

Investigations on the hygienic properties of wood

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Presentation

1. Interactions between bacteria and wood chips
2. Survival of bacteria on and in wooden boards
3. Preliminary test of wooden pallets in practice
4. Characterization of the antibacterial properties of wood



Experimental approach

Sample preparation
(Inoculation of wood chips with *Escherichia coli* pIE639 or *Enterococcus faecium*)

Incubation

Sampling

**Recovery of bacteria by treatment
in a Stomacher-blender**

Cultivation on nutrient agar

Determination of CFU

Direct DNA extraction

Purification/ PCR amplification

Agarose gel electrophoresis

Southern blot

Hybridization



Experimental approach

Samples (wood chips)



Stomacher treatment



Inoculation

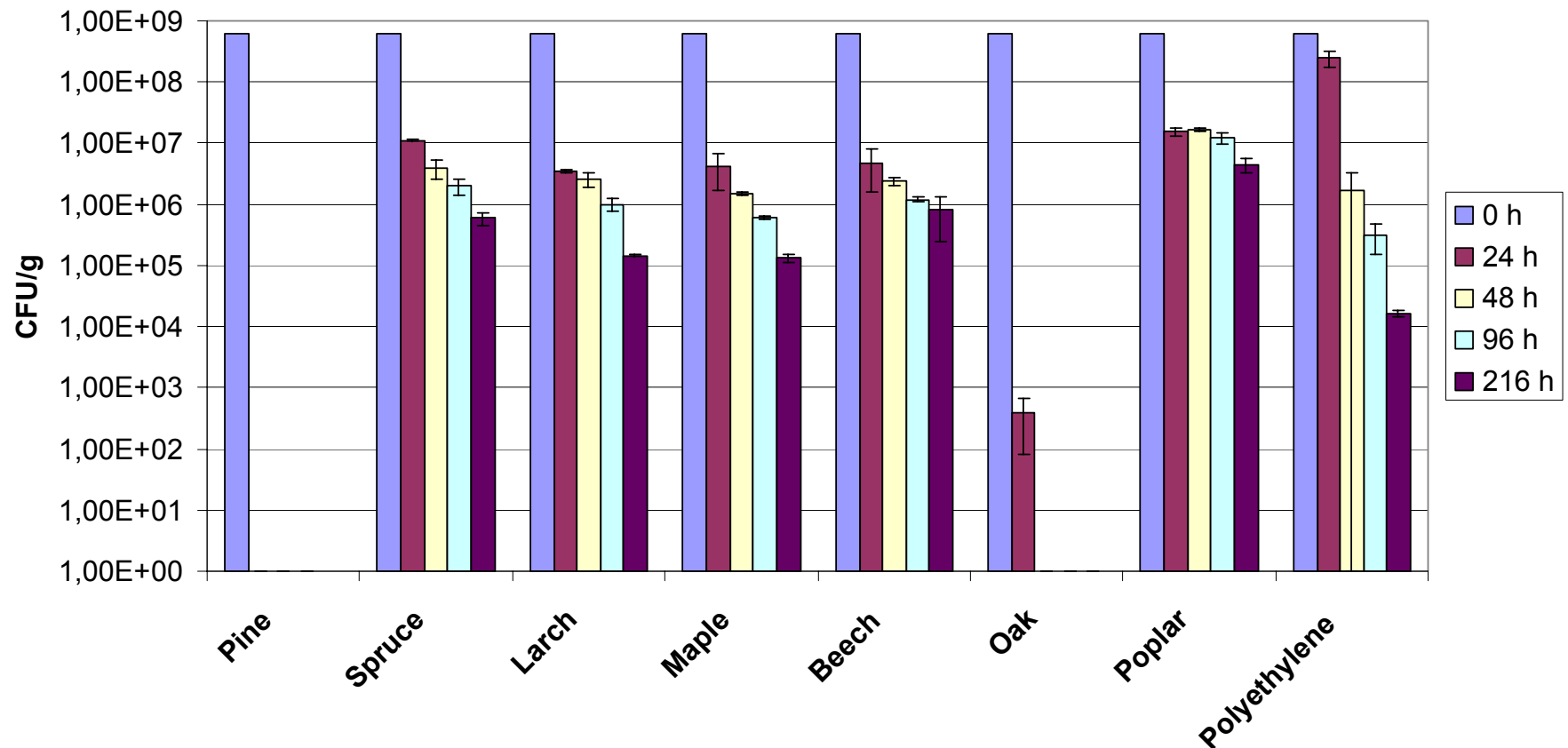


**crude DNA directly extracted
from wood chips**

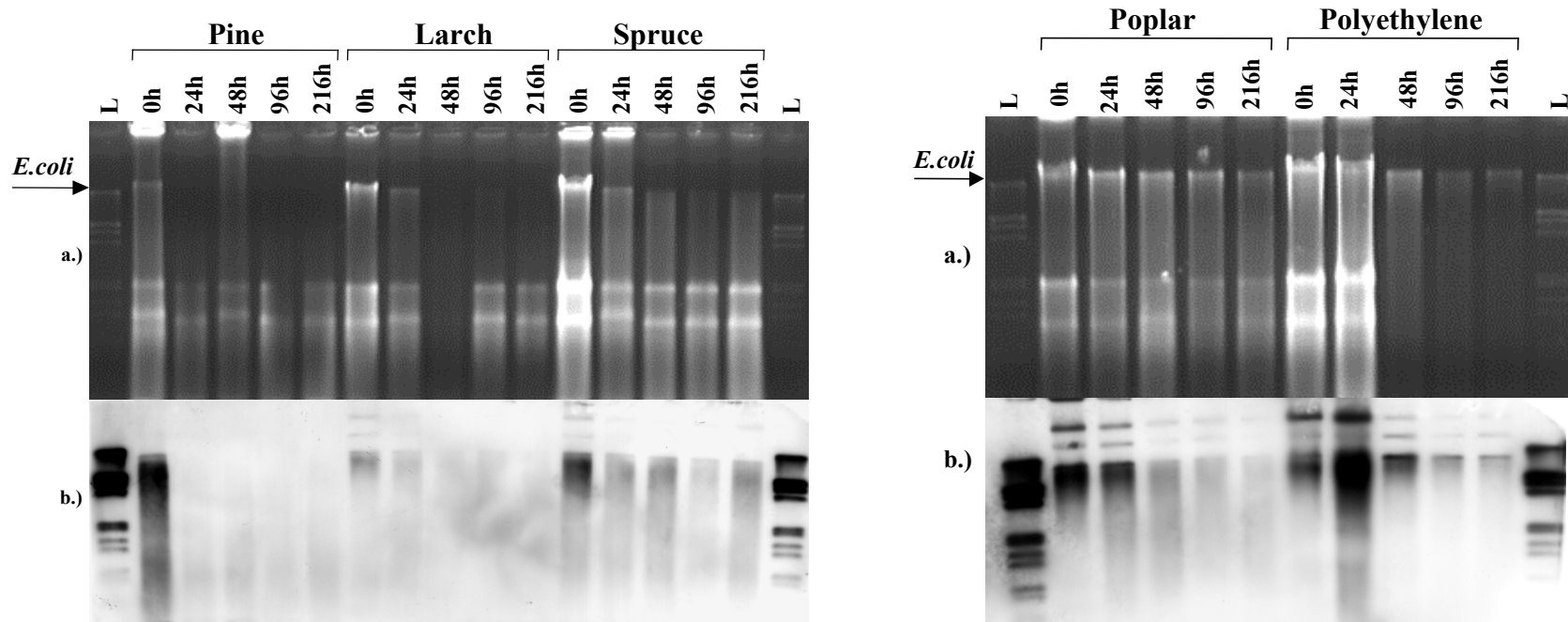


Interactions between bacteria and wood chips

Inoculation of different wood varieties and plastic with
Escherichia coli pIE639



Interactions between bacteria and wood chips

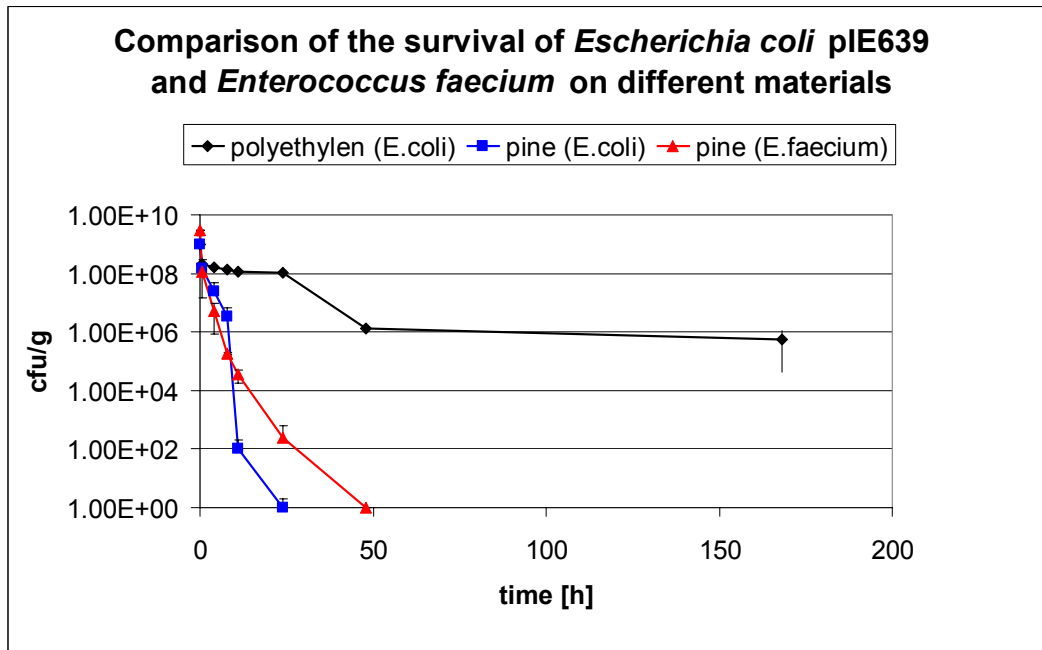


a) crude DNA of *E. coli* pIE639 directly extracted from wood chips on agarose gel

b) Southern blot after hybridization with *sat3*-probe specific for *E. coli* pIE639



