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Survival of bacteria on wood and plastic particles: Dependence on wood species and environmental conditions

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Abstract

The survival of two hygienically relevant bacteria, *Escherichia coli* pIE639 and *Enterococcus faecium*, was followed on wooden sawdust of seven different European woods (pine, spruce, larch, beech, maple, poplar, and oak) versus polyethylene chips by using cultivation-dependent and molecular-based methods in parallel. The survival of the bacteria on wood was dependent on various factors such as the wood species, the type of the inoculated bacterium, the ambient temperature, and humidity. The bacterial titre decreased fastest on pine followed by oak compared to the other woods and plastic. Cultivation-independent analysis employing DNA extraction, Southern blot hybridisation, and PCR-based detection of marker genes of the test bacteria confirmed this result. The decline in bacterial numbers correlated with the decrease of bacterial DNA in the samples. Amounts of DNA of *E. coli* and *E. faecium* recovered from pine and oak-wood sawdust were generally lower compared to the other woods and plastic.

The presented study shows that pine and oak exhibit substantially better hygienic performance than plastic and indicates an antibacterial effect caused by a combination of the hygroscopic properties of wood and the effect of wood extractives.

Keywords: bacteria; DNA extraction; hygiene; plastic; wood.

Introduction

Wood has a long tradition as a natural material used by humans for centuries. Since the 1960s, there has been a continuous debate about the hygienic properties of wood and wooden products, and as a result the use of wood is seen critically in many sectors. Nowadays, it seems to be a general perception that products made of synthetic materials such as stainless steel and plastics are more hygienic and cleaner than those made of natural materials such as wood. In contrast, wood is well-known as a porous material that can absorb and retain bacteria, and thus it is regarded as impossible to be kept completely clean and decontaminated.

Numerous scientific studies have evaluated the hygienic potential of wood compared to plastics and stainless steel and resulted in completely different observations. On the one hand, contamination experiments showed that plate counts from wood were greater than those from all the boards made of plastics or metal tested (Kelch and Palm 1958; Rödel et al. 1994). Furthermore, also after different cleaning procedures, high rates of bacteria were recovered from the wooden surfaces, indicating that those surfaces could not be decontaminated efficiently (Gilbert and Watson 1971; Kampelmacher et al. 1971; Borneff et al. 1988a,b; Abrishami et al. 1994; Rödel et al. 1994). On the other hand, the results published by Ak et al. (1994a,b) indicated that wood is safer in contact with foodstuffs than plastics. After contamination of cutting boards made of nine different hardwoods and plastics with several hygienically relevant gram-negative bacteria, significantly fewer viable bacteria were detected on the wooden boards than on the plastic boards, regardless of new or used status of the boards. In the frame of the Nordic Wood Project "Wood in Food", the hygienic properties of wood, plastic, and steel were compared (Koch et al. 2002). The survival of *Bacillus subtilis* and *Pseudomonas fluorescens*, found in the meat industry, was followed in two sets of experiments: (I) on boards made of beech, oak, and ash, representing commonly used materials for tabletops or (II) on spruce and pine, simulating the usage of pallets. A remarkably great difference in the survival of the bacteria on the surface of the samples was observed between wooden samples and plastic and steel. Oak showed the highest decrease rate in bacterial titre, followed by beech and ash. Bacteria survived longest on plastic followed by stainless steel. In the experiments with pine and spruce, pine performed better than spruce both at low and at high moisture content. A recent study performed by our group (Schönwälder et al. 2000, 2002) showed that the survival of the test bacteria *Escherichia coli* pIE639 and *Enterococcus faecium* depended on different factors such as tree species, the initial inoculum size, and the characteristics of the inoculated bacterium. Pine-wood boards exhibited better hygienic performance than other wooden boards made of spruce, beech, and poplar or plastic boards. The study indicated an antibacterial effect of wood, especially pine, presumably caused by a combination of the hygroscopic properties of wood and wood extractives.

Nevertheless, it is difficult to compare the different studies because the very wide disparities between the

experimental conditions such as type of wood, surface state of wood, orientation of wood fibres in cutting boards, humidity level, and fouling of wood prior to contamination. Also, the origin and type of micro-organisms used to contaminate surfaces, the method of surface contamination, and sampling methods have a strong impact on the obtained results (Carpentier 1997).

A lack of information regarding the optimum conditions for the use of wood has been ascertained (Carpentier 1997), and more information is needed to understand the activities of bacteria in wood in general. The aim of the experiments presented in this study was to evaluate the recent findings and to better define factors that could influence the survival of bacteria on wood. Based on preliminary results obtained by our group (Schönwälder 1999; Schönwälder et al. 2000), the survival of *E. coli* and *E. faecium* on wood and plastic in dependence of wood species, type of the inoculated bacterium, and environmental conditions was followed. For the first time, a combination of cultivation-dependent and DNA-based methods was used for a hygiene study. In contrast to all other hygienic investigations, in the current work small wooden particles (sawdust) of seven different European wood species were investigated to minimise influences caused by compact wooden boards such as capillary properties, orientation of wood fibres, or the water-retention capacity and to achieve results that are more independent of the physical properties of wood according to Schönwälder (1999).

Material and methods

Bacteria and growth conditions

Escherichia coli pIE639 (Tietze et al. 1989) and *Enterococcus faecium* (Klare et al. 1995) were used in the experiments as test bacteria and model organisms for hygienically relevant gram-negative and gram-positive bacteria. Both bacterial strains carry antibiotic resistance genes, which were used as selection and as molecular markers. By addition of antibiotics to the medium, it was possible to follow precisely the behaviour of the test bacteria on the different materials also under semi-sterile conditions. *E. coli* was grown overnight in Luria-Bertani broth (LB) containing 100 μg ml^{-1} cycloheximide, 50 μg ml^{-1} l rifampicin, and 50 μg ml^{-1} streptomycin at 37°C for 16–24 h, *E. faecium* in LB broth containing 25 μg ml^{-1} vancomycin at 37°C for 24–36 h. For molecular detection, the streptothricin acetyltransferase gene (*sat*3) of *E. coli*, coding for nourseothricin resistance and localised on the InQ plasmid pIE639, and the chromosomally localised vancomycin resistance gene (*van*A) of *E. faecium* were used.

