Brief report A reverse chemical ecology approach to explore wood natural durability
Thomas Perrot, Guillaume Salzet, Nadine Amusant, Jacques Beauchêne, Philippe Gerardin, Stéphane Dumarcay, Rodnay Sormani, Melanie Morel-Rouhier, Éric Gelhaye

To cite this version:
Thomas Perrot, Guillaume Salzet, Nadine Amusant, Jacques Beauchêne, Philippe Gerardin, et al.. Brief report A reverse chemical ecology approach to explore wood natural durability. Microbial Biotechnology, 2020, 13 (5), pp.1673-1677. 10.1111/1751-7915.13540. hal-02913021
A reverse chemical ecology approach to explore wood natural durability

Perrot Thomas,1 Salzet Guillaume,1 Amusant Nadine,2 Beauchene Jacques,2 Gérardin Philippe,3 Dumarcay Stéphane,3 Sormani Rodnay,1 Morel-Rouhier Mélanie1 and Gelhaye Eric1

1 Université de Lorraine, INRAE, IAM, Nancy, France.
2 UA, AgroParisTech, UMR Ecofog, CIRAD, CNRS, INRAE, BF701, Kourou, France.
3 Université de Lorraine, INRAE, LERMAB, Nancy, France.

Introduction

Wood biodegradation, an essential step in carbon recycling, is a complex phenomenon which depends on many abiotic and biotic factors occurring at different spatial and temporal scales. Wood natural durability, defined as the natural resistance of wood against biological degradation (Taylor et al., 2002), varies according to wood species, geographic regions, and in response to variations of environmental exposure conditions during tree life. Nevertheless, main wood intrinsic physico-chemical properties are essential to wood decay resistance. For instance, wood density, which is widely used as a functional trait in the field of functional ecology, has been correlated to wood natural durability (Beauchêne, 2012; Lehnenbach et al., 2019), even if this correlation remained weak depending of the considered species (Chambers et al., 2000). Wood components such as extractives are also known to be involved in wood resistance against decay (Valette et al., 2017). These molecules are not covalently linked to cell walls and can thus be extracted using several solvents. Part of these wood extracts possess antimicrobial and insecticidal activities explaining their involvement in wood durability (Rodrigues et al., 2011; Amusant et al., 2014).

On the other hand, in forest ecosystems, wood degradation is mainly mediated by specialized microbial communities and in particular by wood-decaying fungi. These fungi have evolved to efficiently breakdown and mineralize wood components. In the last few years, confirming previous biochemical and microbiological approaches, comparative genomic studies have demonstrated the presence of specialized extracellular and intracellular enzymatic networks in these organisms (Nagy et al., 2017). Extracellular networks comprise oxidative and hydrolytic enzymes, which catalyse synergistically an efficient breakdown of wood polymers. In particular, white-rot fungi possess fungal systems could be used to explore the chemical environment encountered by wood-decaying fungi and also wood natural durability.

Microbial Biotechnology (2020) 0(0), 1–5 doi:10.1111/1751-7915.13540

Funding Information

This work was supported by a grant overseen by the French National Research Agency (ANR) as part of the ‘Investissements d’Avenir’ programme (ANR-11-LABX-0002-01, Lab of Excellence ARBRE) and the Region Lorraine Research Council.
class II peroxidases, which are involved in lignin breakdown (Floudas et al., 2012). Intracellular networks are mainly involved in the import and catabolism of the degraded wood products and in the detoxification of toxic molecules initially present or generated during wood degradation (Morel et al., 2013; Nagy et al., 2017). Within their extended detoxification system, wood-decaying fungi usually possess a larger set of genes encoding glutathione transferases (GSTs). These enzymes are involved in the second step (conjugation) of the detoxification pathways (Morel et al., 2009; Morel et al., 2013) and are largely used as indicators of the stress responses in various organisms (Bass and Field, 2011; Bouzahouane et al., 2018; Fernández-González et al., 2018). Moreover, GSTs exhibit ligandin properties allowing their non-catalytic interactions with potentially toxic wood molecules (Mathieu et al., 2012).

