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Wooden boards affecting the survival of bacteria?

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The survival of bacteria on and in different wooden and plastic boards was examined by microbiological methods. Wood of different tree species and polyethylene were inoculated with *Escherichia coli* pIE639 and *Enterococcus faecium* as hygienically relevant test bacteria. The development of the bacterial titer was evaluated by culturing on agar contact plates and investigating wood shavings. Survival of the test bacteria depended on different factors such as tree species, the initial inoculum size and the characteristics of the inoculated strain. The bacterial titer decreased fastest on pine compared to other woods (spruce, beech, poplar) and plastic. After bacterial infestation only pine wood was germ-free at the surface and in the inner structure after a few hours. The survival of the bacteria on poplar and beech was comparable to their survival on plastic. The study indicated that an antibacterial effect of wood, especially for pine, is caused by the hygroscopic properties of wood and the wood extractives. The antibacterial effect of pine wood was not influenced by the storage time of the wood following harvest or the functional condition of the wood up to a germ load of 10^8 CFU/cm^2 *E. coli* pIE639.

Beinflussen Holzbretter das Überleben von Bakterien?

Das Überleben von Bakterien auf und in verschiedenen Brettchen aus Holz und Kunststoff wurde mit mikrobiologischen Methoden untersucht. Verschiedene Holzarten und Polyethylen wurden mit *Escherichia coli* pIE639 und *Enterococcus faecium* als hygienisch relevante Testbakterien beimpft. Die Entwicklung des Bakterientiters ist durch Abklatschproben und Untersuchung von Hobelspänen verfolgt worden. Das Überleben der Testbakterien war dabei von verschiedenen Faktoren abhängig, wie z.B. von der Holzart, der Animpfdichte und der Art des inokulierten Bakterienstamms. Der Bakterientiter nahm im Vergleich zu anderen Holzarten (Fichte, Buche, Pappel) und Kunststoff am schnellsten auf Kiefernholz ab. Nur Kiefernholz war innerhalb weniger Stunden an der Oberfläche und im Innern des Holzes keimfrei. Das Überleben der Bakterien auf Pappel- und Buchenholz war mit dem Überleben der Bakterien auf Kunststoff vergleichbar. Die Studie deutet auf eine antibakterielle Wirkung von Holz hin, besonders von Kiefernholz, aufgrund der hygroskopen Eigenschaften von Holz und der Wirkung von Holzinhaltsstoffen. Die antibakterielle Wirkung von Kiefernholz wurde durch die Dauer der Lagerung nach der Holzernte und den Gebrauchszustand der Brettchen bis zu einer Keimbelastung von 10^8 CFU/cm^2 *E. coli* pIE639 nicht beeinflusst.

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1 Introduction

Wood has been traditionally used for many centuries in the preparation, packing and transport of food products. However, the hygienic status of wood is still very disputed. The main concern in respect to food-contact surfaces is cross-contamination by bacteria from animal sources (De Boer and Hahne 1990) because wood is a porous and absorbent material which can be penetrated by organic matter along with bacteria. Negative hygienic characteristics of wood have been implied in a large number of publications (Kelch and Palm 1958, Gerigk 1966, Großklaus and Levetzow 1967, Gilbert and Watson 1971, Kampelmacher et al. 1971, Borneff et al. 1988a and b, Rödel et al. 1994). The focus of these studies aimed at determining the hygienic properties of wood in comparison to metal and plastic materials with application in relevant areas of meat and poultry processing and food preparation. For this purpose the germ contents on the surfaces were determined by contact plate and swab methods, and in more deep-seated wood layers by destructive proof procedures. All these studies came to the same conclusions: The germ content on wood surfaces was always higher than the germ content on metal and plastic surfaces. Furthermore, these studies stressed that wood is more difficult to clean and decontaminate than metal and plastic materials. The rejection of wood as an unhygienic material in the foodstuffs sector seemed justified after these investigations, and accordingly regulations and guidelines preventing the use of wood in this sector were implemented on a European-wide basis.

Due to this development the use of wood in food production was continually reduced. Presently wood is increasingly being replaced by plastics in microbiologically sensitive areas.

A study by the Food Research Institute in Wisconsin (Ak et al. 1994a and b) compared wooden and plastic boards and came to the surprising result that wood pos-

sesses substantially better hygienic characteristics than plastic. After contaminating different cutting boards with bacteria, significantly fewer viable bacteria could regularly be recovered from wooden boards than from plastic boards. These results were confirmed by Gehrig et al. (2000) in a recent study investigating hygienic aspects of wooden and plastic boards regarding the risk of food contamination. Previous studies assumed that the detected reduction in bacterial numbers on the wood surfaces is caused by an antibacterial effect of wood based on several physical and chemical properties of wood. The porous structure and hygroscopic characteristics of wood could remove the water needed by the bacteria for their vital functions and multiplication and thus kill them (Kampelmacher et al. 1971, Schulz 1995). In addition, substances present in wood (e.g. polyphenoles) could be responsible for an antibacterial effect (Willaman 1955, Biswas et al. 1981, Laks and McKaig 1988, Field et al. 1989, Schrägle and Müller 1990, Scalbert 1991, Müller et al. 1995).

In preliminary tests the survival of bacteria on chips of seven different European wood species was investigated with the result that bacterial survival depended on the wood species. It was suggested that the hygienic characteristics of the woods cannot be generalized (Schönwälder 1999, Schönwälder et al. 2000, Schönwälder et al., in prep.). The tests indicated certain antibacterial effects and showed that some wood species such as pine, larch and oak possess substantially better hygienic characteristics than plastic. Especially pine wood proved to be a natural material with antibacterial characteristics.