Interactions between bacteria and wood chips



Inoculum: $1 \cdot 10^9$ cfu/g, RT: 21°C, Relative atmospheric humidity: 55 %



Comparison of the survival of *Escherichia coli* pIE639 on pine wood (right) and polyethylene (left) after 24 h



Summary - Interactions between bacteria and wood chips

- Antibacterial effects were found, especially for Pinaceae.
- Recovery of the inoculated strain from pine sawdust was generally lower than that of the other wood varieties.
- Tested bacteria survived longest on plastic.
- On the tested wood Gram-positive bacteria survived longer than Gram-negative bacteria.
- Survival of bacteria on wood depended on wood variety, moisture content of the wood, temperature and humidity of the environment and on the characteristics and density of the inoculated strain.
- The molecular detection showed that the decrease of CFU numbers correlated with the decrease of DNA on wood.
- Some bacteria, especially *Enterobacteriaceae*, are more sensitive to wood ingredients than others .
- Wood ingredients from different wood types influence different bacterial species.



Survival of bacteria on and in wooden boards

Experimental approach

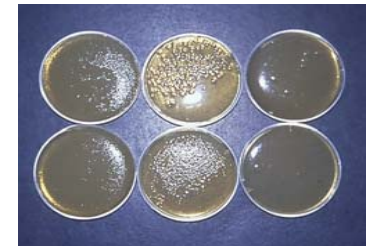
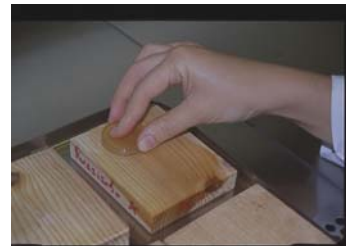
Boards: pine, spruce, larch, poplar, beech, polyethylene

Bacteria: *Escherichia coli* pIE639
Enterococcus faecium

Inoculation: The inoculum was deposited directly on the board surface and spread with the pipette.
The wooden boards were soaked in the inoculum for 15 min.

