European Journal of Wood and Wood Products (2019) 77:319–322
https://doi.org/10.1007/s00107-019-01390-2

BRIEF ORIGINAL

Direct screening method to assess antimicrobial behavior of untreated wood

Muhammad Tanveer Munir[1] · Florence Aviat[2] · Hélène Pailhories[3] · Matthieu Eveillard[3] · Mark Irle[1] · Michel Federighi[4] · Christophe Belloncle[1]

Received: 30 October 2018 / Published online: 31 January 2019

Abstract
The objective of the present study was to develop a simple and direct screening method to assess antimicrobial activities of wood material. Wood samples consisted of sawdust and wood discs cut in transversal (RT) and tangential (LT) direction, from four different wood species which were tested against four important bacteria in hospital hygiene. Area of inhibition was observed around sawdust embedded wells and wooden discs placed on agar. This study showed that wood disc diffusion can be used to easily and rapidly screen antimicrobial properties of untreated wood, while sawdust diffusion needs further work to do this.

1 Introduction

Wood is an organic renewable material, which is widely used in food and construction industry. It has been shown to have hygienic benefits for the food industry (Aviat et al. 2016). Therefore, the demonstration of its potential hygienic properties could be an additional advantage for the use of wood in such constructions of hygienically important places.

The agar diffusion is one of the most commonly adopted methods for testing the antimicrobial properties. In the case of wood, it is generally used after a solvent extraction and serial dilutions. It adds extra steps, requiring more time and handling of chemicals, which may be harmful for operators. Moreover, it is not suitable for testing physical interaction of bacteria and wood in solid form. After an extensive literature research, it would seem that there are only two research papers using direct wood disc diffusion method (Laireiter et al. 2013; Pailhoriès et al. 2017). As far as the authors' know, there are no specific studies using this method to screen the different types of wood for their antimicrobial properties.

The aim of this study was to determine the potential of a direct agar diffusion method that tests untreated wood in the form of solid wooden discs (transversal and longitudinal section) or sawdust in wells to screen their antimicrobial properties against some important bacteria in hospital hygiene.

2 Materials and methods

2.1 Wood species

The selected wood species were European fir (*Abies alba*), American red oak (*Quercus rubra*), European oak (*Quercus* spp.) and European beech (*Fagus sylvatica*). Samples were cut from heartwood of trees grown around Nantes, France. The samples were conditioned to ~ 12% moisture content in a climatic chamber.

2.2 Wood discs

For the preparation of wood discs, wood logs were initially cut into boards of 3–4 cm thickness, and freed from sapwood. Then, the boards were further cut by an electric saw (Altendorf-F45, Minden, Germany) into thinner (2.5 mm)

✉ Michel Federighi
michel.federighi@oniris-nantes.fr

[1] Laboratoire Innovation Matériau Bois Habitat Apprentissage (LIMBHA), Ecole Supérieure du Bois, 7 rue Christian Pauc, 44000 Nantes, France

[2] Your ResearcH-Bio-Scientific, 307 la Gauterie, 44430 Le Landreau, France

[3] ATOMycA, Inserm Equipe Avenir, Inserm U892, CNRS 6299, Université Bretagne-Loire, Centre Hospitalier Universitaire, 4 rue Larrey, 49933 Angers Cedex, France

[4] UMR INRA 1014 SECALIM, Oniris, route de Gachet, CS 40706, 44307 Nantes Cedex 03, France

sheets with respect to transversal (RT) and tangential (LT) sections. These wood sheets were used to prepare uniformly sized circular wood disks (diameter 9 mm) using a laser cutting machine (Trotec-SP500 C60, Wels, Austria).

2.3 Sawdust

The earlier prepared wood sheets were ground to flour with a maximum particle size of 500 µm using a grinder (Retsch ZM200, Haan, Germany).

2.4 Sterilization of samples

The sawdust and half of the discs were packed in sterile plastic bags and then, sterilized by irradiation at 25 kGy (Ionisos, Sablé sur Sarthe, France). The other half of the discs was packed and not treated by gamma irradiation.

2.5 Bacterial strains

A set of four reference strains of American Type Culture Collection (ATCC) provided by Centre Hospitalier Universitaire (CHU), Angers, France, was used in this study: *Staphylococcus aureus* ATCC 29213 (sensitive to vancomycin and colistin), *Escherichia coli* ATCC 25922 (vancomycin resistant and colistin sensitive), *Pseudomonas aeruginosa* ATCC 27853 (vancomycin resistant and colistin sensitive) and *Enterococcus faecalis* ATCC 29212 (colistin resistant and vancomycin sensitive).

2.6 Agar diffusion

For disc diffusion method, bacterial suspensions adjusted to a density of 0.5 MacFarland were inoculated by streaking on Mueller-Hinton agar plates (BioRad, Marnes La Coquette, France), according to the recommendations of the Antibiogram Committee of the French Society of Microbiology (CASFM). The sterilized disks were placed directly on agar plates (12 × 12 cm). Sixteen samples can be used on each plate and incubated at 37 °C for 24 h. Vancomycin (5 µg) and colistin (25 µg) antimicrobial susceptibility test discs (diameter 6 mm) were used (Oxoid, Basingstoke, UK) as control.

