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Pine heartwood and glass surfaces: easy method to test the fate of bacterial contamination

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Abstract

The survival of two bacteria, *Escherichia coli* and *Listeria monocytogenes* was observed on pine heartwood and glass surfaces by using a simple test method. The development of the number of bacterial cells was evaluated by titration after vortexing the samples in BHI broth and culturing the resulting broth on agar plates. The bacterial count decreased clearly faster on pine heartwood than on glass surfaces. This result was confirmed by studying the wooden samples also one day after to exclude possible adherence of the bacterial cells on the porous surface. This study confirms the results of several other studies that suggest wood to have antibacterial properties.

Kiefernkernelholz und Glasoberflächen: eine einfache Methode zur Prüfung von Bakterienbefall

Zusammenfassung

Das Überleben von zwei Bakterienarten, *Escherichia coli* und *Listeria monocytogenes*, auf Kiefernkernelholz und Glasoberflächen wurde mit einer

einfachen Prüfmethode untersucht. Mittels Titrierung wurde die Entwicklung der Anzahl der Bakterienzellen untersucht, nachdem die Proben in einer BHI-Nährlösung geortext wurden, die anschließend auf Agarplatten kultiviert wurde. Die Keimzahl nahm auf Kiefernkernelholz deutlich schneller ab als auf Glasoberflächen.

Dieses Ergebnis konnte auch durch Untersuchungen der Holzproben am darauf folgenden Tag bestätigt werden, um ein mögliches Anhaften der Bakterienzellen auf der porösen Oberfläche auszuschließen. Mit dieser Studie konnten die Ergebnisse früherer Untersuchungen bestätigt werden, die Holz antibakterielle Eigenschaften zuschreiben.

Introduction

Whether wood surfaces are hygienic or not has been widely discussed especially in regard to cutting boards. Several researchers have found wood more difficult to clean than plastic boards and therefore a more hygienically unfavorable material (Gilbert and Watson 1971; Abrishami et al. 1994; Gough and Dodd 1998). On the other hand, there are several studies suggesting that wood has antibacterial properties against *Listeria monocytogenes*, *Salmonella* serotype Typhimurium (Ak et. al. 1994 a, b), *Enterococcus faecium* (Milling 2005a), enterobacteria, enterococci and streptococci (bacteria from chicken manure) (Milling 2005b), *Bacillus subtilis* and *Pseudomonas fluorescens* (Koch et. al. 2002). The reasons behind antibacterality are not well understood; it is supposed to be a combination of several factors such as wood surfaces drying and chemical composition (Schönwälder et al. 2002). Some of the chemical components have been studied separately. Lindberg et al. (2004) have found the concentration of pinosylvin to correlate with antibacterality against *B. coagulans*, *Burkholderia multivorans* and *Alcaligenes xylosoxydans*. Mourey and Canillac (2002) have found α -pinene to inhibit the growth of *L. monocytogenes*. Also the resin of Norway spruce (*Picea abies*) has been found to have antibacterial effect on several Gram-positive bacteria (Sipponen et. al. 2007).

The purpose of this study was to investigate the antibacterial effect of wood by comparing wooden and glass surfaces using a simple test method. Preferably, a

method was wanted, which could be standardized and which was easy to repeat. To be able to study wooden surfaces it was chosen to use solid wood pieces instead of sawdust. *Escherichia coli* and *L. monocytogenes* were used as hygienically relevant Gram-positive and Gram-negative test bacteria, respectively. Both bacterial species are also common human pathogens.

Materials and methods

Wood and glass cylinders

The wood material used in these tests was heartwood of Scots pine (*Pinus sylvestris*). The test samples were cut within six months from sawing and used within one week from manufacturing the test samples. The samples were made by drilling cylinders with a diameter of 15 mm and a surface area of circa 176.7 mm². The thickness was 10 mm. Glass cylinders of same diameter and surface area were ordered from a company making laboratory equipment. The thickness of glass samples was 3mm.

Bacterial strains

L. monocytogenes 1/2a (IH 83616 [isolated from the blood sample of a patient]) and *E. coli* (MG 1655; Guyer et al. 1981) were stored in skimmed milk at -70°C. Prior to use, *L. monocytogenes* and *E. coli* were subcultured at least twice on sheep blood and Drigalski-Conradi agar plates, respectively. The plates were incubated at 37°C overnight. Bacterial colonies were moved directly from agar to physiological NaCl solution, and the concentration was adjusted to 1.5x10⁸ CFU/ml using a photometer (Gene-Trak Systems, Hopkinton, MA, USA). This was equivalent to photometer reading 0.13 corresponding to McFarland 0.5. Serial dilutions of the bacterial suspensions were then prepared in physiological NaCl to correspond to the concentrations of 1.5x10⁶, 1.5x10⁴ and 1.5x10² CFU/ml. These and the original concentrations were used in preliminary tests to find the most suitable one for the experiments. The concentration 1.5x10⁶ CFU/ml was chosen for final tests.