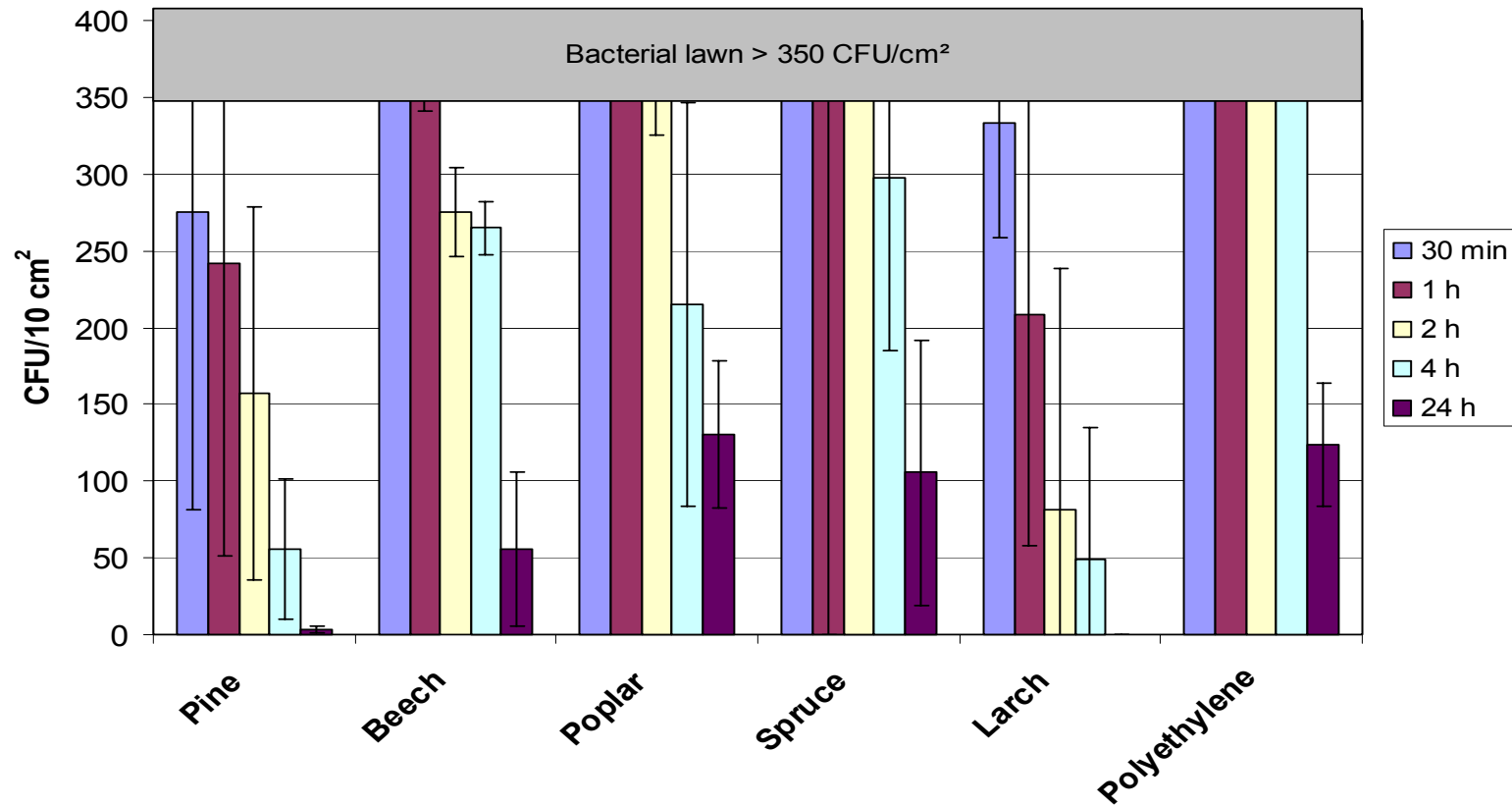


Sampling: agar contact plates
measuring of wood shavings



Survival of bacteria on wooden boards

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

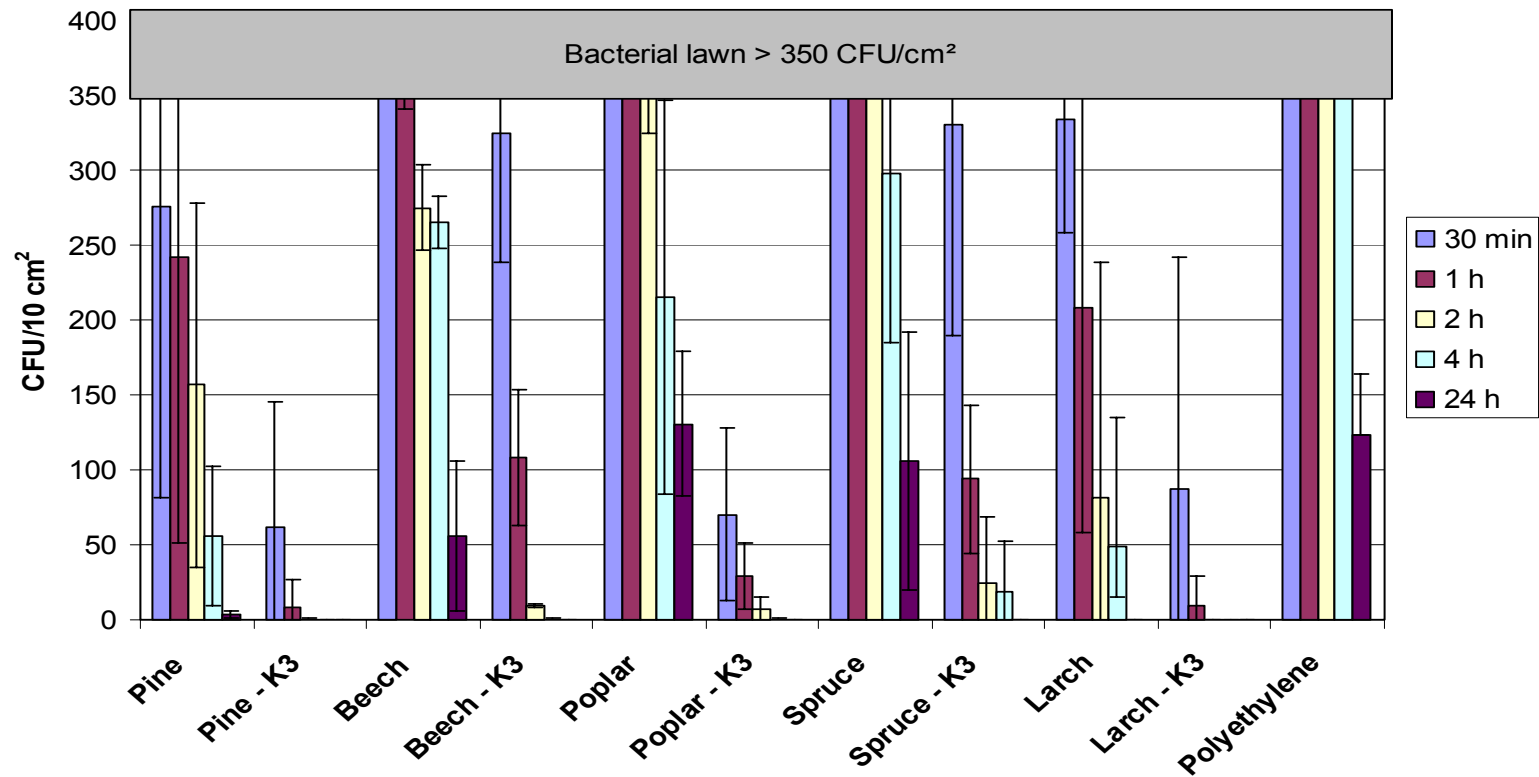


Recovery via agar contact plates, Initial inoculum size: 1×10^6 CFU/cm², RT: 21 °C