The main objective of the work presented here was to follow the survival of bacteria on the surface and the inner structure of wooden boards. A further aim was to determine whether the results obtained in the preliminary tests (Schönwälder 1999, Schönwälder et al. 2000) with wood chips are comparable to the behaviour of the bacteria on and in compact wooden boards.

2
Material and methods

2.1
Bacteria

Experiments were performed using *Escherichia coli* pIE639 (Tietze et al. 1989) and *Enterococcus faecium* (Klare et al. 1995). These bacteria were used as test germs and model organisms for hygienically relevant Gram-negative and Gram-positive bacteria. *E. coli* pIE639 carries a streptothricin acetyltransferase gene (*sat3*) localized on the InQ plasmid pIE639. *E. faecium* possesses a chromosomal localized vancomycin resistance gene. The inoculum used to contaminate the wooden and plastic samples was grown in Luria-Bertani broth (LB). Antibiotics were added to the cultivation media because both bacterial strains have antibiotic resistance genes which functioned as selection markers. Therefore it was possible to follow precisely the behaviour of the test organisms on the different materials also under semi-sterile conditions. *E. coli* pIE639 was grown overnight in LB–broth containing 100 μg/ml cycloheximide, 50 μg/ml rifampicin and 50 μg/ml streptomycin at 37°C for 16–24 h, *E. faecium* in LB–medium containing 25 μg/ml vancomycin at 37°C for 24–36 h.

2.2
Boards

The wood tested included Scots pine (*Pinus silvestris* L.), Norway spruce (*Picea abies* Karst.), European larch (*Larix decidua* Mill.), Beech *(Fagus silvatica* L.) and Black poplar (*Populus nigra* L.). The coniferous species and beech represent commonly used European tree species. Poplar was selected as a representative of fast-growing, non-durable deciduous species. Trees of a diameter at breast height (dbh) of 15 to 25 were taken from forests in the immediate vicinity of Braunschweig in Northern Germany. The trees were cut into 2 cm thick boards longitudinally and these were cut into 10-cm square blocks (100 cm^2, 2 cm thick). In addition, boards from both new and used wooden pallets were also provided by the company "Gustav Wilms Holzverpackungen" (Bad Essen-Barkhausen, Germany). Boards and blocks were selected randomly for each experiment. The boards were new or used and untreated or treated according to a special wash and dry procedure K3 (European Patent Office: EP 1005964A1) of the above company. Not all wood species were used for all the various experiments with boards, details of which are given in the appropriate results section. As reference material new plastic boards (polyethylene – 100 cm^2, 0.5 cm thick) were used.

2.3
Inoculation of boards

Before each experiment, each test surface was sterilized to avoid any contamination from the sample. Wooden boards were dried for at least 12 h at 103 °C, polyethylene boards were sterilized under UV light for 2 h. In order to characterize the behaviour of the bacteria on and in the wood, the blocks were treated with an overnight culture of the test bacteria. Before inoculation the desired cell density of the inoculum, respectively the initial inoculum size, was adjusted if necessary by diluting the overnight culture with saline (0.85%). Two different methods were used to inoculate the boards.

Method 1: The inoculum (2 ml) was deposited directly on the board surface and spread with a Drigalsky spatula.

Method 2: The wooden boards were soaked for 15 minutes in the inoculum. The volume of liquid absorbed by the sample differed between the individual wood species and was determined by weighing the blocks before and after the inoculation. The inoculated samples were incubated at room temperature (21–23 °C). All experiments were repeated at least twice with 2–4 replicates per experiment.

2.4
Sampling and measuring methods

The germ content on the surfaces of all tested materials was determined by using the RODAC plate sampling (agar contact plate method). For this purpose Petri dishes were filled completely with nutrient agar and a contact area (10 cm^2) slightly curved upwards developed. Bacteria were

recovered by pressing the agar plate directly onto the surfaces of the tested boards for 10–20 seconds. The results were recorded as colony counts per sample.

In addition, a destructive procedure was applied to follow the germ development in deeper wood layers. The wood surface was planed off approx. 1 mm. The wood shavings (1–3 g) were transferred to sterile bags and an extraction buffer (0.85% NaCl, 0.1% Bacto-Trypton; 0.1% Tween 20 [Merck, Darmstadt; Germany]) was added in a 1:10 relation. The wood shavings 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* pIE639 was cultivated on Plate Count Agar containing 100 μg/ml cycloheximide, 50 μg/ml rifampicin and 50 μg/ml streptomycin, *Enterococcus faecium* on Plate Count Agar (Merck) containing 25 μg/ml vancomycin. After an incubation period of 24–72 h at 37 °C the grown colonies were counted to determine the titer of culturable bacteria per gram of wood (CFU/g).

3 Results

3.1 Survival of *E. coli* pIE639 in dependence on different inoculum levels

First the survival of *E. coli* pIE639 was evaluated on the surface of different materials. *E. coli* pIE639 was applied by method 1 on unwashed and washed pine-heartwood, beech wood and plastic surfaces. 100 cm^2 areas of each test surface were separately treated with 2 ml cell suspension with different cell densities (10^4–10^8 CFU/cm^2). The development of the bacterial titer was registered by employing agar contact plates. The results of these experiments are represented in Fig. 1. The decrease in bacterial numbers on the surface was strongly dependent on the absorption capacity of the material. After inoculation of the untreated wooden boards according to method 1 the samples required approx. 1–2 h for the complete absorption of the inoculum into the material and drying of the surface. The germ reduction started after drying and fewer viable bacteria could be recovered from the wooden surface compared to plastic. However, the rate of the germ reduction depended on the wood species. On the impermeable plastic the bacterial film was visibly dry after approx. 3–4 h following the application of 2 ml inoculum onto an area of 100 cm^2. In contrast to wood, the earliest reduction in bacterial numbers on plastic began 12–24 h after drying the surface. Furthermore, the reduction of bacteria was dependent on initial cell density. The higher the initial inoculum size and the germ load, the longer the culturable bacteria could be detected on the different wooden (unwashed and washed) and plastic surfaces.