Molecular mechanisms governing wood durability remain largely to be unravelled and could be explored through the adaptation of organisms involved in wood decay. In the last few years, it was suggested that fungal detoxification systems could give insights about the chemical environment encountered by wood-decaying fungi (Deroy et al., 2015; Perrot et al., 2018). In this study, we propose a reverse chemical approach-like to explore this hypothesis. Chemical ecology is defined by Leal 'as the study of the chemical languages, cues and mechanisms controlling interactions among living beings, including communication among individuals of the same species and between organisms and their environment' (Leal, 2017). From the molecular knowledge of olfaction systems, reverse chemical ecology approaches have been developed to study the behavioural active compounds of various organisms and in particular of insects (Zhu et al., 2017; Choo et al., 2018). For instance, interactions between odorant-binding proteins and ligands have been used to screen potential semiochemicals (Li et al., 2018). In the context of wood durability, detoxification systems can be used in a similar approach. Previous studies have indeed demonstrated that GSTs of wood-decaying fungi could be used as molecular targets to identify wood molecules with antioxidative and antimicrobial properties (Schwartz et al., 2018; Perrot et al., 2018).

To test the hypothesis that GSTs could be indicators of wood durability, a reverse chemical ecology approach was developed using a set of enzymes from the world widespread white-rot fungus *Trametes versicolor* and wood extracts from neotropical forest of French Guiana. The obtained results support the initial hypothesis and demonstrate that such reverse chemical ecology approach could be useful to predict wood durability.

### Results and discussion

**Interactions between GSTs and wood extracts**

To set up the experimental design, heartwoods of 17 species from French Guiana tropical forest have been selected (Table 1). Data obtained with the heartwoods from *Andira coriacea*, *Bagassa guianensis*, *Dicorynia guianensis*, *Hymenaea courbaril*, *Peltophyge venosa*, *Sextonia rubra* and *Tabebuia serratifolia* have been previously published (Perrot et al., 2018). Heartwoods from *Abarema junpumba*, *Boca prouacensis*, *Hirtella bicornis*, *Oxandra asbeckii*, *Parkia nitida*, *Parkia pendula*, *Pouteria decorticans*, *Protium gallicum*, *Swartzia canescens* and *Vouacapoua americana* were from commercial origin (Degrad Saramaca’s sawmill, Kourou, French Guiana) or harvested as described in Lehnebach et al. (2019). All these woods belong to the DEGRAD database (Beauchêne, 2012). The DEGRAD database contains wood density and wood durability data for more than 300 tree species from French Guiana tree species. Among the 17 chosen woods, four are classified as very durable (x < 10%), five as durable (10% < x < 25%), five moderately durable (25% < x < 45%) and three non-durable (x > 45%) (Table 1). From the 17 selected species, molecules from corresponding heartwoods have been sequentially extracted using four solvents exhibiting different polarities (dichloromethane, acetone, toluene/ethanol, and water). From this step, we obtained a collection of 68 wood extracts.

Glutathione transferases, as other drug metabolizing enzymes, possess the ability to bind structurally unrelated molecules (Atkins, 2019), suggesting that they could be good candidates to test the proposed reverse chemical approach. In particular, six GSTs belonging to the omega class from *Trametes versicolor* (*TvGSTOs*) have been shown to be able to bind wood polyphenolic compounds known to possess antimicrobial activity (Schwartz et al., 2018; Perrot et al., 2018). The interactions between these *TvGSTOs* and their ligands were quantitatively measured through a thermal shift assay. This assay allows to determine modification of the protein thermal stability (ΔTd) due to ligand binding (Deroy et al., 2015; Schwartz et al., 2018).

Using this approach, interactions between the 68 extracts and the 6 *TvGSTOs* were then followed (Table S1). Each *TvGSTO* exhibits a specific pattern of interactions with the tested extracts. For further analysis, a ‘GST reactivity’ value (ΣΔTd) has been calculated for each extract, adding the ΔTd absolute values (using reduced centred data) obtained with the six *TvGSTOs* (Table 1, Table S1). Each tested wood was then defined by four values corresponding to the sum of interactions between *TvGSTOs* and the mixtures obtained after
Glutathione transferases for wood durability

Table 1. Wood durability (%mass loss), wood density and GST reactivity obtained from the 17 woods of French Guiana forest.