Material

The tested wood included Scots pine (*Pinus silvestris* L.), Norway spruce (*Picea abies* Karst.), European larch (*Larix decidua* Mill.), black poplar (*Populus nigra* L.), sycamore maple (*Acer pseudoplatanus* L.), beech (*Fagus silvatica* L.), and pedunculate oak (*Quercus robur* L.). Trees of a diameter of 15 to 25 cm at breast height (dbh) were taken from forests in the immediate vicinity of Braunschweig in Northern Germany. The trees were cut into 2-cm thick boards longitudinally, air-dried at ambient temperatures for 6–12 months, and converted to sawdust using a circular saw. Experiments were done with mixed samples of heartwood and sapwood in heartwood-forming species (oak, pine, and larch) and with mixed sapwood samples taken across the wood diameter in the other species. The proportion of heartwood averaged 60–70% in pine, larch, and oak samples. As reference material, polyethylene chips (5×10 mm, 0.5 mm thick) were used.

Determination of dampness

A characteristic factor of wood is its water content. To determine the moisture content (or dampness), two replicates of sawdust (1 g) of each wood type were kiln-dried in an oven at 103°C for at least 12 h until a constant mass was reached and cooled down afterwards in the desiccator. Finally, the dry weight was determined, and the moisture content based on the dry weight was calculated from the difference of the measured values. The actual moisture content of the wooden and plastic samples was checked during the experiments according to the samplings done.

Inoculation

Before each experiment, the sawdust was dried and disinfected for at least 12 h at 103°C; polyethylene chips were treated under UV light for 2 h. After drying, the moisture content was standardised to 2% of dry weight. In order to characterise the interactions between wood particles and bacteria, 25 g or 50 g sawdust of the appropriate woods and polyethylene chips were treated by spraying with a defined volume of the test bacteria to achieve the desired initial inoculum size. Before inoculation, the cell density of the inoculum was adjusted. The overnight culture of the bacteria was centrifuged for 10 min at 2000 *g* and the resulting pellet was resuspended in the required volume of sterile 0.85% (w/v) saline. All experiments were performed with two replicates per treatment. In the following, the inoculation performed in the different experiments is described.

Survival of *E. coli* and *E. faecium* on wooden particles of different wood species

The dried sawdust of pine, larch, spruce, maple, beech, poplar, and oak and polyethylene chips as reference material were treated with 0.5 ml g^{-1} of an appropriately diluted cell suspension of the test bacteria to achieve an initial inoculum level of 5×10^8 CFU g^{-1} of *E. coli* pIE639 or 1×10^6 CFU g^{-1} of *E. faecium*, respectively. We decided to work with different initial cell densities of *E. coli* compared to *E. faecium* because in preliminary tests it turned out that approximately this amount of the test bacteria can be killed on pine wood within 24 h (Schönwälder 1999). Since pine wood so far has shown the best antibacterial characteristics (Schönwälder et al. 2002), we used the possible decreasing rates of pine wood as reference value. The initial moisture content of the wooden material was 50% after inoculation. The samples were incubated at 21°C. In order to compare the survival on both test strains directly, an additional experiment was performed. Pine-wood sawdust and plastic splinters from polyethylene were treated with 0.5 ml g^{-1} of a higher concentrated suspension of *E. coli* and *E. faecium* to achieve an initial cell density of 1×10^9 CFU per gram of wood or plastic and were incubated at 21°C.

Survival of *E. coli* on pine wood in dependence of the temperature

To examine whether survival of the test bacteria on pine-wood particles and plastic chips is dependent on the ambient temperature, samples of both materials were inoculated with 0.5 ml of *E. coli* to achieve an initial germ load of 5×10^8 CFU g^{-1} and

an initial moisture content of 50% of the wooden material. The samples were incubated at 4°C (relative humidity (RH) 100%), 21°C (RH 60%), and 37°C (RH 23%).

Extraction of bacterial cells and bacterial counts

At fixed time intervals, wood particles and plastic chips (3 g) were transferred to sterile plastic bags and an extraction buffer (0.85% (w/v) NaCl, 0.1% (w/v) Bacto-Tryptone, 0.1% (v/v) Tween 20 obtained from Merck, Darmstadt, Germany) was added in a 1:10 relation. The samples were mechanically treated in a Stomacher lab blender (Seward Medical, London, UK) for 3 min at 260 rpm to dislodge the adhering bacteria. Serial dilutions were plated onto appropriate solid growth media. *Escherichia coli* was cultivated on Plate Count Agar (Merck) containing 100 μg ml^{-1} cycloheximide, 50 μg ml^{-1} rifampicin, and 50 μg ml^{-1} streptomycin; *Enterococcus faecium* on Plate Count Agar containing 25 μg ml^{-1} vancomycin. After an incubation period of 24–72 h at 37°C, the grown colonies were counted to determine the titre of culturable bacteria per gram of sample (CFU g^{-1}).

DNA extraction

The cell suspension obtained after treatment in the Stomacher blender was centrifuged at 10 000 *g* for 30 min. Total community DNA was extracted from the bacterial pellet according to the protocol of van Elsas and Smalla (1995) originally developed for DNA extraction from soil. Briefly, the DNA was extracted by lysozyme, bead beating, and alkaline sodium dodecyl sulphate treatments followed by phenol and chloroform extractions. The crude DNA was purified by glass milk (GENECLEAN®SPIN kit, BIO101, Vista, CA, USA) according to the manufacturers' instructions.

PCR amplification

PCR amplifications were carried out with 1 μl of purified DNA extracts obtained as described above. PCR products of the *sat*3 gene of *E. coli* were generated as described by Pukall et al. (1996). The *van*A gene of *E. faecium* was amplified according to Klare et al. (1995).