Survival of bacteria on wooden boards

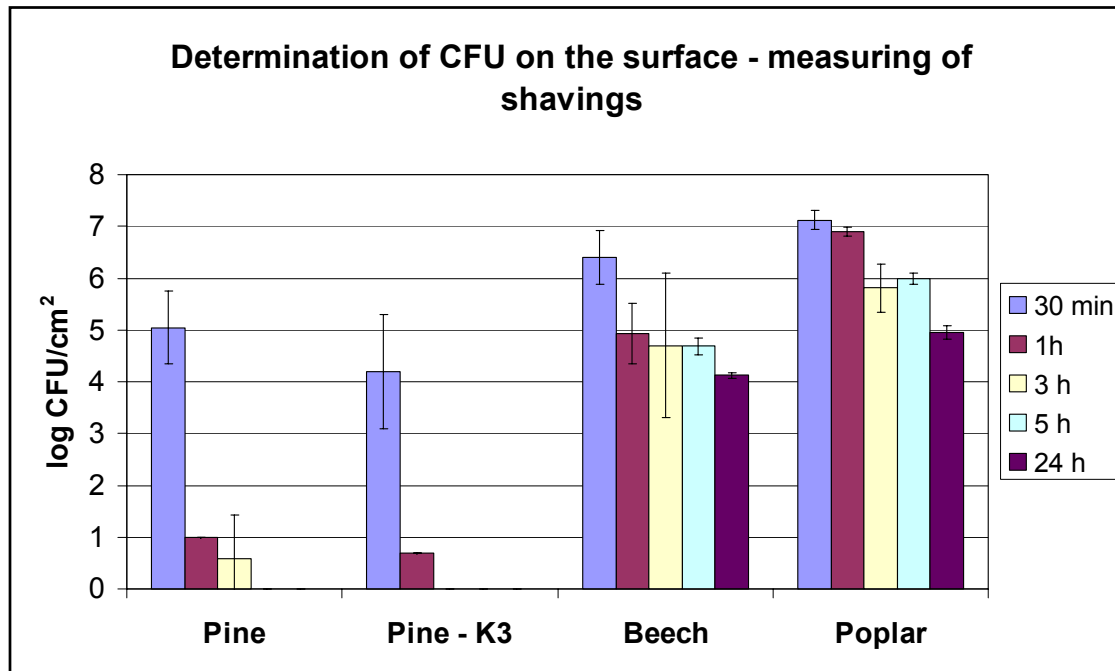
Survival of *E. coli* pIE639 on new wooden and plastic boards before and after treatment K3



Recovery via agar contact plates, Initial inoculum size: 1×10^6 CFU/cm², RT: 21 °C



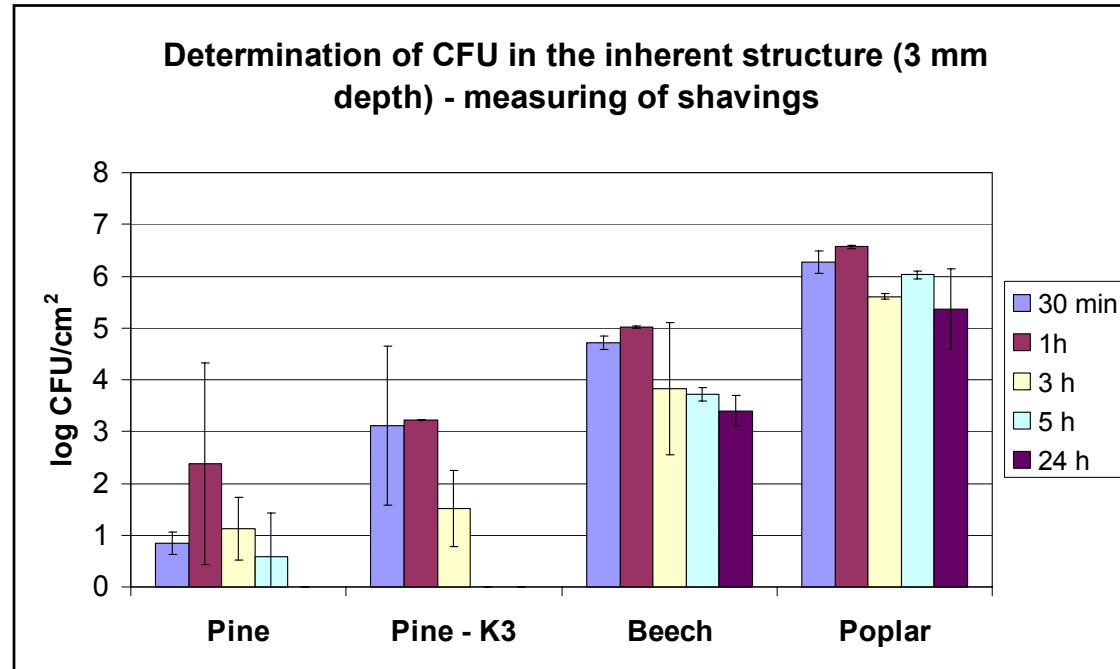
Survival of bacteria on and in wooden boards