Cultivations

100 µl of bacterial solution was placed on wooden and glass samples which is equivalent to 1.5×10^5 CFU per sample. Five parallel samples were used for each of the five incubation times. The samples were placed in large petri dishes, the five parallel samples in each of them. 25 wood as well as 25 glass samples were inoculated with both *L. monocytogenes* and *E. coli*, 100 samples altogether. After incubation at 25 °C for 1h, 2h, 4h, about 1d (22h for *L. monocytogenes* and 28h for *E. coli*) and about 2d (44h for *L. monocytogenes* and 52h for *E. coli*), the samples were tested for recoverable bacteria. To remove all adhered bacterial cells from the surface of the samples, they were vortexed in 15 ml BHI (brain heart infusion) broth for 5sec. Immediately afterwards, 400 µl of the broth with *L. monocytogenes* or *E. coli* was spread onto sheep blood or Drigalski-Conradi agar plates, respectively. After overnight incubation at 37 °C, the CFUs on the plates were counted. In cases where the bacterial colonies formed a lawn on the plates and, thus were impossible to count separately, the CFUs were estimated to correspond to the original number of cells at 0h (i.e., 1.5×10^5). The average and the standard deviation of the five parallel results were calculated, and the results are shown as CFU/plate in a logarithmic scale.

Because wood is accused of collecting bacteria tightly adhered to its porous surface, the wood samples were further tested. The BHI broth tubes with wood samples in BHI broth were also studied after incubation overnight at 37 °C where the bacterial cells were expected to multiply even if they were tightly adhered to the surfaces. The next day, 400µl of the broth containing samples with *L. monocytogenes*, was spread again onto sheep blood agar and incubated at 37 °C overnight. Only the samples of 2, 4 and 22 hours were tested this way in order to see the potential difference of the results compared with those after vortexing. Also the test tubes with *E. coli* were incubated at 37 °C overnight, and the bacterial growth was investigated directly by looking for turbidity or clarity of the broth. The results are shown as + or – for growth or no growth, respectively.

Results

To define optimal bacterial concentration and incubation times for the final method, preliminary examinations were carried out. Based on these results (data

not shown), the concentration 1.5×10^6 CFU/ml corresponding to 1.5×10^5 CFU/sample was chosen. At this concentration, the CFUs of both *L. monocytogenes* and *E. coli* started decreasing after two hours of incubation on wood samples.

It was clearly shown that bacterial numbers of both strains decreased more quickly on wooden surfaces compared to the glass surfaces. (Fig. 1 and 2). The difference between the reductions is considerable for the 2h and 4h incubation at 25 °C for both bacterial strains. For *L. monocytogenes* the difference is considerable also for 22h and 44h incubation times. Already after 2 hours of incubation a reduction of viable counts of bacterial cells could be seen on wooden surfaces with both test bacterial strains. On average, there were 300 CFUs/plate of *L. monocytogenes* and 350 CFUs/plate of *E. coli* meaning 0.2% of the original 1.5×10^5 CFU/sample. After 4 hours there was a log 2 reduction on wooden surfaces and no change in the count of bacterial cells on glass surfaces for both bacterial strains. The number of *L. monocytogenes* was on average 12 CFUs/plate and the number of *E. coli* was on average 19 CFUs/plate. The results from the parallel wood samples varied more than the results from the parallel glass samples (Fig. 1 and 2).

On glass samples, the reduction of both bacteria was only seen after about one day of incubation at 25 °C (Fig. 1 and 2). The number of *L. monocytogenes* on glass surfaces (on average 23 CFU/plate) was greater than for *E. coli* (on average less than one CFU/plate) and neither could be found on wooden surfaces. *L. monocytogenes* survived longer on glass surfaces than *E. coli*.