For sawdust testing, 8 mm wells were created inside Mueller-Hinton agar plates with an adapted perforating-punch (Jeulin, Evreux, France). Bacterial strains were then inoculated on these plates as described previously. Afterwards, the wells were filled with sawdust and plates were incubated at 37 °C for 24 h. The inhibition zones, as defined by CASFM and EUCAST (Antibiogram Committee of the French Society of Microbiology and European Committee on Antimicrobial Susceptibility Testing), were checked after 24 h of incubation (Fig. 1). The tests for discs and sawdust were carried out in triplicates, and all the experiments

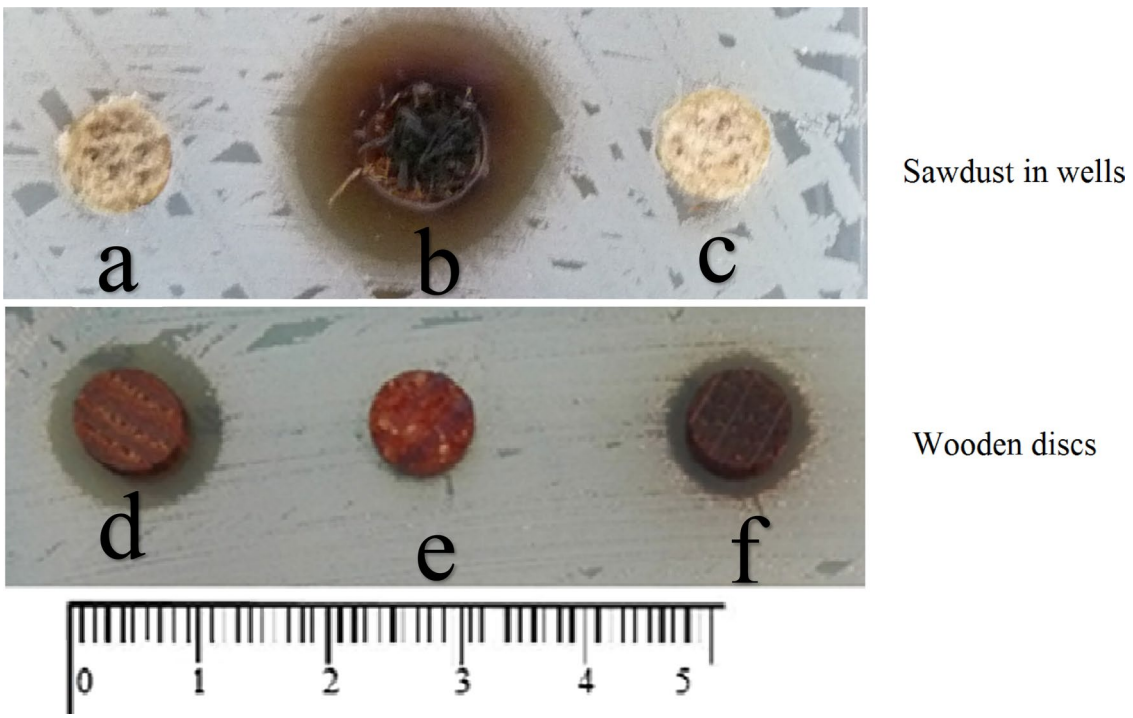

Fig. 1 Area of inhibition around wooden discs (9 × 2.5 mm) and sawdust (500 µm particles size) on agar plate inoculated with *S. aureus* ATCC 29213; b, d, f are positive results, while a, c, e are negative. The manual scaling is used to measure the area of inhibition around test samples

were conducted in the bacteriology laboratory of Institute of Health Biology in CHU, Angers. When an inhibition zone of more than 0.5 mm was observed on all replicates, the result was considered as positive (+) and if not they were labelled as negative (−).

3 Results and discussion

The results of the different tests with discs [tangential (LT) and transversal (RT) cuts], sterilized or non-sterilized, and sterilized sawdust are presented in Table 1. The results from gamma irradiated and non-irradiated samples showed no difference in the results regarding the antimicrobial activity. All the tested wood species showed antimicrobial effects against *S. aureus*, two against *P. aeruginosa* and one against *E. faecalis*. No differences between the activity of sterilized and non-sterilized discs were evident.

Two species showed a different response according to the orientation of the cut. The RT seems to be more active, as can be seen in the case of American oak against *P. aeruginosa* and European oak against *E. faecalis*. Similar differences have been reported in a previous study by Pailhoriès et al. (2017). The possible explanation of this effect maybe that the wood is an orthotropic material with a specific organization of the cells in the three planes and probably RT cutting has more exposed surface area and higher diffusion of extractives into agar, ultimately causing the difference in effect of LT and RT cuts against different bacteria (Munir et al. 2018).