Southern blotting und hybridisation

The quality and quantity of crude DNA received after the extraction was analysed in an agarose gel (0.8% w/v). Subsequently, the DNA was blotted from the agarose gel matrix onto an uncharged nylon membrane (Amersham, Freiburg, Germany) by capillary transfer (Sambrook et al. 1989). For molecular detection, Digoxigenin-labelled probes specific for the streptothricin acetyltransferase gene (*sat*3) in *E. coli* pIE639 and the vancomycin resistance gene (*van*A) in *E. faecium* were used. In order to prepare the probes, PCR products of the *sat*3 and the *van*A gene, respectively, were generated as described above, excised from an agarose gel (1.0% w/v), eluated and purified by using the Qiaex II gel extraction kit (Qiagen, Hilden, Germany), and labelled by a standard random priming according to the instructions of the DIG DNA labelling kit (Roche, Mannheim, Germany). Hybridisation at 42°C, washing and detection of DNA hybrids was carried out as recommended by Roche.

Inhibitory potential of aqueous wood extracts

Wood extracts were prepared by adding 20 g of wood sawdust to 180 ml sterile water, incubated at 80°C for 2 h and sterile filtered through a 0.2 μm cellulose acetate filter (Sartorius, Göttingen, Germany). The woods tested were pine, spruce, larch, beech, poplar (as described before), and, in addition, pure pine heartwood. One hundred microlitres of each wood extract were transferred in a 96-well microtitre plate and supplemented with an overnight grown culture of the test bacteria. The final cell density in the wells was 1×10^{9} CFU ml^{-1} *E. coli* and 1×10^{6} CFU ml^{-1} *E. faecium*, respectively, according to the inoculum levels used to contaminate wood dust. Pure water and tannic acid (5% w/v, 10% w/v; Sigma, Steinheim, Germany) were used as controls. Each approach was repeated four times. Plates were incubated overnight at 37°C. A tetrazolium salt as indicator of viability (Rodriguez et al. 1992), 2,3,5-Triphenyltetrazolium chloride (0.1 mg ml^{-1}; Merck) was added to the wells. After a further incubation of 30 min at 37°C in the dark to allow colour development, bacterial growth was determined by measuring the optical density at 595 nm with a microtitre plate reader (model V_{max}; Molecular Devices Corp., Menlo Park, CA, USA).

Results

Survival of *E. coli* pIE639 and *E. faecium* on wooden particles of different wood species

The survival of *E. coli* and *E. faecium* was followed on sawdust of several wood species using cultivation-based and cultivation-independent methods. First, wooden and plastic samples were inoculated with initial cell densities of 5×10^{8} CFU g^{-1} *E. coli* or 1×10^{6} CFU g^{-1} *E. faecium*, respectively. Samples of the inoculated woods and plastic chips were taken at fixed time intervals and plate counts were done. A very different survival behaviour of the test bacteria on the different wood species and plastic was found (Figures 1a,b). A dramatic reduction in bacterial numbers of *E. coli* was detected on pine and oak wood. Already after 24 h, no culturable bacteria could be detected on pine, and on oak after 48 h. These results correspond to a log 9 reduction in bacterial numbers of *E. coli* within 24 h on pine and within 48 h on oak, respectively. However, a decline in *E. coli* numbers, corresponding to a log 2 germ reduction on spruce, larch, maple, poplar, and beech, became apparent only during the first 24 h. Afterwards, the bacterial titre remained constant over several days at high levels of 10^{6}–10^{5} CFU g^{-1}. The bacterial titre on the plastic chips developed in a similar way compared to spruce, larch, maple, poplar, and beech and also remained at levels of 10^{6}–10^{4} CFU g^{-1} during the whole sampling time of 9 days. However, 1–2 orders of magnitude fewer culturable *E. coli* could be recovered from the plastic chips compared to the sawdust of spruce, larch, maple, beech, and poplar after 9 days. A drastic decrease of the *E. faecium* titre was observed on pine, oak, and larch. After 24 h, a log 6 reduction in CFU numbers was obtained, and no culturable bacteria could be recovered from these woods anymore. In the other woods, the germ reduction was fastest in the first 24 h. The bacterial numbers continued to decline and resulted in a complete germ reduction of *E. faecium* on spruce and maple after 7 days. *E. faecium* survived longer on beech and poplar, where 10^{1}–10^{2} CFU per gram wood dust were recovered even after 7 days. However, *E. faecium* survived longest and in larger quantities on the plastic chips compared to all woods tested. Within the first days a very stable bacterial titre was observed on plastic. The first reduction in bacterial numbers began 4 days after inoculation and resulted only in a log 2 reduction after 7 days. All samples dried equally fast. The moisture content of all different