| Wood species                          | %mass loss | Densitya | Durability Class | ΣΔTd A | ΣΔTd D | ΣΔTd TE | ΣΔTd W |
|---------------------------------------|-----------|----------|-----------------|--------|--------|---------|--------|
| Abarema Jupunba                       | 51.5      | 0.68     | ND              | -3.86  | -3.24  | -4.95   | -3.13  |
| Andira coreacea                       | 24        | 0.92     | D               | 0.85   | 9.94   | 0.35    | -4.53  |
| Bagassa guianensis                    | 23.9      | 0.61     | D               | 8.18   | -0.52  | 6.02    | 3.94   |
| Bocconia pinnata                      | 5         | 1.20     | VD              | 5.59   | 3.45   | 5.70    | 1.13   |
| Dicoria guianensis                    | 17.6      | 0.78     | D               | -1.89  | 1.96   | -0.50   | -1.51  |
| Hirtella bicolora                     | 23.8      | 0.96     | D               | -2.31  | -2.46  | -3.95   | 0.30   |
| Hypolda guianensis                   | 48.3      | 0.89     | ND              | 5.55   | 5.18   | 1.79    | -1.01  |
| Oxandra asbeckii                      | 27.4      | 1.03     | MD              | -3.47  | -2.50  | -3.17   | -2.67  |
| Peltogyne venosa                      | 44.4      | 0.49     | MD              | 4.58   | 0.32   | 0.22    | -1.58  |
| Porotheria decoricanse                | 25.7      | 0.88     | MD              | -0.31  | -1.26  | -0.54   | -1.89  |
| Proteus ubiquilis                     | 9         | 0.87     | VD              | 1.35   | -3.75  | 4.01    | 0.65   |
| Sextonia rubra                        | 28.7      | 0.75     | MD              | -3.50  | -2.45  | -2.61   | -2.14  |
| Swartzia canesensis                   | 37.1      | 1.00     | MD              | -2.20  | -3.25  | -3.85   | -0.88  |
| Tabebuia capitata                     | 6.8       | 1.20     | VD              | -1.39  | 5.80   | -1.94   | -2.45  |
| Vouacapoa americana                   | 9         | 0.90     | VD              | 1.20   | 4.51   | 1.72    | 0.48   |

ΣΔTd A: GST reactivity obtained with the acetonic extract of the considered wood species. ΣΔTd D: GST reactivity obtained with the dichloromethane extract of the considered wood species. ΣΔTd TE: GST reactivity obtained with the dichloromethane extract of the considered wood species. ΣΔTd W: GST reactivity obtained with the dichloromethane extract of the considered wood species. ‘GST reactivity’ (ΣΔTd) for each extract has been calculated adding the absolute values (using reduced centered data) obtained with the six TvGSTo. Durability classes: VD very durable; D durable; MD moderately durable; ND non durable.
a. Values extracted from the DEGRAD database.

GSTs and wood durability

Wood durability is usually estimated from long-term soil bed tests (XP CEN/TS, 2006, 2006; Meyer et al., 2014). To constitute the DEGRAD database, soil tests have been performed incubating wood blocks in French Guiana soils in 2010/2012 in controlled laboratory conditions as mentioned in Amusant et al. (2014). Mass losses (%) have been measured after 6 incubation months. Data from DEGRAD database were used to quantify wood durability of the seventeen species used in this experiment. 68% of the variability (P < 0.006) of the measured mass losses could be explained by a model (linear regression) set-up from the four ‘GST reactivity variables’ as shown in Table 2 (%mass loss = 19.6 ΣΔTdA - 1.6 ΣΔTdD - 6.3 ΣΔTdTE - 4.5 ΣΔTdW; R² = 0.679, P < 0.006). This significant correlation supported the hypothesis that ‘GST reactivity’ could reflect at least partially the wood durability of the considered species.

GST reactivity and wood density

Despite a poor correlation and a considerable variability, wood density (WD) could be used as an indicator of wood durability (Chave et al., 2009; Chambers et al., 2000; Larjavaara and Muller-Landau, 2010). We postulated that ‘GST reactivity’ and WD should be linked to explain the durability of the tested species. From the same data set (17 heartwoods; Table 1), WD and ‘GST

Table 2. Correlation (Pearson coefficient) between GST reactivities, wood durability (%mass loss) and wood density.

| Variables | %mass loss | Wood density | ΣΔTd A | ΣΔTd D | ΣΔTd TE | ΣΔTd W |
|-----------|-----------|--------------|--------|--------|---------|--------|
| %mass loss| 1         | -0.657 (p < 0.004) | -0.144 | -0.129 | -0.464  | -0.398 |
| Wood density| 1         | 0.009        | 0.142 | 0.205 | 0.012   |
| ΣΔTd A     | 1         | 1            | 0.296 | 0.881 | 0.585 (p < 0.001) |
| ΣΔTd D     | 1         | 1            | 0.232 | 0.292 | 0.604 (p < 0.010) |
| ΣΔTd TE    | 1         | 1            | 0.604 | 1     |