There was a significant difference in bacterial recoveries from wood and plastic. Bacterial numbers decreased faster on the wood surfaces than on the plastic surface. This effect becomes particularly obvious at higher germ loads. If the surfaces were subjected to 10^6 CFU/cm^2 or more, the bacterial titer decreased fastest on the pine wood surface, followed by the beech surfaces. On the plastic surface the test bacteria were detectable for the longest time, i.e. the bacteria survived here longest and in larger quantities. If only small concentrations of bacteria (10^3–10^4 CFU/cm^2) were applied, the bacteria behaved quite similarly on the different wooden and plastic materials, i.e. after very short time intervals no or only very few test germs were detectable on all surfaces.

3.2 Influence of washing the wood on its antibacterial effect

To improve and standardize the absorption capacity of the wood a special washing and drying procedure (K3) was developed by "Gustav Wilms Holzverpackungen" (Bad Essen-Barkhausen, Germany). After this treatment the inoculum is usually absorbed promptly into the wood. Treating the wood with the K3 process led to the improvement of the germ reduction on surfaces of all tested wood species (Fig. 1 and 2). Pine-heartwood treated with this procedure before the first inoculation showed visually very good absorptive properties and the fastest and most effective germ reduction on the surface. With initial inoculum sizes of 10^4 or 10^5 CFU/cm^2 no *E. coli* pIE639 could be recovered from the surface already 30 min after application. The rate of the germ reduction decreased with the increase of the initial inoculum size, but even with initial cell densities of 10^6 CFU/cm^2 no bacteria were detectable on treated pine wood after two hours. A further increase of the initial cell density led to a noticeable delay in germ reduction on the surface, and the onset of reduction was detectable only after approx. 2 h. Larch wood came close to pine in terms of germ reduction, whereas spruce, beech and poplar were not as satisfactory, in that order.

3.3 Survival of *E. coli* pIE639 and *E. faecium* on wooden and plastic boards

These trials aimed to examine if the survival of bacteria on different materials is dependent on the type of the inoculated strain. Boards of pine-heartwood, beech and poplar were inoculated with *E. coli* pIE639 or *E. faecium* by method 1. Areas (100 cm^2) of each test surface were applied with 2 ml inoculum or equivalent with 1×10^6 CFU per cm^2 test surface. From Fig. 3 it is evident that the decline in CFU observed was tendentious independently of the type of the test bacteria, i.e. the CFU of *E. coli* pIE639 and *E. faecium* recovered from pine wooden boards were generally lower than the bacterial recovery from beech and poplar. However, *E. faecium* always survived longer than *E. coli* pIE639. The higher the germ load, the longer the culturable bacteria of both types could be detected on the different surfaces and the more time was needed for the reduction of the bacterial titer. Once again the bacterial titer of both strains decreased fastest on the pine wood surface.

3.4 Development of the number of bacteria on the surface and in the inner structure of wooden boards

In order to check whether the germ reduction observed at the surface is due to a translocation of bacteria into deeper