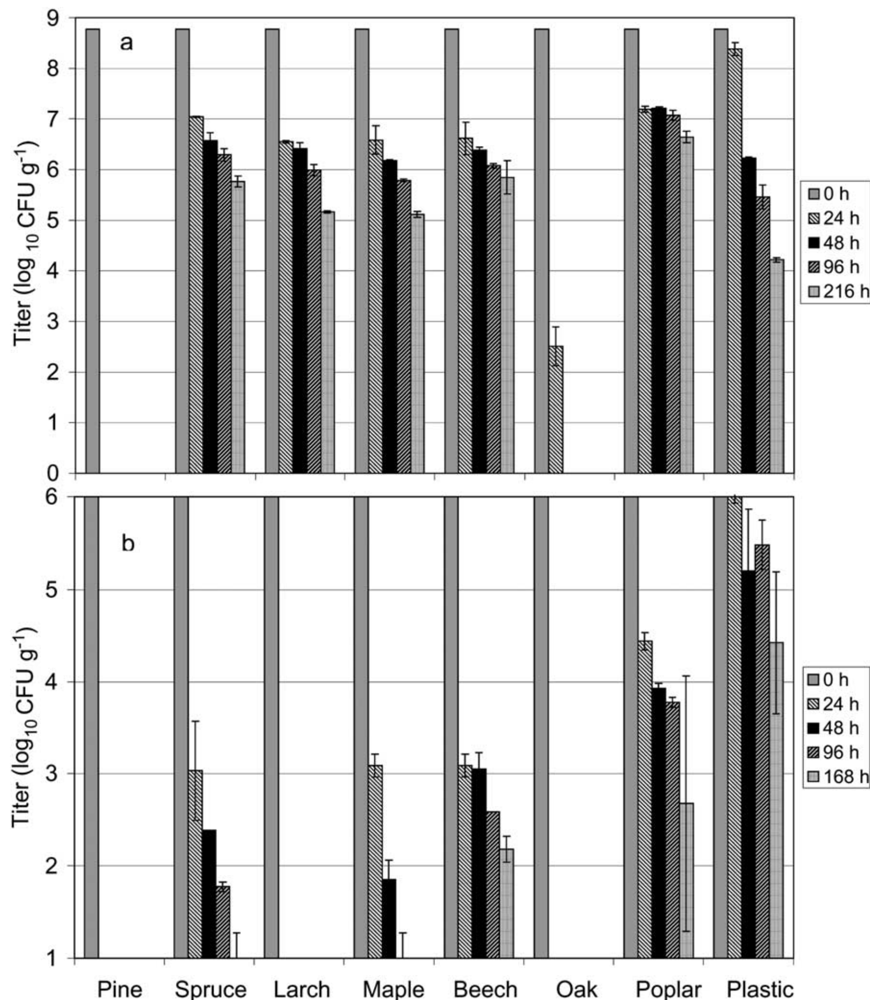

Figure 1 Survival of *E. coli* pIE639 (a) and *E. faecium* (b) on wooden sawdust and plastic chips. Initial inoculum size: *E. coli*, 5×10^8 CFU g^{-1}; RT: 21°C; relative humidity (RH) 55%; *E. faecium*, 1×10^6 CFU g^{-1}; RT: 21°C; RH 55%.

woods tested was around 5–8% after 12 h. The plastic chips were completely dried after this time (data not shown).

In addition, the survival of the test bacteria was followed on pine-wood sawdust and plastic splinters from polyethylene after both samples were inoculated with the same cell density of 1×10^9 CFU g^{-1} (data not shown). Again, the bacterial titre of both strains decreased considerably faster on pine wood compared to plastic. However, the gram-positive *E. faecium* always survived longer than the gram-negative *E. coli* on both kinds of material. Immediately after inoculation, a drastic germ reduction started on pine-wood. After 24 h and 48 h, respectively, no culturable *E. coli* or *E. faecium* were recovered from pine-wood sawdust. In contrast, the decline in CFU on plastic resulted only in a log 3 reduction of bacterial numbers of *E. coli* within 7 days. Almost no decrease in bacterial numbers of *E. faecium* was observed on plastic; rather, the gram-positive bacteria could be recovered at very high levels of more than 10^4 CFU per gram of polyethylene chips also after 7 days.

In parallel, a cultivation-independent detection of the inoculated bacteria was performed. Results concerning the persistence of *E. coli* on the different materials are presented in Figure 2. The molecular detection showed that the decrease of CFU numbers on pine correlated with the decrease of *E. coli* DNA on wood. It was shown that the DNA contents of *E. coli* on pine wood decreased rapidly, and that no DNA of the test organism could be detected after 24 h. In contrast, only a slight reduction of the DNA contents of *E. coli* was observed on spruce, larch, maple, beech, poplar, and plastics according to the bacterial numbers determined. The molecular detection of *E. coli* DNA on oak was not successful. Although numerous culturable bacteria were recovered from oak wood after the first 24 h, no corresponding DNA signal became apparent after hybridisation (Figure 2a). The initial DNA contents on maple and beech detected after hybridisation did not correlate with the amount of extracted DNA in the agarose gel (data not shown). The intensity of corresponding PCR signals obtained after 48 h was lower for pine and oak wood samples compared to the other wood species and plastic when fewer amplification cycles were used (Figure 2b). PCR products of the *sat*3 gene of *E. coli* were obtained for all wood species tested at all samplings with increasing PCR cycles up to 35 only. The DNA tended to decline independently of the type of bacteria. Also, amounts of DNA of *E. faecium* recovered from pine and oak-wood sawdust were generally lower compared to the other woods and plastic (data not shown).