Values in bold are significant (p < 0.05).
reactivity’ are not significantly correlated (P > 0.05). In contrast, as expected, WD could be an indicator of wood durability estimated by soil bed tests (r = -0.657, P = 0.004). Using a linear regression, a model was then constructed using both ‘GST reactivity’ and WD. This model explained more than 81% (P < 0.006) of the mass loss variability [% predicted mass loss = 45 – [30 * WD] + [5*ΣΔTdA - 1.3*ΣΔTdD - 4.6*ΣΔTdTE - 4.5*ΣΔTdW]; R² = 0.818, p < 0.006.

Fig. 1. Wood durability model set-up from GST reactivities and wood density (WD). The multiple linear regression model was set up using Xlstat giving the following equation: % predicted mass loss = 45 – [30 * WD] + [5*ΣΔTdA - 1.3*ΣΔTdD - 4.6*ΣΔTdTE - 4.5*ΣΔTdW]; R² = 0.818, p < 0.006.

Conflict of interest
None declared.

References
Amusant, N., Nigg, M., Thibaut, B., and Beauchene, J. (2014) Diversity of decay resistance strategies of durable tropical woods species: Bocoa prouacencsis Aublet, Vouacapoua americana Aublet, Inga alba (Sw.) Wild. Int Biodeterior Biodegrad 94: 103–108.
Atkins, W.M. (2019) Mechanisms of promiscuity among drug metabolizing enzymes and drug transporters. FEBS J. doi: 10.1111/febs.15116
Bass, C., and Field, L.M. (2011) Gene amplification and insecticide resistance. Pest Manag Sci 67: 886–890.
Beauchene, J. (2012) Durabilité naturelle des bois de guyane. URL https://agritrop.cirad.fr/582599/1/Projet%20Degrad%20WP%20durabilite%20des%20bois%20rapport.pdf
Bouzahouane, H., Barou, C., Sleimi, N., and Oualli, K. (2018) Multi-biomarkers approach to the assessment of the southeastern Mediterranean Sea health status: preliminary study on Stramonita haemastoma used as a bioindicator for metal contamination. Chemosphere 207: 725–741.
Chambers, J.Q., Higuchi, N., Schimel, J.P., Ferreira, L.V., and Melack, J.M. (2000) Decomposition and carbon cycling of dead trees in tropical forests of the central Amazon. Oecologia 122: 380–388.
Chave, J., Coomes, D., Jansen, S., Lewis, S.L., Swenson, N.G., and Zanne, A.E. (2009) Towards a worldwide wood economics spectrum. Ecol Lett 12: 351–366.
Choo, Y.-M., Xu, P., Hwang, J.K., Zeng, F., Tan, K., Bhagavalthy, G., et al. (2018) Reverse chemical ecology approach for the identification of an oviposition attractant for Culex quinquefasciatus. Proc Natl Acad Sci USA 115: 714–719.
Deroy, A., Saiag, F., Kebbi-Benkeder, Z., Touahri, N., Hecker, A., Morel-Rouhier, M., et al. (2015) The gstimore reflects the chemical environment of white-rot fungi. PLoS ONE 10: e0137083.

Fernández-González, A.J., Valette, N., Kohler, A., Dumarcay, S., Sormani, R., Gelhaye, E., and Morel-Rouhier, M. (2018) Oak extractive-induced stress reveals the involvement of new enzymes in the early detoxification response of Phanerochaete chrysosporium: early fungal responses to oak extractives. Environ Microbiol 20: 3890–3901.

Floudas, D., Binder, M., Riley, R., Barry, K., Blanchette, R.A., et al. (2012) The paleozoic origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes. Science 336: 1715–1719.

Larjavaara, M., and Muller-Landau, H.C. (2010) Comparison of decay progress and resistance under various laboratory and field conditions. Int Biodeter Biodegrad 63: 3711–3725.

Lehnebach, R., Bossu, J., Va, S., Morel, H., Amusant, N., Nicolini, E., and Beauchêne, J. (2019) Wood density variations of legume trees in French Guiana along the shade tolerance continuum: heartwood effects on radial patterns and gradients. Forests 10: 80.

Li, Q.L., Yi, S.C., Li, D.Z., Nie, X.P., Li, S.Q., Wang, M.-Q., Leal, W.S. (2017) Reverse chemical ecology at the service of conservation biology. Proc Natl Acad Sci USA 114: 12094–12096.