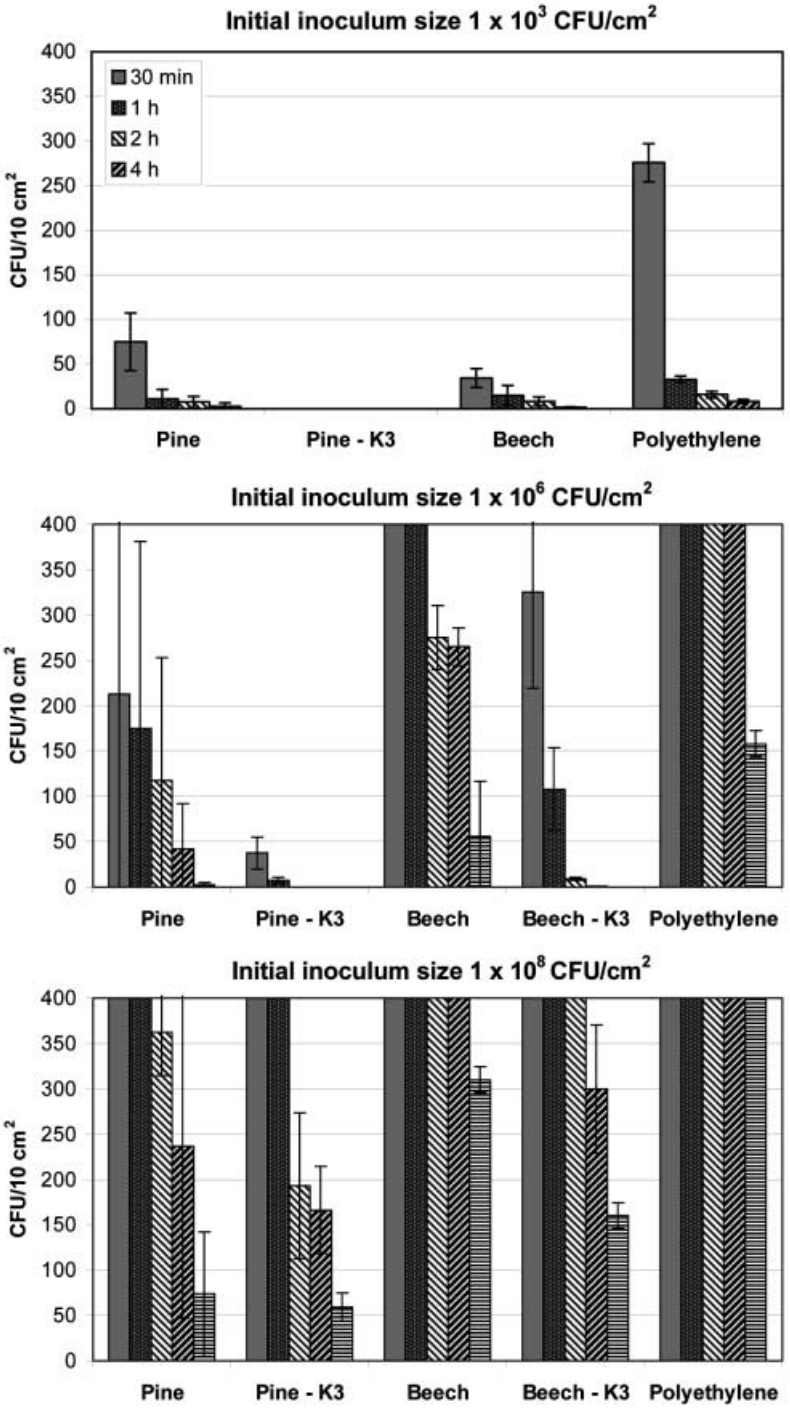


Fig. 1. Survival of *E. coli* pIE639 on new wooden and plastic boards depending on the initial inoculum size. Recovery of bacteria from the surfaces via agar contact plates, RT = 21 °C, rel. humidity = 55%

Bild 1. Überleben von *E. coli* pIE639 auf neuen Brettchen aus Holz und Plastik in Abhängigkeit von der Animpfdichte. Detektion der Bakterien auf der Oberfläche mittels Abklatschplatten, RT = 21 °C, rel. Luftfeuchte = 55%

layers, wooden blocks (unwashed and washed pine-heartwood, beech and poplar, size 5 cm × 10 cm × 2 cm) were placed into a bacterial suspension of *E. coli* pIE639 (5×10^7 CFU/ml) for 15 min (method 2). Different absorption properties were observed after this time period. Visually it could be determined that the blocks from washed pine (K3) and poplar were completely impregnated by the suspension. On the other hand the beech blocks were infiltrated only to approx. 5 mm depth and the unwashed pine blocks up to approx. 10 mm depth by the inoculum. The blocks were weighed before and after the inoculation and so the absorbed volume of the *E. coli* suspension, respectively the germ load per cm^2 block surface, were determined. On average, this procedure resulted in an average germ load of 3–5 × 10^6 CFU/cm^2. The development of the bacterial titer was followed on the surface and in 3 mm depth after sawing the block laminate-wise in longitudinal direction. The CFU were determined by agar contact plates and additionally by testing wood shavings from these depths.

A dramatic reduction in bacterial numbers was detected during the first 30 min on both unwashed and washed pine wood surfaces with agar contact plates (Fig. 4.1). On beech and poplar blocks only a slow reduction of the bacterial titer was to be observed and after 24 h viable bacteria were still detectable on the surface.

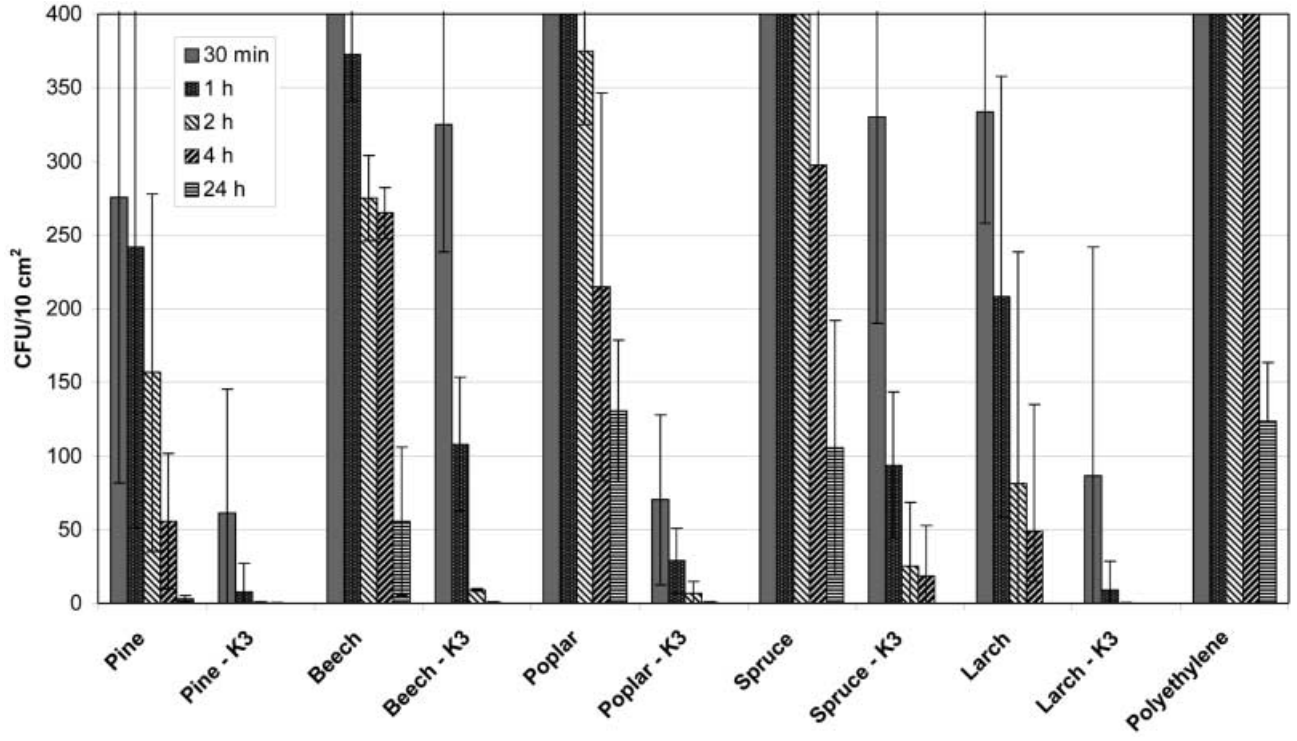


Fig. 2. Survival of *E. coli* pIE639 on new wooden and plastic boards before and after K3 treatment. Recovery of bacteria from the surfaces via agar contact plates, initial inoculum size = 1×10^6 CFU/cm^2, RT = 21 °C, rel. humidity = 50%