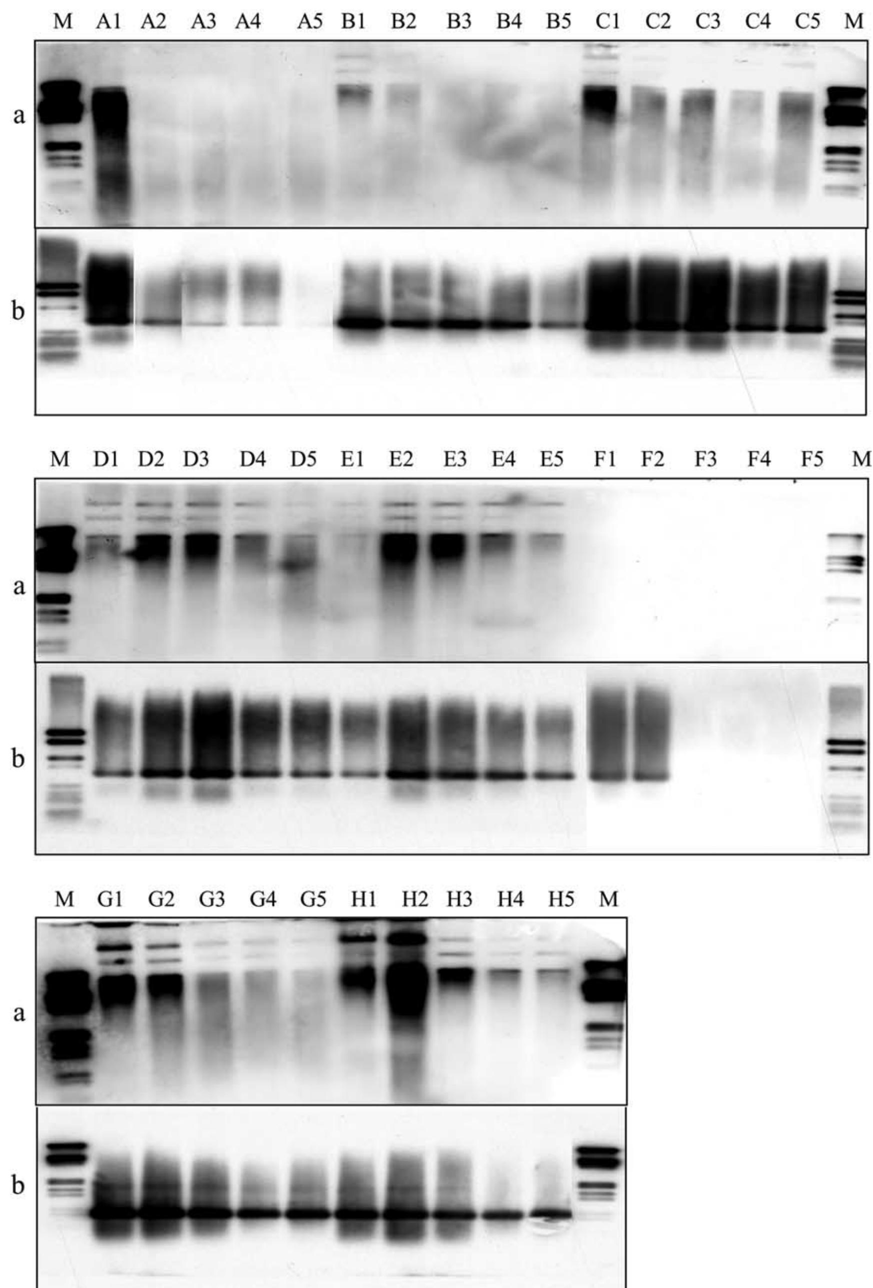

Figure 2 Detection of *E. coli* pIE639 DNA on wooden sawdust and plastic chips. Initial inoculum size: 5×10^8 CFU g^{-1}; RT: 21°C; RH 55%. (a) Southern blot of the extracted crude DNA after hybridisation with *sat*3-probe. (b) Southern blot of the PCR-amplified DNA (852 bp) after 23 amplification cycles and hybridisation with *sat*3 probe. M, DNA molecular weight marker III, DIG-labelled (Roche, Mannheim, Germany); A, pine; B, spruce; C, larch; D, maple; E, beech; F, oak; G, poplar; H, polyethylene; samples were analysed after 1, 1 h; 2, 24 h; 3, 48 h; 4, 96 h; 5, 216 h.

Further experiments to characterise the interactions between wood and bacteria in more detail were performed with pine wood only, because this wood showed the best antibacterial properties.

Survival of *E. coli* on pine wood in dependence of the ambient temperature and humidity

The experiment aimed to test whether survival of the test bacteria on pine wood particles and plastic chips is dependent on the ambient temperature. Figure 3a shows that the number of culturable bacteria decreased faster on both materials at higher temperatures and corresponding lower humidities. The recovery of *E. coli* from pine sawdust was generally lower compared to the recovery of *E. coli* from plastic chips at all temperatures. After 24 h, no culturable bacteria could be detected on pine wood at 37°C, while on plastic chips almost 10^6 CFU g^{-1} were determined. In contrast, if the pine wood samples were incubated at 4°C, a complete decrease of bacteria required more than 6 days. The germ reduction clearly slows down with decreasing temperatures, yet a clear decrease of the germs on pine took place also at 4°C. A decline in bacterial numbers on plastic chips was only observed at 21°C and 37°C. In contrast, the bacterial titre on plastic remained almost unaltered at high levels of 10^7 CFU g^{-1} over 6 days at 4°C.

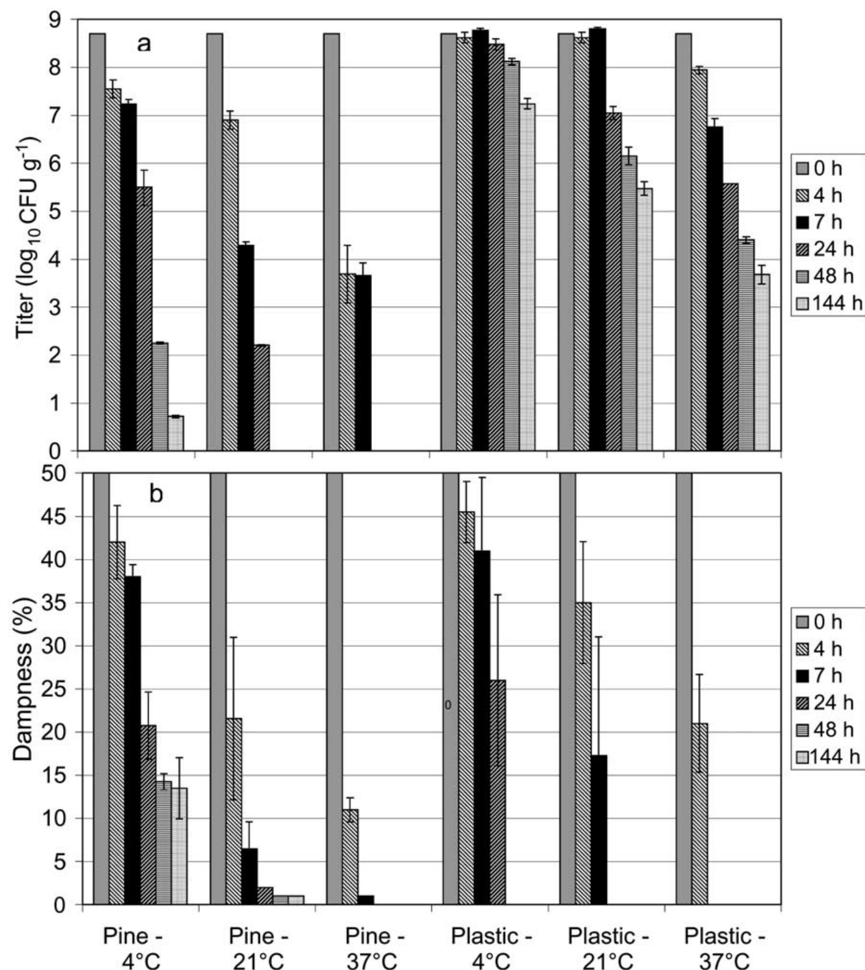

Figure 3 Survival of *E. coli* pIE639 on pine-wood sawdust and plastic chips (a) and drying process of pine-wood sawdust and plastic chips (b) depending on the ambient temperature. (a) Initial inoculum size: 5×10^{8} CFU g^{-1}; incubation at 4°C (RH 100%), 21°C (RH 60%), and 37°C (RH 23%). (b) Initial moisture content: 50%; incubation at 4°C (RH 100%), 21°C (RH 60%), and 37°C (RH 23%).