To study the possibility of bacteria tightly adhering to the wood surfaces, the BHI test tubes with samples were further tested after overnight incubation at 37 °C. There was one of the 15 tested wood samples with *L. monocytogenes* that showed growth even though in the original test after 22 hours at 25 °C there had been no bacterial growth. None of the other tested wood samples with *L. monocytogenes* showed growth on the following day unless there had been growth the first day

(Table 1). Subculturing after overnight incubation at 37 °C was not necessary for the BHI tubes *E. coli*: if there was no bacterial growth, the BHI broth was clear and if there was bacterial growth, the broth was turbid. None of the tested wooden specimens with *E. coli* showed growth on the following day unless there had been growth the first day. (Table 1)

Discussion

The survival of *L. monocytogenes* and *E. coli* was studied on wooden and glass surfaces, and bacterial counts on each surface were compared. The number of bacterial cells used was 1.5×10^5 CFU per sample. This number was high enough to show reliably the reduction of the bacteria on both surfaces. However, it was not too high to demand several sets of serial dilutions to see this reduction. In addition, to increase the reliability of the study design, five parallel samples were monitored for each experiment. Also, five different incubation times were used. Throughout the tests, the difference between wood and glass surfaces could be seen only after 2h incubation at 25 °C. The normal variation of room temperature was avoided by using the incubator with controlled temperature. In these controlled conditions, the bacterial count clearly declined faster on wooden surfaces compared to glass surfaces.

There is a wide range of studies comparing wooden and plastic cutting boards. This study has brought the hygienic properties of wood on a more general level. The comparison was made between wood and glass. Glass is considered a neutral material, which should not affect the bacterial growth in any way. This gave the opportunity to study the antibacterial effect of wood by comparing it with a material without any effect. The most common methods used in similar studies are a contact plate method (Ak. et al. 1994 a, b; Park and Cliver 1996; Gough and Dodd 1998; Schönwälder et al. 2002; Koch et al. 2002; Milling et al. 2005b; Boursillon and Riethmüller 2007) where agar medium is pressed directly onto the test surface and a swab method (Gilbert and Watson 1971; Park and Cliver 1996; Miller et al. 1996; Welker et al. 1996; Koch et al. 2002) where the test surface is swept with a swab or a sponge, and the bacterial count from the swab is then studied. Vortexing was chosen in the current study to avoid the possible effect of adherence. Destructive methods have been used by Park and Cliver (1996) and

Schönwälder et al. (2002). This allows using solid wood samples and excludes the effect of adherence. However, it is faster and easier to use small enough samples to be tested entirely, as was done in this study. Abrishami et al. (1994) used 8.9x8.9 cm² wood samples which were placed into a cylindrical jar in buffer liquid and shaken for 2-3 minutes, but the authors did not explain how the shaking was done in the cylindrical jar. Schmidt (1989) also used a similar method rinsing the solid wood samples in succession in a plastic bag with 50 ml physiological salt solution. However, vigorous vortexing is presumably more effective than rinsing.

L. monocytogenes presents hygienically relevant Gram-positive bacterial species that often causes contaminations, for example in fish production factories (Miettinen et. al. 2001). *E. coli* was chosen as a Gram-negative reference bacterium to indicate potential faecal contamination. First of all, the study aimed at developing a simple test method that could be used in future studies to reveal the reasons behind the antibacterial properties of wood. Pine was chosen because of its known antibacterial properties (Milling et. al. 2005 a,b), and since it is one of the most common wood species sawn in Finland. To study wooden surfaces, solid blocks were used instead of sawdust (Milling et. al. 2005 a,b).

Wood has been accused of adherence of bacteria tightly on the surface because of its porous structure (Abrishami et al.1994; Gough and Dodd,1998). To study other possible effects on the antibacterial properties, adherence to the surface had to be eliminated. For this reason, the samples were placed in the test tubes with BHI broth and shaken thoroughly with Vortex instead of using the contact method (Ak et. al. 1994 a, b; Gough and Dodd 1998; Schönwälder et. al. 2002). The samples were also left overnight in BHI broth and investigated the next day to find out if there had been viable adhered bacteria on those samples where the first cultivation produced no bacteria. This showed very good correlation. Namely, if the cultivation directly from BHI broth had given 0 CFU as a result, there was no growth later either. Instead, if there had been any CFUs in previous cultivation, the next day's results also showed bacterial growth. This clearly shows that the porous structure of wood does not account for the faster decrease of bacterial count on wood compared to glass.