Bild 2. Überleben von *E. coli* pIE639 auf neuen Brettchen aus Holz und Plastik vor und nach K3-Behandlung. Detektion der Bakterien auf der Oberflache mittels Abklatschplatten, Animpfdichte = 1×10^6 CFU/cm^2, RT = 21 °C, rel. Luftfeuchte = 50%

The analysis of the wood shavings was more sensitive and characterizes the behaviour of the bacteria on and in the wooden blocks more exactly. The determination of the CFU of the shavings from the surface confirmed that after 24 h on beech and poplar blocks the titer of *E. coli* pIE639 was still very high (Fig. 4.1). The analysis of the wood shavings resulted only in a log2-reduction in bacterial numbers. In contrast, no *E. coli* pIE639 were recovered from the inner structure of washed pine wood (K3) already after 3 h, and after 5 h on unwashed pine wood. These results correspond to a log5 reduction within 3 h, respectively 5 h. The presumed translocation of the bacteria into deeper wood layers takes place according to these results. A complete absorption of the inoculum into the wood structure and the distribution of the bacteria took approx. 1 h. Within the first hour the bacterial titer increased in 3 mm depth and more or less strongly decreased thereafter, depending on the wood species (Fig. 4.2). Washed pine wood (K3) made an exception in this respect, since the absorption and germ distribution took place immediately and a decline of bacterial numbers was observed already after 30 min.

The tests showed clearly that a transfer of bacteria into deeper wood layers takes place and that these bacteria are fixed there. Within the beech and poplar blocks bacteria remained culturable during a 24 h period and could be

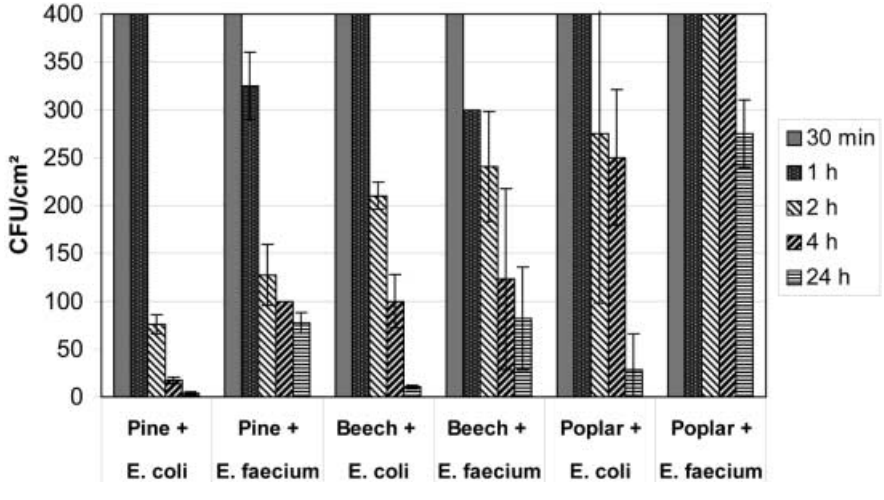
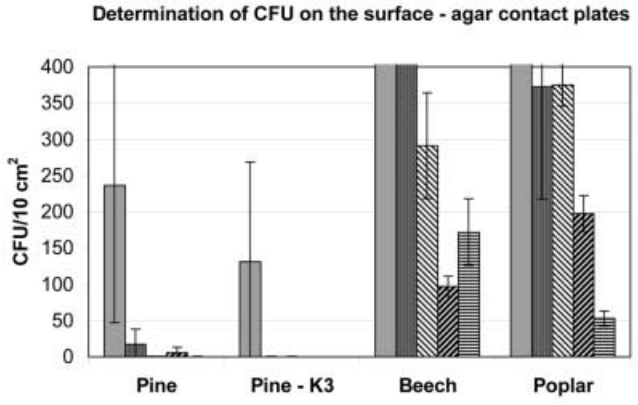
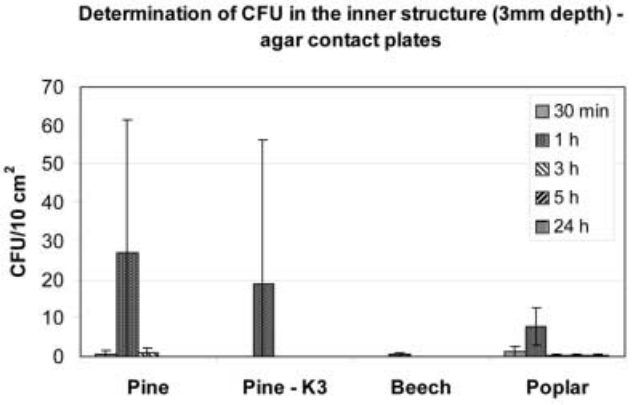
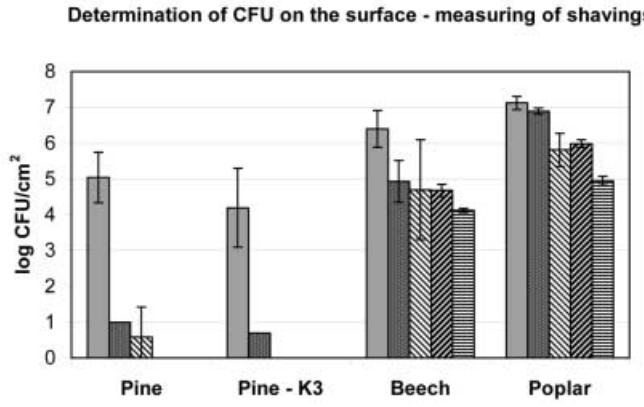