Although wood and plastic dried equally well at ambient temperatures of 37°C (RH 23%) and 21°C (RH 60%) within 24 h, bacterial numbers decreased conspicuously faster on pine wood than on plastic. The drying process substantially slowed down at a temperature of 4°C and a corresponding humidity of almost 100%, and the pine-wood sawdust dried more slowly than the plastic chips (Figure 3b). Nevertheless, a clear germ reduction on pine started already after 4 h at 4°C. In contrast, almost no decline in CFU was observed on plastic chips during the first 48 h. Even 6 days after inoculation, the analysis of the plastic chips resulted only in a log 1 reduction in bacterial numbers (Figure 3a).

Testing for inhibitory potential of aqueous wood extracts

In order to clarify the mechanism of the detected antibacterial effect, the survival of the test bacteria in the aqueous wood extract was analysed. An inhibition of bacterial growth of *E. coli* pIE639 became apparent only in the aqueous extracts of pine according to the inhibition capacity of a 5% (w/v) solution of tannic acid (Figure 4a). However, clear differences between the inhibitions of *E. coli* in extracts made of pure pine heartwood and extracts made of pine with a heartwood content of only about 60% were not detected. Aqueous extracts of spruce, larch, beech, and poplar supported a level of bacterial growth comparable with that in water. Growth of *E. faecium* was completely suppressed in the extracts from pure pine heartwood, larch, and in the tannic acid suspensions. The lower heartwood content in the mixed pine wood sample caused a slight reduction of the inhibition of *E. faecium*. Some inhibition was also observed in the extract of spruce, while the growth of *E. faecium* in the extract made of poplar and beech was not suppressed (Figure 4b). The intensity of the inhibitory activity of the aqueous wood extract correlated with the results of plate counts and DNA analysis.

Discussion

The most important finding of the study was that the hygienic potential of wood cannot be generalised, because the survival of bacteria was completely different on the different wood species tested. In the experiments presented, the survival of *Escherichia coli* pIE639 and *Enterococcus faecium* on wood depended on various factors such as the wood species, the type of the inoculated bacterium, the ambient temperature, and humidity.

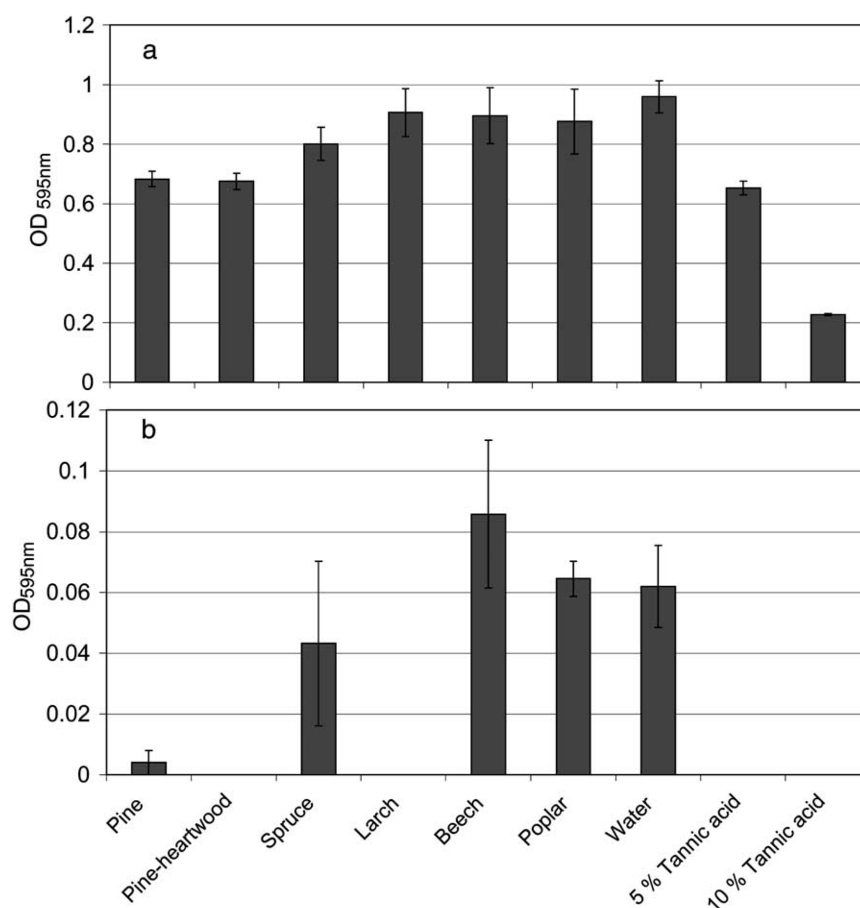

Figure 4 Growth of *E. coli* pIE639 (a) and *E. faecium* (b) in aqueous wood extracts, tannic acid, and water after incubation overnight at 37°C. Initial cell densities: *E. coli*, 1×10^9 CFU ml^{-1}; *E. faecium*, 1×10^6 CFU ml^{-1}.