Initial inoculum size: 5×10^6 CFU/cm² *E.coli* pIE639, RT: 21 °C



Survival of bacteria on and in wooden boards

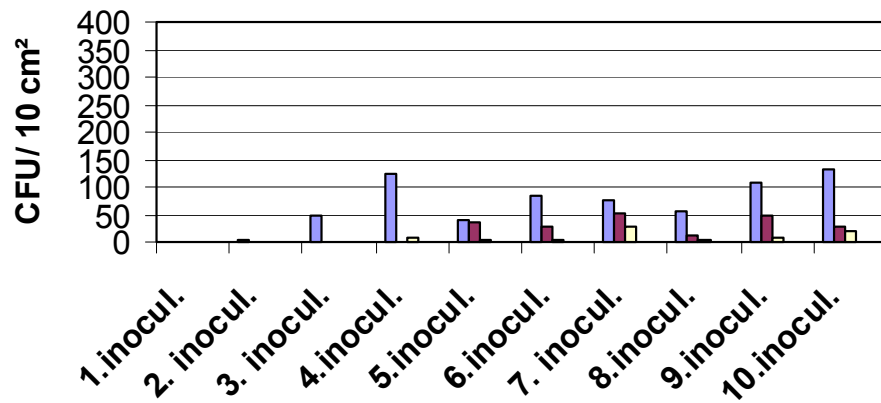


Initial inoculum size: 5×10^6 CFU/cm² *E. coli* pIE639, RT: 21 °C

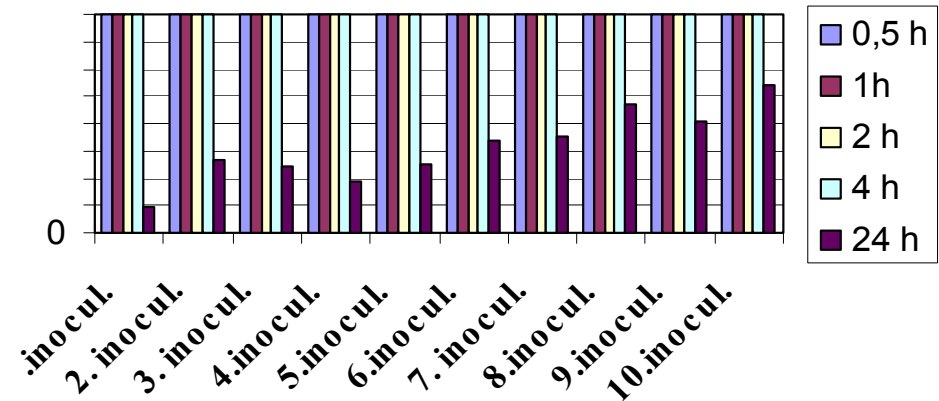


Survival of bacteria on wooden boards

Multiple inoculation of pine wood boards - K3 with
E. coli pIE 639



Multiple inoculation of plastic boards with
E. coli pIE 639

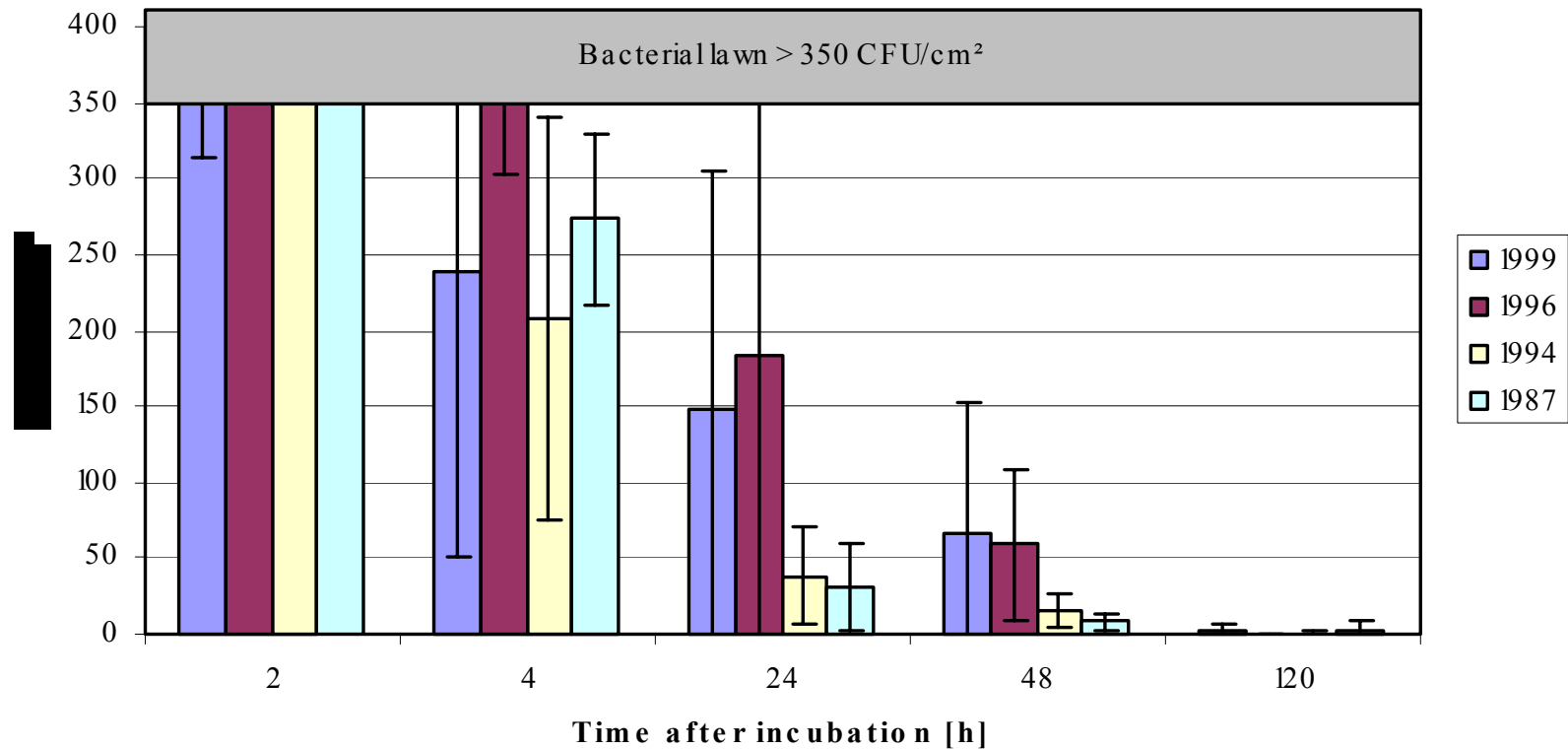


Recovery via agar contact plates, Initial inoculum size: 1×10^6 CFU/cm²,
Inoculation each 3 – 5 days, RT: 21 °C