The method developed in this study is easy to use and the results clearly showed differences in the survival of bacteria on wooden and glass surfaces. Thus, this method will be very helpful when studying the nature of the antibacterial properties of wood. Five parallel samples, as used in this study, showed reliably the different effect of the two matrices, wood and glass, on the reduction of the bacterial counts of two hygienically important bacterial species, *L. monocytogenes* and *E. coli*. However, wood as a natural material is very heterogeneous and to get robust results several parallel tests are necessary. Pine heartwood was chosen for this study, and these results can not be generalized for all the wood species. Each species needs to be tested separately, and for some species there can even be differences between heartwood and sapwood, since their chemical composition might differ from each other (Bertaud and Holmbom, 2004).

The antibacterial properties of wood might bring some new viewpoints regarding the use of wood as an interior material. These properties have already been exploited in e.g. wooden pens, bed mats, bath mats and different skin treatment products (www.wilms.com). Natural anti-microbial properties offer an interesting possibility for product development.

It would also be important to study the hygienic properties of wood because it has a reputation of being difficult to clean and unhygienic. This might lead to unfavorable choice of material e.g. when designing public buildings. For this purpose, more research on the effect of different surface coatings is necessary. The material used in this study was new; the wood was only half a year old. Normally, the surface materials, like wall panels, are in use for years. Thus it would be important to research the effect of the age of wood.

Wood's activity against bacteria leads the researcher to other similar questions. How does wood affect impurities in indoor air or our own bodies? How do fungi producing toxins grow on wooden surfaces compared with competing materials? In addition, the question of what causes this interesting antibacterial effect needs to be answered in more research.

Conclusion

The aim of this study was to compare the hygienic properties of wood and glass using a test method which is easy to repeat. The results clearly showed bacterial count to decrease faster on wood than glass surfaces. By using a method where the samples are vigorously vortexed, the adherence of the bacterial cells on the wood surface is shown to be an inadequate explanation for these results. Clearly, pine heartwood has antibacterial effects but to better understand the reasons behind it more research needs to be done.

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Figure 1. Amount of CFUs of *L. monocytogenes* recovered from the wooden and glass surfaces after six different incubation times. The average of five parallel samples is shown and the error bars show the standard deviation.

Figure 2. Amount of CFUs of *E. coli* recovered from the wooden and glass surfaces after six different incubation times. The average of five parallel samples is shown and the error bars show the standard deviation.

Table 1. Occurrence of the bacterial growth of *L. monocytogenes* and *E. coli* on wooden surfaces in the two cultivations. The first +/- in slash shows whether there was any bacterial growth in the test after vortexing and the second +/- in slash shows whether there was bacterial growth one day later in the BHI broth incubated at +37 C overnight.

Bacterial strain	Incubation time	Parallel samples				
		1	2	3	4	5
<i>L.monocytogenes</i>	2h	+/+	+/+	+/+	+/+	+/+
<i>L.monocytogenes</i>	4h	-/-	+/+	+/+	+/+	n/a ^a
<i>L.monocytogenes</i>	22h	-/-	-/-	-/-	-/+	-/-
<i>E.coli</i>	2h	+/+	-/-	-/-	+/+	+/+
<i>E.coli</i>	4h	-/-	-/-	-/-	+/+	+/+
<i>E.coli</i>	28h	-/-	-/-	-/-	-/-	-/-

^a)n/a, not available

Abb. 1

Anzahl der koloniebildenden Einheiten (CFUs) von *L. monocytogenes* auf den Holz- und den Glasoberflächen nach sechs unterschiedlichen Inkubationszeiten. Angegeben sind der Mittelwert aus fünf Proben und die Standardabweichung.

Abb. 2

Anzahl der koloniebildenden Einheiten (CFUs) von *E. coli* auf den Holz- und den Glasoberflächen nach sechs unterschiedlichen Inkubationszeiten. Angegeben sind der Mittelwert aus fünf Proben und die Standardabweichung.

Tabelle 1

Bakterielles Wachstum von *L. monocytogenes* und *E. coli* auf Holzoberflächen in den zwei Kulturen. + oder - vor dem Schrägstrich gibt an, ob bakterielles Wachstum nach dem Vortexen auftrat und + oder - nach dem Schrägstrich gibt an, ob nach einer weiteren eintägigen Inkubationszeit bei 37°C in der BHI-Lösung bakterielles Wachstum auftrat