First of all, plate counts showed that the test bacteria had remarkably different abilities to survive on the different wood species. The rate of germ reduction was always fastest on pine-wood sawdust followed by oak compared to spruce, maple, beech, poplar, and polyethylene. In previous studies performed by our group, the survival of *E. coli* and *E. faecium* was followed on wooden sawdust (Schönwälder 1999) and on compact wooden boards (Schönwälder et al. 2002). In those studies, pine also exhibited strong antibacterial characteristics, comparable to the currently presented results. In the current study, *E. coli* survived longer on spruce, maple, beech, and poplar than on plastic, while in contrast *E. faecium* survived longer on the plastic chips compared to the wooden samples. Furthermore, on larch wood a drastic decrease in bacterial numbers of *E. faecium* comparable with that on pine and oak became apparent, whereas the survival of *E. coli* was not suppressed on larch dust. Contrary to that finding, we demonstrated in the earlier study that the germ-reducing properties of larch wooden boards against both *E. coli* and *E. faecium* were almost as good as for pine wood, but a high variation concerning the antibacterial effect was observed (Schönwälder et al. 2002). The antibacterial effect of pine wood was reproducible when the survival of the test bacteria was followed by plate counts as described before on eight independent pine wood samples (ratio of heartwood to sapwood: 70:30) originating from different areas of Germany and Russia. The decrease in bacterial numbers of applied bacteria on pine-wood sawdust resulted always in at least 5×10^8 CFU g^{-1} of *E. coli* and 5×10^6 CFU g^{-1} of *E. faecium*, respectively, within 24 h for all pine wood samples tested (data not shown).

Differentially, antibacterial effects were observed by Ak et al. (1994a) only after a contamination of wooden boards with high levels (10^8 CFU 25 cm^{-2}) of *E. coli* on three successive days without intermediate cleaning. Bacterial recoveries from cherry and maple were significantly fewer than from basswood, birch, and butternut. But, in general, significantly fewer viable bacteria could be recovered from the wooden surfaces compared to plastic regardless of the wood species and the type of the inoculated strain (Ak et al. 1994a,b). Gehrig et al. (2000) found no differences in bacterial survival rates on boards of maple and beech. The study by Koch et al. (2002) revealed that oak showed the best result in elimination of *Pseudomonas fluorescens* and *Bacillus subtilis* on the surface of boards compared to beech and ash. Again, the survival rates of bacteria on plastic and stainless steel were always higher than on wooden boards. Furthermore, it was concluded that pine revealed a better hygienic performance than spruce after testing both wood species in a second set of experiments (Koch et al. 2002). Earlier research repeatedly demonstrated that more micro-organisms were present on wooden boards compared to metal or plastic surfaces after contact with food products (Kelch and Palm 1958; Gilbert and Watson 1971; Kampelmacher et al. 1971; Borneff et al. 1988a,b).

However, those studies did not provide sufficient results regarding important details of the experimental design, because the wood species used in the experiments was not mentioned.

The gram-positive *E. faecium* survived noticeably longer on both pine wood dust and plastic chips compared to the gram-negative *E. coli,* confirming the findings of Schönwälder et al. (2002). Differences in the cell wall structure of gram-positive bacteria compared to gram-negative ones might explain this result. Quite possibly, the higher number of layers in the cell wall of gram-positive bacteria compared to that of gram-negative bacteria could protect the former against desiccation and wood components with antibacterial potential for a longer time. In contrast, experiments performed by Koch et al. (2002) indicated the slightly higher survival rates of the gram-negative *P. fluorescens* compared to the gram-positive *B. subtilis* on boards made of ash, beech, oak, plastic, and stainless steel. Ak et al. (1994a) demonstrated almost similar survival behaviour of three different gram-negative strains (*E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella typhimurium*) on hard maple boards. In addition, earlier research conducted by our group with wooden boards (Schönwälder et al. 2002) and with pine dust (Schönwälder 1999) revealed that the rate of germ reduction was strongly influenced by the initial inoculum size. The more bacteria were applied, the longer culturable bacteria could be recovered.

Furthermore, it was shown that moisture content of the test material and humidity are key factors for germ development on different materials, and an obvious delay in germ reduction was observed with increasing humidity and moisture content, respectively. Nevertheless, the decline in bacterial numbers on pine wood was faster than on plastic in a humid environment, too. In addition, higher temperatures accelerated the drying process and thus also the decrease of culturable bacteria. Already, results obtained in preliminary experiments indicated that the survival of bacteria on pine sawdust was strongly dependent on the moisture content of wood. The higher the initial moisture, the longer the drying process and the longer bacteria could survive (Schönwälder 1999). Gehrig et al. (2000) performed a contamination experiment of wooden cutting boards made of maple and beech versus polyethylene boards. Results showed very high numbers of *E. coli* on both wood and plastic in a humid environment. In a drier environment, noticeably fewer bacteria were recovered from wood samples than from plastic. Comparison of wet and dry boards of pine and spruce by Koch et al. (2002) revealed a higher survival of bacteria on the wet surface. But, in both cases, the amount of bacteria decreased with time. The effect of temperature (RT, 4°C) and humidification on the survival of *E. coli* K12 Hfr on wooden and plastic boards was also tested by Ak et al. (1994b). In contrast to the other studies mentioned, multifactorial analysis of variance showed no significant differences in the survival rates of *E. coli* on wood among wood types, between temperatures, or between humidification conditions, but clear differences between recoveries of bacteria from wooden and plastic surfaces were demonstrated here also. Humidification was only critical on plastic at room temperature and led to increased recovery of bacteria.