Survival of bacteria on wooden boards

Survival of *E. coli* pIE639 on new and used wooden boards

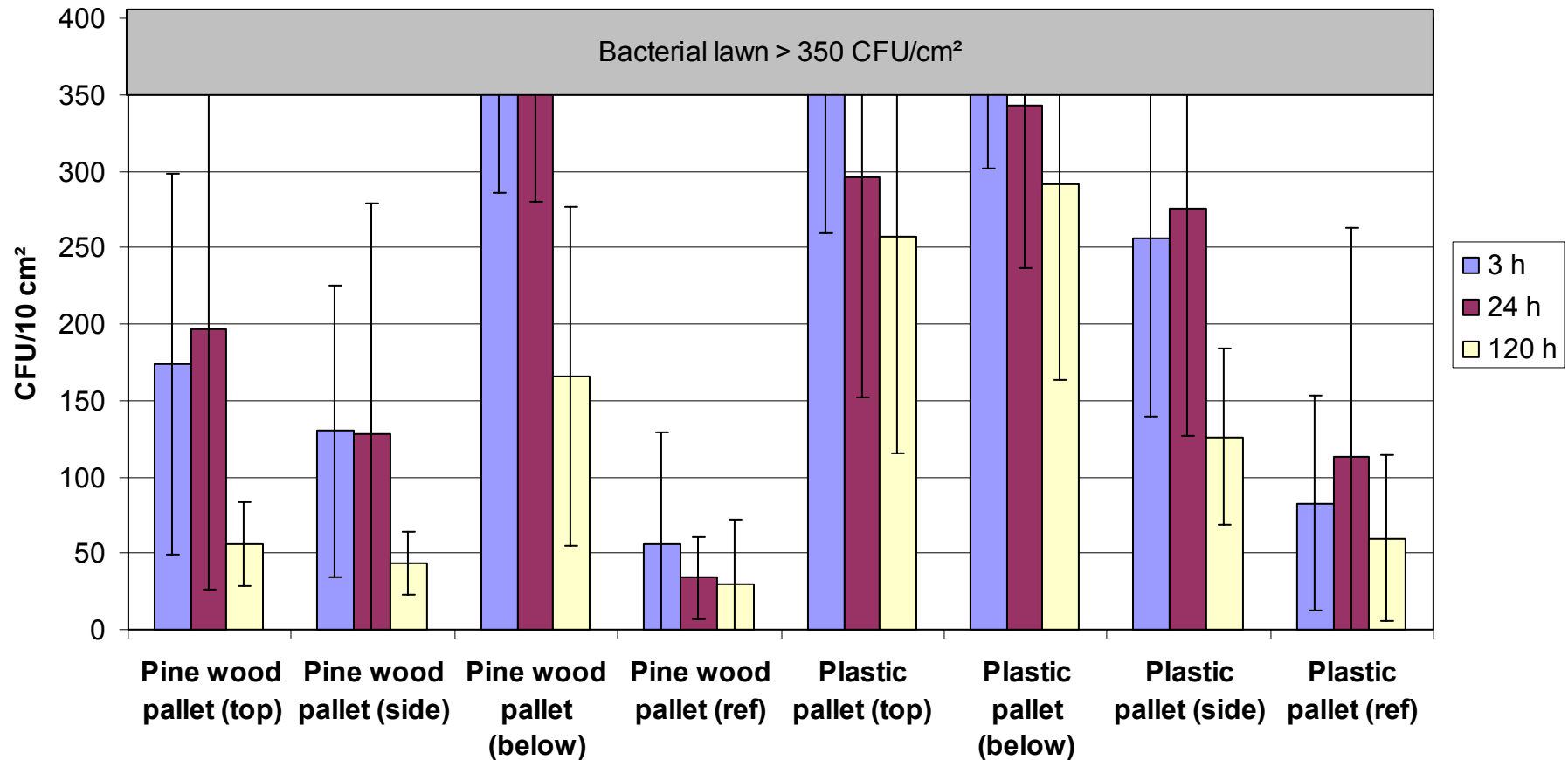


Recovery via agar contact plates, Initial inoculum size: 1×10^8 CFU/cm², RT: 21 °C



Survival of bacteria on wooden boards

Pallets in use



Summary – Survival of bacteria on and in wooden boards

- The antibacterial effect of pine could be confirmed.
- The antibacterial effect of pine was stable during a number of contaminations.
- Bacterial survival and hygienic properties of wood depended on a number of factors such as wood species, the initial inoculum size and the characteristic of the inoculated strain.
- There was a significant difference in bacterial recoveries from pine wood and plastic. On pine wood the bacteria had the lowest survival compared with other woods (spruce, poplar, beech) and plastic.
- On the tested wood Gram-positive bacteria survived longer than Gram-negative bacteria.