Fig. 3. Comparison of the survival of *E. coli* pIE639 and *E. faecium* on new wooden and plastic boards. Recovery of bacteria from the surfaces via agar contact plates, initial inoculum size = 1×10^6 CFU/cm^2, RT = 21 °C, rel. humidity = 55%

Bild 3. Vergleich des Überlebens von *E. coli* pIE639 und *E. faecium* auf neuen Brettchen aus Holz und Plastik. Detektion der Bakterien auf der Oberfläche mittels Abklatschplatten, Animpfdichte = 1×10^6 CFU/cm^2, RT = 21 °C, rel. Luftfeuchte = 55%







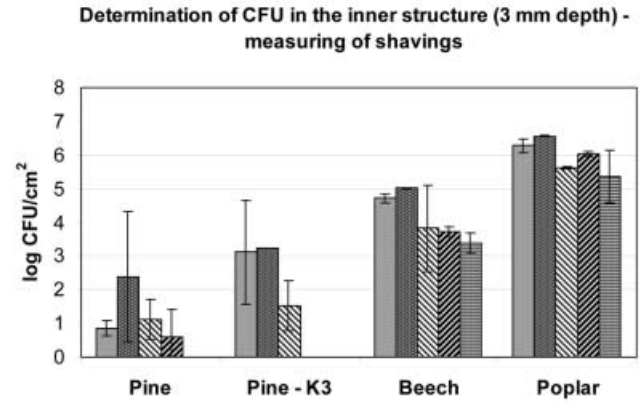


Fig. 4.1. Survival of *E. coli* pIE639 on new wooden boards. Recovery of bacteria from the surfaces via agar contact plates and investigating wood shavings, initial inoculum size = 5×10^6 CFU/cm^2, RT = 21 °C, rel. humidity = 50%
Bild 4.1. Überleben von *E. coli* pIE639 auf neuen Holzbrettchen. Detektion der Bakterien auf den Oberflächen mittels Abklatschplatten und Untersuchung von Hobelspänen, Animpfdichte = 5×10^6 CFU/cm^2, RT = 21 °C, rel. Luftfeuchte = 50%

Fig. 4.2. Survival of *E. coli* pIE639 in new wooden boards. Recovery of bacteria from the inner structure of wood via agar contact plates and investigating wood shavings, initial inoculum size = 5×10^6 CFU/cm^2, RT = 21 °C, rel. humidity = 50%
Bild 4.2. Überleben von *E. coli* pIE639 in neuen Holzbrettchen. Detektion der Bakterien im Holzinneren mittels Abklatschplatten und Untersuchung von Hobelspänene, Animpfdichte = 5×10^6 CFU/cm^2, RT = 21 °C, rel. Luftfeuchte = 50%

recovered at high concentration levels. However, in the pine wood blocks a reduction of the bacteria was observed also in the inner structure. After 5 h no culturable bacteria could be recovered from the inner structure of washed pine wood blocks (K3), and this was also the case in the unwashed pine blocks after approx. 7–8 h.

3.5
Development of the number of bacteria on the surface of used pine boards and pine boards of different age

The survival of *E. coli* pIE639 on the surface of used pine wood boards and boards of different age was analyzed to test the effect of the wood age (following felling) and the functional condition on the antibacterial properties. The older boards originated from pallets which were used since 1987, 1994 and 1996 compared with new and unused pine wood boards from 1999. Boards were inoculated with 1×10^6 CFU/cm^2 *E. coli* pIE639 by method 1. The test surface (100 cm^2) was treated with 2 ml inoculum by method 1. The development in bacterial numbers was followed by agar contact plates taken of the surface at determined time intervals. The result of this experiment is represented in Fig. 5. The germ-reducing effect of pine wood was independent of the functional condition and age of the wood up to a germ load of 1×10^8 CFU/cm^2. A decrease in *E. coli* cell numbers was observed on the surface of all samples. The antibacterial effect and the effectiveness of the germ reduction was comparable for new and old pine wood boards.

4
Discussion

A literature review by Carpentier (1997) summarized the most important studies concerning sanitary quality and hygienic properties of cutting boards and came to the conclusion that not enough work has been done in this area. Experimental factors varied from one study to another and this could explain the different survival rates of bacteria in wood and plastic determined by the different studies. This is one reason for carrying out the work presented here.