Most investigations into the hygienic properties of wood employed cultivation-dependent techniques such as agar contact plates, swabbing, rinsing, soaking, and destructive methods (Kampelmacher et al. 1971; Abrishami et al. 1994; Ak et al. 1994a,b; Miller et al. 1996; Prechter et al. 2002; Schönwälder et al. 2002). It has been proposed that bacteria can survive stressful conditions such as limited nutrient availability, osmotic stress, oxygen limitation, and large variations in temperature and pH by entering a viable but nonculturable (VBNC) state. These cells are metabolically active but cannot be cultured on laboratory media by standard bacteriological methods (Roszak and Colwell 1986; Winfield and Groisman 2003). Even by desiccation of the wood, the test organisms are exposed to heavy stress. During drying, some bacteria die while others are only impaired in their life activity, without being killed. However, these bacteria can be vitalised if they are returned into a favourable environment (Kampelmacher et al. 1971). Furthermore, it was shown that bacteria such as *E. coli* form dormant cells in order to survive also under unfavourable living conditions with limited nutrient offer (Perez-Rosas, 1988; Barcina et al. 1989). Del Mar Lleò et al. (2003) reported the survival of stressed vancomycin-resistant enterococci in the environment by entering the VBNC state. In order to exclude that the detected reduction in bacterial numbers, particularly on pine and oak wood, is mainly caused by viable but nonculturable cells caused by desiccation stress, it was necessary to use molecular methods capable of detecting nonculturable bacteria. After recently published studies had demonstrated the eligibility of DNA-based methods for direct analysis of wood-inhabiting fungi (Jasalavich et al. 2000; Vainio and Hantula 2000) and reported the successful isolation of functional RNA from woody branches (Lewisohn et al. 1994; Melichar et al. 2000), we decided to use DNA extraction and PCR for the first time in a hygiene study to facilitate a reliable and sensitive cultivation-independent analysis of selected bacteria in wood.

The cultivation-independent analysis showed that the decrease in CFU numbers correlated with the decrease of DNA on wood. Especially on pine wood, a rapid decline of extracted DNA became apparent. These results indicate that the decrease in bacterial numbers on wood was not due to the ability of the test bacteria to enter the VBNC state or due to the transfer of the bacteria into the wood and close adsorption of the bacteria to the wood structure as argued by several authors (Kampelmacher et al. 1971; Ruosch 1981; Abrishami et al. 1994; Rödel et al. 1994; Lorentzen et al. 2000). Rather, the applied bacteria were killed completely due to interaction with wood.

Molecular detection was dependent on the wood species and could not be successfully accomplished for beech, maple, and oak. Results indicate some sort of inhibition during DNA extraction of oak and Southern blot hybridisation of beech, maple, and oak. It is assumed that molecular detection was probably disturbed by co-extracted polyphenolic wood components, because even humic acids were shown to interfere with DNA hybridi-

sation, restriction enzyme digestion, and PCR amplification (Smalla et al. 1993; Tebbe et al. 1993). Inhibiting wood components might be co-extracted directly from remaining wooden particles in the cell pellet, washed-out during mechanical treatment of the samples in the Stomacher blender, or might have been incorporated into the cell walls of the bacteria during incubation. DNA and enzymes become affected by polyphenolic components (Scalbert 1991; Field and Lettinga 1992), so that the DNA is no longer available for molecular detection, and enzymes used for DNA extraction and hybridisation are inhibited. In preliminary tests, direct extraction of bacterial DNA from wood and DNA extraction after prior washing and preparing a cell pellet as introduced in this study was tested. Both methods resulted in comparable findings. The amount of extracted DNA correlated with the bacterial numbers recovered from the different woods. However, a higher quality and less inhibited DNA was obtained after DNA extraction from the cell pellet compared to the direct DNA extraction from wood. Southern blot hybridisation of DNA extracts was improved by prior purification of the crude extracts with glass milk as performed before PCR amplification (data not shown).

Results obtained in this study indicate that the detected germ reduction on wood is caused by an antibacterial effect of wood based on several physical and chemical properties of wood. The porous structure and hygroscopic characteristic of wood leads to desiccation of bacteria. Most bacteria are desiccation-sensitive and require a water potential of -2.8 MPa or less for growth in wood. This is significantly above the moisture content of air-dried wood stored indoors, so that properly dried wood does not offer bacteria enough water for growth and multiplication (Bavendamm 1974; Schmidt 1994). However, the present study revealed that desiccation of wooden material cannot be the only reason for the effects observed. During the experiments, all woods and plastic dried rapidly within 24 h, but on pine and oak a much higher reduction of culturable bacteria as well as a faster decrease of bacterial DNA on pine became apparent.

In addition, polyphenolic substances present in wood (e.g., tannins or flavonoids) could be responsible for an antibacterial effect (Field et al. 1989; Scalbert 1991; Field and Lettinga 1992; Cowan 1999; Rauha et al. 2000). It was clearly shown that wood ingredients extracted by hot water treatment are involved in the decrease of bacteria on wood. *Escherichia coli* and *E. faecium* were efficiently killed in aqueous extracts from pine. However, while *E. faecium* was drastically decreased in the aqueous extracts made of larch, no sign of inhibition was observed for *E. coli* in larch extracts. Results indicate different susceptibility of different bacteria towards wood extracts of the same species. Even differentially inhibitory effects of extractives (flavonoids) towards bacteria were observed by Rauha et al. (2000); for example, naringenin belonging to the group of flavonoids showed strong antibacterial activity against *Staphylococcus* sp., but *E. coli* and *Pseudomonas aeruginosa* were inhibited to a lesser degree. Moreover, the same study confirmed inhibitory characteristics of pine, since ether extracts made of the pine phloem suppressed the growth of *E. coli* and *Staphylococcus aureus* (Rauha et al. 2000). Our data contradict the statement of Ak et al. (1994b) that antibacterial substances in wood are not water-soluble. In addition, Miller et al. (1996) also demonstrated a considerable level of inhibitory activity of aqueous extracts made of white ash against *E. coli* O157:H7.

Conclusions

The hygienic properties of wooden and plastic particles in contact with test bacteria were investigated employing a combination of classic microbiological and DNA-based methods. The experiments revealed that the survival of *E. coli* and *E. faecium* was dependent on factors such as humidity and ambient temperature, and on the type of the test bacteria on both wooden dust and plastic chips. Furthermore, it must be stressed that different wood species displayed completely different hygienic performance. In the past, wood has obviously been unfairly classified as unhygienic. Some wood species like pine and oak showed excellent antibacterial characteristics, efficiently killed applied bacteria, and had clear hygienic advantages compared to other woods and plastics. Further research has to be performed under conditions closer to practice (e.g. humid air, effect of organic matter, and naturally occurring microbial communities) to evaluate whether the hygienic situation in several areas such as in private households, transportation of foodstuff, or animal husbandry could be improved by a careful selection of suitable wood species and appropriate handling based on the results obtained.

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