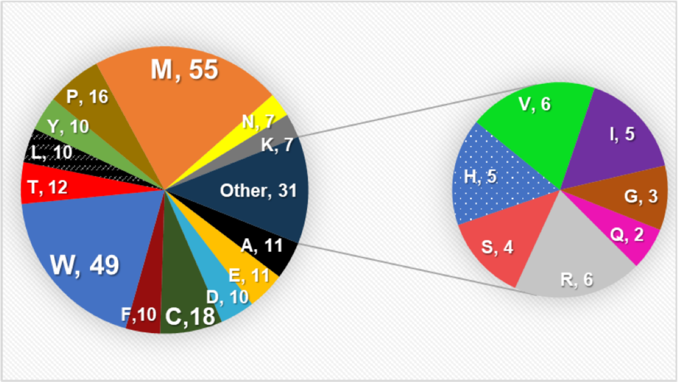
4. Results

T. versicolor



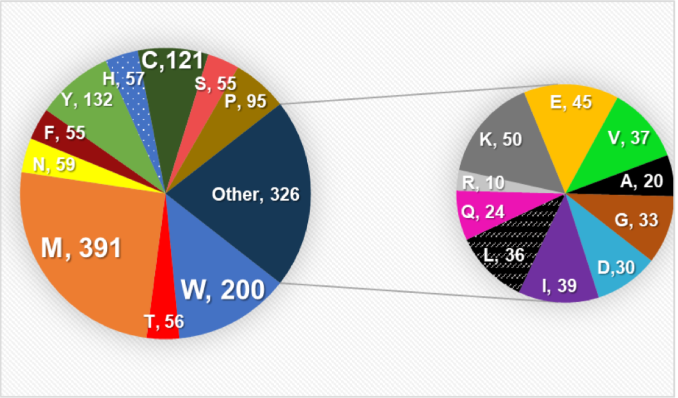
Total residues: 1543

P. placenta



Total residues: 257

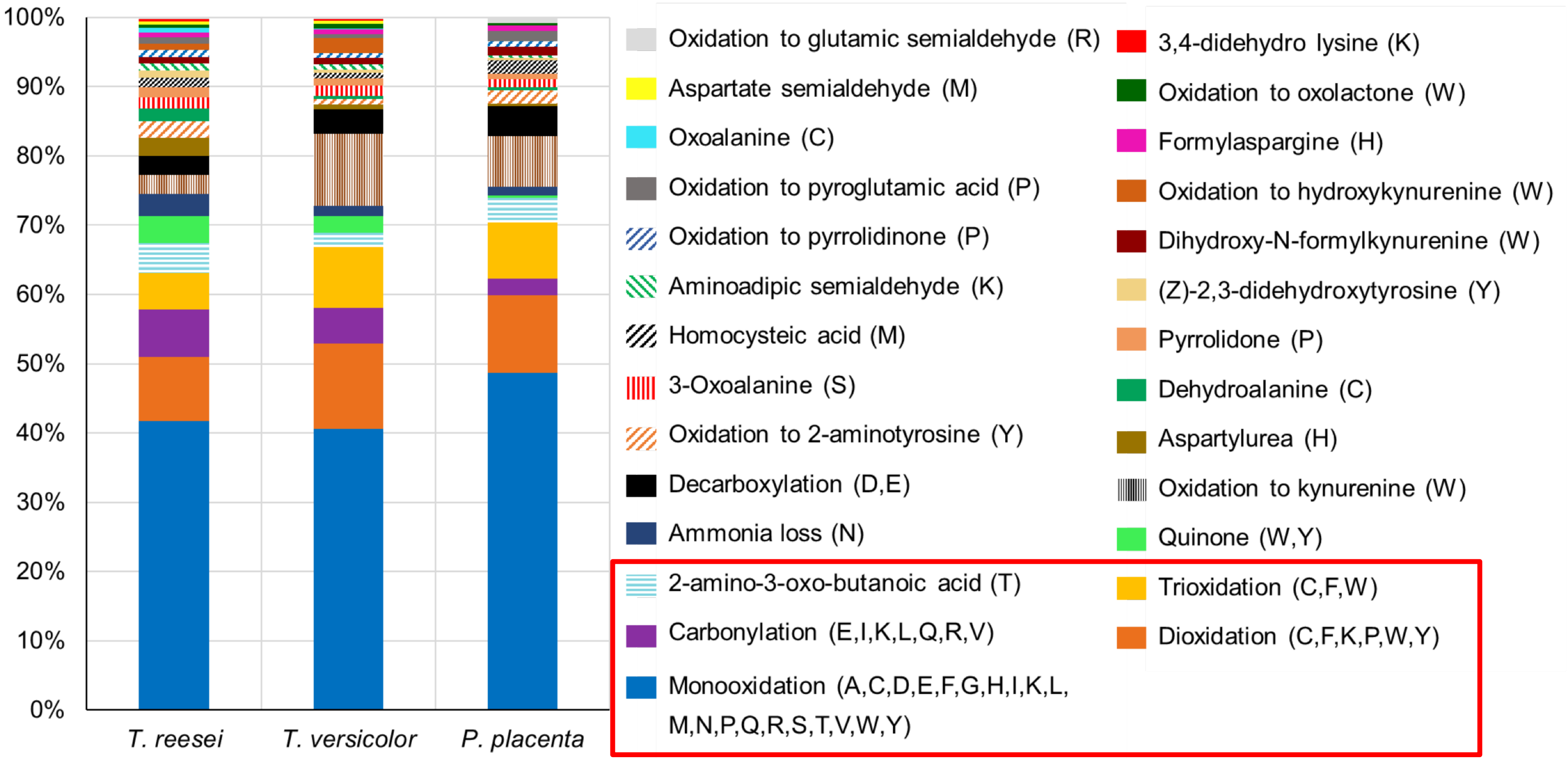
T. reesei



Total residues: 1545

Distribution of <u>significantly</u> oxidized residues in *T. reesei, T. versicolor,* and *P. placenta* (log2FC >2.0 for all peptides)

4. Results



Distribution of the type of significant oxidative found in *T. reesei*, *T. versicolor*, and *P. placenta* after the Fenton reaction treatment of enzyme extracts (log2FC >2.0 for all peptides).

4. Results

ROS-tolerant enzymes in *P. placenta*

- Pectinase
- α-L-arabinofuranosidase
- α-D-galactosidase

Number of enzymes bearing significant oxidative modifications

Enzyme \ Fungus	*T. versicolor*	*T. reesei*	*P. placenta*
Pectinase	4	0	0
α-L-arabinofuranosidase	2	1	0
α-D galactosidase	1	2	0

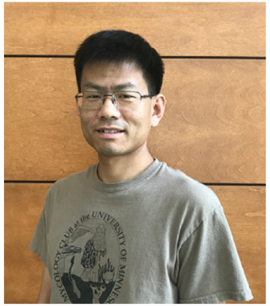
5. Conclusions

- GHs in *P. placenta* show tolerance of ROS.
- Specific oxidative modifications of GHs in *T. reesei* and *T. versicolor* seem to be the key to their sensitivity.
- W, M, and C are the most affected residues (Monooxidation)

Acknowledgments

Grazie mille a Tutti voi!!

Jonathan



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