The survival of *E. coli* pIE639 and *E. faecium* on and in compact wooden boards confirmed the results which were obtained in the preliminary tests on interactions between bacteria and wood chips (Schönwälder 1999). Survival of bacteria depended on the wood species, the initial inoculum size and the characteristics of the inoculated strain in the presented study too. The antibacterial effect of pine

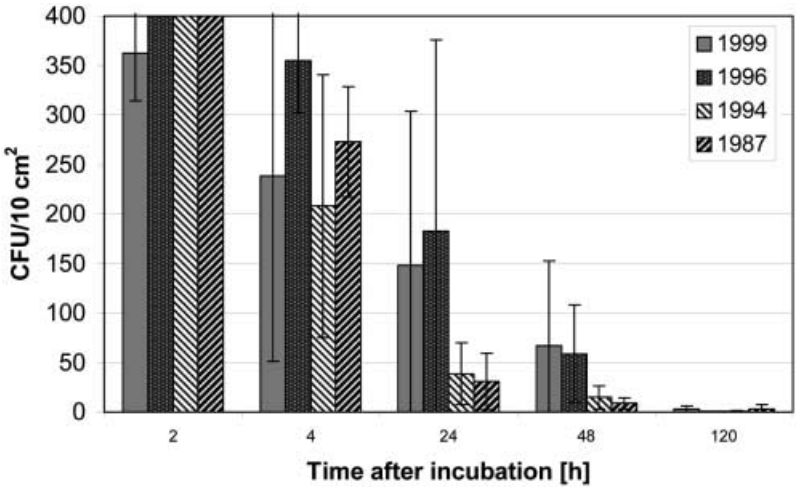


Fig. 5. Survival of *E. coli* pIE639 on new and used wooden boards. Recovery of bacteria from the surfaces via agar contact plates, initial inoculum size = 1×10^8 CFU/cm^2, RT = 21 °C, rel. humidity = 55%
Bild 5. Überleben von *E. coli* pIE639 auf neuen und gebrauchten Holzbrettchen. Detektion der Bakterien auf der Oberfläche mittels Abklatschplatten, Animpfdichte = 1×10^8 CFU/cm^2, RT = 21 °C, rel. Luftfeuchte = 55%

wood found in previous tests with sawdust could be confirmed (Schönwälder et al., in prep.), because on this wood the bacteria had the lowest survival rate compared with spruce, beech, poplar and plastic. Larch wood, however, came close to pine wood in respect to reduction of bacteria, but a high variation regarding the antibacterial properties could be observed (data not shown). The behaviour of the bacteria on beech and poplar was comparable to that of bacteria on plastic.

The Gram-positive test bacteria *E. faecium* could be recovered for a longer period of time from the wood surfaces than the Gram-negative test bacteria *E. coli*. Differences in the composition and structure of the cell wall of Gram-positive bacteria compared to Gram-negative bacteria could make them more resistant to antibacterial wood ingredients or desiccation. A decrease in bacterial numbers was observed on differently old and used pine wood. An influence of the wood age or the functional condition on the antibacterial effect of pine wood could not be determined up to a germ load of 10^8 CFU/cm^2 *E. coli* pIE639.

Some authors (Kampelmacher et al. 1971, Rödel et al. 1994) argue that the detected decline in CFU on the wood surface is exclusively due to a transfer of the bacteria into deeper wood layers, because wood fibres possess capillary properties and a high water-retention capacity. The tests performed in this study showed clearly that bacteria are indeed translocated into the inner part of the wooden boards. Penetration depends on the orientation of wood fibres. If the fibres run perpendicular to the wood surfaces, bacteria can penetrate several centimetres into the wood, e.g. in chopping blocks (Kampelmacher et al. 1971). On the other hand, Park and Cliver (1996) found that bacteria applied to the wood surfaces did not penetrate to a great depth, and Ak et al. (1994b) observed bacteria only up to 15 µm below the surface of wooden cutting boards, which were probably cut longitudinally. In the present study it could be shown that especially in beech and poplar the bacteria settled directly under the surface and were not transported to a great depth into the wood. Generally the test organisms were detected in larger quantities only in the upper 5 mm of all tested wood blocks (data not shown). The bacteria in beech and poplar remained viable over a 24 h period and could be recovered and cultured on suitable growth media. However, previous studies have shown that such absorbed bacteria were unlikely to return to the surface, where they could contaminate food (Ak et al. 1994a, Park and Cliver 1996). In contrast to beech and poplar wood, pine wood appears to be germ-free even within the wood for a short period of time. No culturable bacteria could be recovered in deeper wood layers of pine, depending on the initial inoculum size, after a few hours.

Many authors have pointed out repeatedly that the ability of wood to reduce germs decreases when material such as fat, protein abrasion and a serum effect clogged the wood pores and limited the bacterial transfer into the wood (Kelch and Palm 1958, Großklaus and Levetzow, 1967, Kampelmacher et al. 1971, Ak et al. 1994a and b, Rödel et al. 1994). Scott and Bloomfield (1990) proved that the presence of soil promotes the growth and multiplication of bacteria on dirty laminated working surfaces. Furthermore, the study by Gehrig et al. (2000) showed that humidity is a key factor for germ development on different materials. A humid environment promoted the development of bacteria colonizing wood surfaces and plastic. Contrary to these findings, the studies by Ak et al. (1994a and b) found that a reduction of CFU in a bacterial mixture of *Pseudomonas*, *Escherichia*, *Proteus* and *Micrococcus* occurred regardless of the relative humidity in which the contaminated wood was kept. But drying was important for the survival of bacteria on plastic, because in a humid environment the reduction of bacteria decreased (Ak et al. 1994b). Nevertheless, the better hygienic characteristics of pine wood compared to plastic as a material for transportation pallets has also been demonstrated in preliminary tests under the conditions of practice which include humid air, effect of organic matter and contamination with naturally occurring bacteria from meat (Schönwälder et al. in prep.).

The hygienic characteristics of the wood depended strongly on the penetration and absorption capacity (liquid absorption) of the material, because the faster the bacteria are transferred into the wood, the faster the surfaces are free of bacteria. It could be observed that the absorption capacity of different woods was very variable. In many cases the liquid remained on the wood surface as

if a film prevented its absorption, and sometimes it lasted 1–2 h before this liquid was at all taken up by the wood.