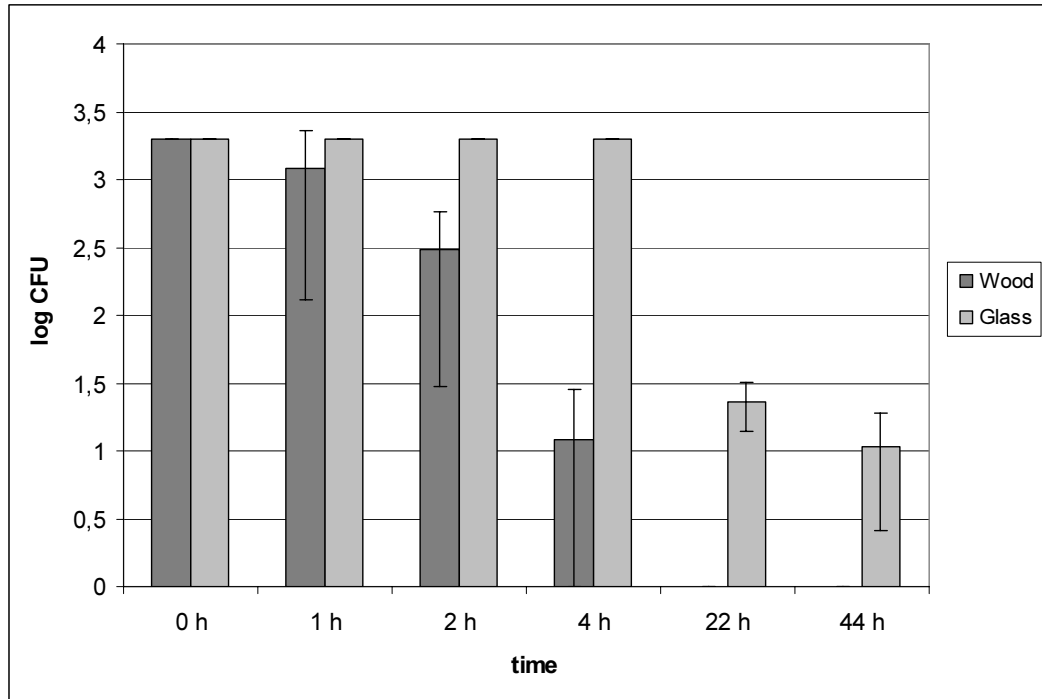


Figure 1.

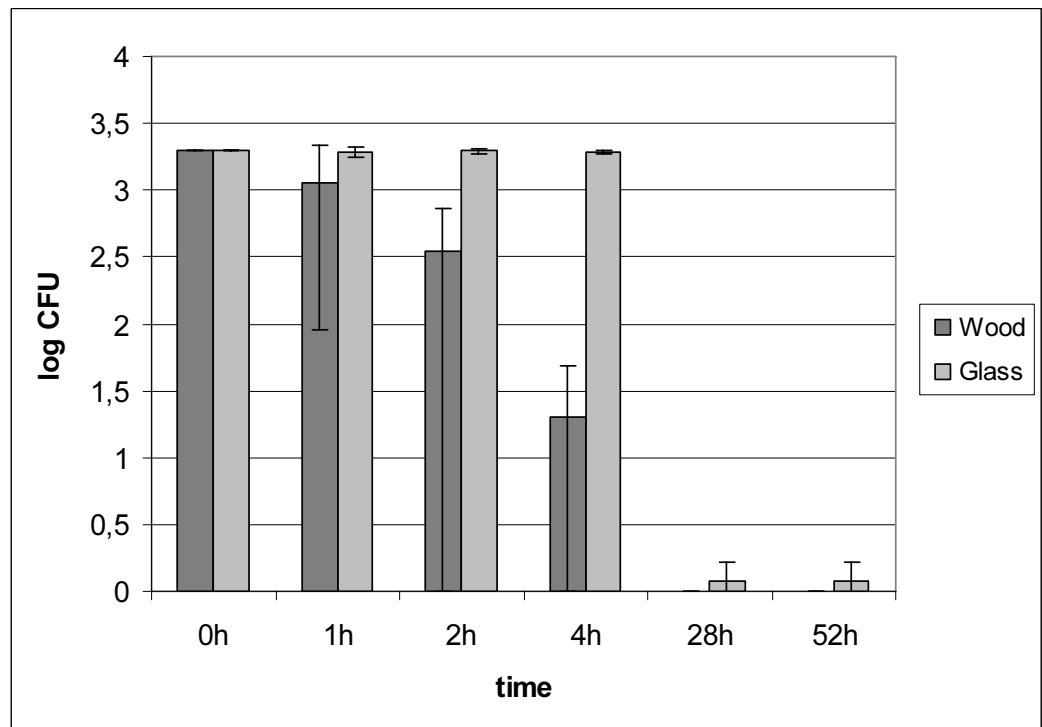


Figure 2.