In this study, the European oak species showed positive activities against a gram-negative (*P. aeruginosa*) and a gram-positive bacteria (*E. faecalis*). These results show the higher antimicrobial potential of oak using similar experimental conditions. The results are different between the two *Quercus* species, which is probably because of anatomical and chemical differences among different species of the same plant genus (Rowell 2012).

The method described here uses a quick laser cutting which allows for creating uniform discs in all specific dimensions of wood. This cutting does not require intensive manipulation of the samples. In addition, the presence of negative results shows that there are no new antimicrobial chemicals formed during the laser cutting process, which could induce false positive results in the experiment. The results were confirmed by using manually prepared wooden

Table 1 Presence (+) or absence (−) of inhibition zone in all replicates, for four bacterial strains, around 9 mm diameter irradiated and non irradiated wood discs (LT and RT cuts) and wells filled with irradiated sawdust

Wood	Test[a]	*P. aeruginosa*	*S. aureus*	*E. faecalis*	*E. coli*
European fir	Disc LT γ	−	+	−	−
	Disc LT	−	+	−	−
	Disc RT γ	−	+	−	−
	Disc RT	−	+	−	−
	Saw dust γ	−	−	−	−
American oak	Disc LT γ	−	+	−	−
	Disc LT	−	+	−	−
	Disc RT γ	+	+	−	−
	Disc RT	+	+	−	−
	Saw dust γ	−	−	−	−
European oak	Disc LT γ	+	+	−	−
	Disc LT	+	+	−	−
	Disc RT γ	+	+	+	−
	Disc RT	+	+	+	−
	Saw dust γ	−	+	−	−
Beech	Disc LT γ	−	+	−	−
	Disc LT	−	+	−	−
	Disc RT γ	−	+	−	−
	Disc RT	−	+	−	−
	Saw dust γ	−	−	−	−
Vancomycin[b]	Impregnated paper disc	−	+	+	−
Colistin[b]	Impregnated paper disc	+	+	−	+

+ (antimicrobial effect) or − (no effect)

[a]γ is for the gamma irradiated samples

[b]Gram negative bacteria (i.e. *E. coli* and *P. aeruginosa*) are sensitive to colistin and resistant to vancomycin. *S. aureus* is sensitive to both while *E. faecalis* is sensitive to vancomycin and resistant to colistin

discs, and no significant difference was seen in antimicrobial activity, even manual cutting took more preparation time than laser cutting (data not shown). It is important to mention that the extractives method does not extract all kinds of molecules from wood, only some are dissolved in solvent and thus tested for antimicrobial potential. In comparison, the wooden discs use all the available chemical and physical properties of wood, which allows diffusion of real antibacterial chemicals of wood.

The sawdust well-diffusion experiment results are also given in Table 1. There was only one positive result, which is in the case of European oak sawdust against *S. aureus*. It was contrary to the hypothesis of experiment which expected that the wood extractives will be more available and efficient in sawdust as shown by Milling et al. (2005). According to the authors, there are two explanations for this difference in the results between sawdust and wood discs. First, the contact between agar and wood might have been limited to a small surface of wood particles close to the cylinder surface of the agar well, decreasing the diffusion. The second reason of these unexpected results can be attributed to the unequal quantity of test material used in the current study. In fact, about 30 mg of sawdust was used in each well and every wooden disc weighed 120–150 mg. Further studies are needed to more precisely describe these differences in the results.

4 Conclusion

According to the aim of this study, it was found that the agar diffusion with wooden discs cut in the RT plane can be used as a quicker screening method for potent antimicrobial woods. Moreover, this method is simple because it does not require any sample sterilization and/or specific chemical handling.

Acknowledgements The study was funded by CODIFAB (http://www.codifab.fr/).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Aviat F, Gerhards C, Rodriguez-Jerez J et al (2016) Microbial safety of wood in contact with food: a review. Compr Rev Food Sci Food Saf 15:491–505. https://doi.org/10.1111/1541-4337.12199

Laireiter CM, Schnabel T, Köck A et al (2013) Active anti-microbial effects of larch and pine wood on four bacterial strains. BioResources 9:273–281. https://doi.org/10.15376/biores.9.1.273-281

Milling A, Kehr R, Wulf A, Smalla K (2005) Survival of bacteria on wood and plastic particles: dependence on wood species and environmental conditions. Holzforschung. https://doi.org/10.1515/HF.2005.012

Munir MT, Belloncle C, Pailhoriès H et al (2018) Survival of nosocomial pathogens on maritime pine and European fir wood. In: Proceedings of the 5th international conference on processing technologies for the forest and bio-based products industries, Freising/Munich, Germany, pp 213–217

Pailhoriès H, Munir MT, Aviat F et al (2017) Oak in hospitals, the worst enemy of *Staphylococcus aureus*? Infect Control Hosp Epidemiol 38:382–384. https://doi.org/10.1017/ice.2016.304

Rowell RM (2012) Handbook of wood chemistry and wood composites. CRC Press, Boca Raton