The wood treatment by the washing and drying procedure (K3) developed by "Gustav Wilms Holzverpackungen" (Bad Essen-Barkhausen, Germany) contributed substantially to the improvement of the germ reduction rate on all kinds of wood surfaces. The absorption rate was influenced positively, resulting in a faster transfer of the bacteria and a more rapid decline of the bacterial surface contamination compared to unwashed wood. On plastic surfaces bacteria are able to form a kind of biofilm and are fixed by the drying process (Dhaliwal et al. 1992, Holal et al. 1990), enabling them to remain viable on the plastic surface during a long period depending on the initial inoculum size. Of course, plastic surfaces can be decontaminated only by cleaning procedures.

One reason for the detected antibacterial effect may be that the porous and hygroscopic wood leads to desiccation of bacteria. The hygroscopic properties of wood prevent that water is available for microorganisms below a certain moisture content. Most bacteria are even more desiccation-sensitive than fungi and require a water potential (Mpa) of minus 2.8 or less for growth in wood. This is significantly above the moisture content of "dry" wood stored in rooms, so that properly dried wood does not offer bacteria enough water for growth and multiplication (Bavendamm 1974, Schmidt 1996).

But the withdrawal of available water cannot be the only reason for the effects observed. Although all woods dried rapidly, pine wood showed a much higher reduction of the titer of viable bacteria than other woods. Also the reduction of the bacterial titer on plastic boards did not correlate with the drying process. This supports the hypothesis that apart from the hygroscopic effect wood extractives (e.g. tannins) are also responsible for the detected reduction of bacteria. Tannins are natural wood preservatives found in high concentration in the bark and the wood of some tree species (Fengel and Grosser 1975). Wood particularly rich in extractives such as pine, larch (Schönwälder 1999) and oak (Ak et al. 1994a and b, Rödel et al. 1994) also possesses excellent antibacterial characteristics.

It could be shown that the recovery and the microbiological results are strongly dependent on the sampling strategy. The ability to detect bacteria using agar contact plates is limited due to the hygroscopic properties and the porous structure of wood. The bacteria are fixed to the wood and the efficiency of microbial recovery depends on the mechanical treatment involved. Other authors stated that recovery of bacteria by rinsing the surface is more sensitive than the recovery by agar contact plates, agar sausages and swabbing (Gilbert 1970, Kampelmacher et al. 1971, Niskanen and Pohja 1977). It has been shown that destructive methods like scraping give the best bacteria recovery rates for wood and a partial revival of sublethally damaged cells takes place (Kampelmacher et al. 1971, Ruosch 1981, Rödel et al. 1994). Therefore, the present study also investigated wood shavings from different depths. With the help of the extraction buffer, the mechanical handling of the wood shavings displaced the attached bacteria completely from the wood and it was possible to quantify the CFU. Although the detection of bacteria on and in wood is more sensitive with destructive methods, the results of both sampling methods correlated and permitted the same conclusions regarding the behaviour of the bacteria on the surface and the inner wood structure. For this reason we decided to use agar contact plates in the present study, because this measuring method is easy to perform, not time-consuming and suitable for a screening of hygienic conditions on different surfaces.

The selection of a suitable method for the detection of bacteria on different surfaces depends on the detection purpose, the detection sensitivity required and the nature of the surface, because the percentage recovery of bacteria is a function of the nature of the surface, i.e. recovery from stainless steel and plastic is easier and higher than from wood. There is a multitude of measuring methods in the area of hygiene. On the one hand microbiological methods such as agar contact plates, agar sausages, dip slides, swabbing, rinsing and a prior scraping have been used (Gilbert 1970, Kampelmacher et al. 1971, Niskanen and Pohja 1977, Ruosch 1981, Schmid 1989, Ak et al. 1994a and b, Rödel et al. 1994, Park and Cliver 1996, Gibson et al. 1999, Gehrig et al. 2000). On the other hand non-microbiological methods like ATP luminometry and protein estimates have assumed a leading position in hygienic monitoring (Davidson et al. 1999, Corbitt et al. 2000). However, all methods have advantages and disadvantages (Griffith et al. 1997) and as yet, no standard protocol has been adopted by industry for surface hygiene monitoring (Davidson et al. 1999).

The rapid decrease of CFU recovered from wood was interpreted by other authors (Lorentzen et al. 2000) as an indication that wood is unhygienic because the germs are difficult to remove. However, using cultivation-independent techniques such as direct DNA extraction followed by Southern blot hybdridization or PCR we could unequivocally demonstrate a decrease of bacterial DNA indicating that the bacteria are killed due to the interactions with wood properties (Schönwälder, in prep.).

It must be concluded that the hygienic characteristics of wood cannot be generalized, since different wood types possess different hygienic characteristics. For example, bacteria can survive on spruce, beech and poplar for long periods of time without damage, whereas they are efficiently killed on pine. In summary, only pine wood was able to regain its initial hygienic status after germ infestation within a fewer hours, so that the wood was germ-free at the surface and within. For this reason pine wood possesses clear hygienic advantages compared to other woods and plastic. It has thus been shown to be a natural raw material with antibacterial characteristics, able to kill applied bacteria by active substances (extractives) and regain its hygienic status after contamination. Completely new fields of wood use are conceivable in the future for pine wood as a naturally antibacterial material with strongly germ-reducing characteristics.

The results of the study necessitate a careful reappraisal of existing guidelines for the application of wood products in the foodstuff sector. In the past, wood has apparently unjustly been generally condemned to be an unhygienic

raw material. Through a careful selection of wood, appropriate handling and using new innovative cleaning strategies (mechanical treatment, microwave decontamination (Park and Cliver 1996)) wood products can certainly contribute to the improvement of the hygienic situation within many areas.

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