Meyer, L., Brischke, C., Melcher, E., Brandt, K., Lenz, M.-T., and Soetbeer, A. (2014) Durability of English oak (Quercus robur L.) – Comparison of decay progress and resistance under various laboratory and field conditions. Int Biodeter Biodegrad 86: 79–85.

Morrel, M., Ngadin, A.A., Droux, M., Jacquot, J.-P., and Gelhaye, E. (2009) The fungal glutathione S-transferase system. Evidence of new classes in the wood-degrading basidiomycete Phanerochaete chrysosporium. Cell Mol Life Sci 66: 3711–3725.

Morrel, M., Meux, E., Mathieu, Y., Thuillier, A., Chibani, K., Harvengt, L., et al. (2013) Xenomic networks variability and adaptation traits in wood decaying fungi: fungal xenomic networks. Microb Biotechnol 6: 248–263.

Nagy, L.G., Riley, R., Bergmann, P.J., Krizsán, K., Martin, F.M., Grigoriev, I.V., et al. (2017) Genetic bases of fungal white rot wood decay predicted by phylogenomic analysis of correlated gene-phenotype evolution. Mol Biol Evol 34: 35–44.

Perrot, T., Schwartz, M., Saiag, F., Salzet, G., Dumarcay, S., Favier, F., et al. (2018) Fungal glutathione transferases as tools to explore the chemical diversity of amazonian wood extractives. ACS Sustain Chem Eng 6: 13078–13085.

Rodrigues, A.M., Amusant, N., Beauchêne, J., Eparvier, V., Lemenâger, N., Baudassé, C., et al. (2011) The termiticidal activity of Sextonia rubra (Mez) van der Werff (Lauraceae) extract and its active constituent rubrynone. Pest Manag Sci 67: 1420–1423.

Schwartz, M., Perrot, T., Aubert, E., Dumarcay, S., Favier, F., Gérardin, P., et al. (2018) Molecular recognition of wood polyphenols by phase II detoxification enzymes of the white rot Trametes versicolor. Sci Rep 8: 8472. doi: 10.1038/s41598-018-26601-3

Taylor, A.M., Gartner, B.L., and Morrell, J.J. (2002) Heartwood formation and natural durability – A review. Wood Fiber Sci 34: 587–611.

Valette, N., Perrot, T., Sormani, R., Gelhaye, E., and Morel-Rouhier, M. (2017) Antifungal activities of wood extractives. Fungal Biology Reviews 31: 113–123.

Zhu, J., Arena, S., Spinelli, S., Liu, D., Zhang, G., Wei, R., et al. (2017) Reverse chemical ecology: olfactory proteins from the giant panda and their interactions with putative pheromones and bamboo volatiles. Proc Natl Acad Sci USA 114: E9802–E9810.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Assays was performed as described in (Deroy et al., 2015). The experimental procedure was performed in 96-well microplate (Harshell, Bio-Rad) and the measurements were carried out with real time PCR detection system (CFX 96 touch, Bio-Rad). The assays were achieved as follows: 5 μL of Tris-HCl (150 mM) pH 8.0 buffer, 2 μL of wood extracts at an initial concentration of 1 mg.mL-1 in DMSO, 2 μL of proteins (final concentration of either 10 or 20 μM depending on the corresponding assays), 2 μL of SYPRO® orange diluted 62 fold (Sigma) and 14 μL of ultra-pure water. The microplate was centrifuged 30 s at 4000 g. The fluorescence was measured (excitation at 485 nm and emission at 530 nm) each minute starting with 3 min at 5 °C and increasing temperature from 5 to 95 °C with a step of 1 °C.min-1. The denaturation temperature (Td), which corresponds to the temperature where the protein is 50% unfolded, was determined using the first derivative of the obtained data in the presence or in the absence of potential ligands. As reference, experiments were conducted by adding DMSO only, allowing the determination of Td ref. The corresponding values are the average of three technical repetitions, standard deviation remaining in all cases below 10%. Then, the difference between the denaturation temperature of the protein incubated with wood extracts and with DMSO only (Td ref) were calculated in order to obtain the thermal shift (ΔTd). Absolute values of ΔTd were then reduced centred. Solvent : A: Acetone; D: Dichloromethane; TE: Toluene/Ethanol; W: Water.

© 2020 The Authors. Microbial Biotechnology published by John Wiley & Sons Ltd and Society for Applied Microbiology.