- The antibacterial effect and the effectiveness of the germ reduction was comparable on new and used pine wood boards.
- The decrease of CFU on the surface of pine wood boards correlated with the decrease of CFU in the inner structure of pine wood.



Characterization of the antibacterial properties of wood

Reasons for the antibacterial effect may be:

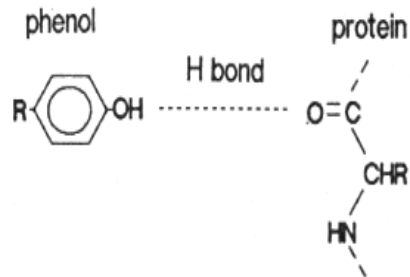
1. That the porous and hygroscopic wood take the moisture away from the bacteria, which they need to survive (desiccation). - **physical reason**
2. Wood ingredients (polyphenols or tannins) cause the antibacterial effect. - **chemical reason**



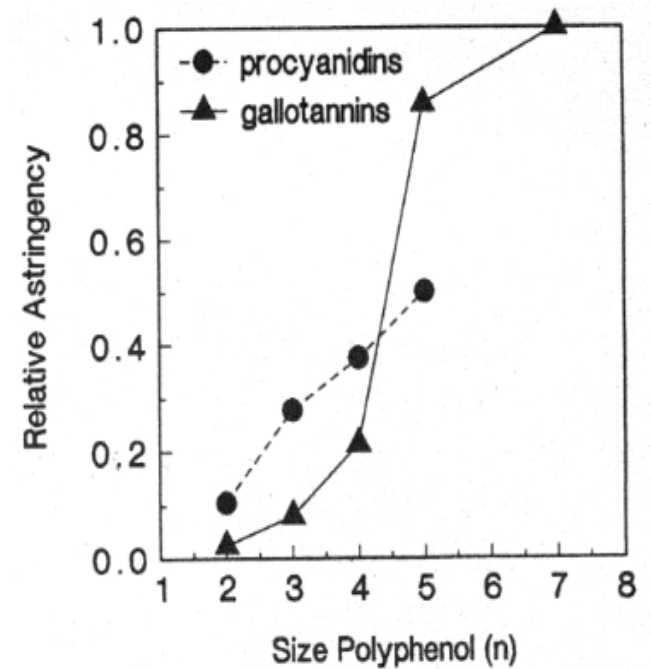
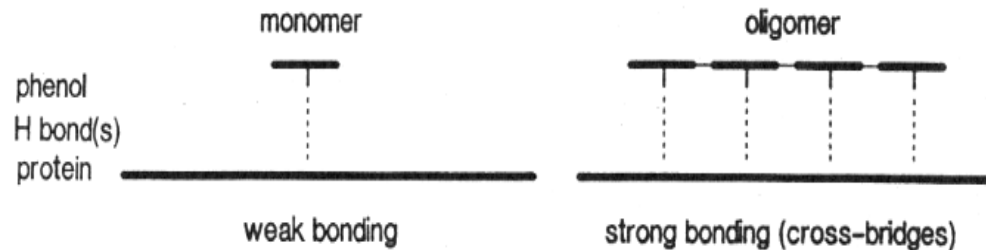
Mechanisms of tannin toxicity

1. Astringency: enzyme inhibition and substrate deprivation

A. Hydrogen Bond



B. Strength Hydrogen Bonds

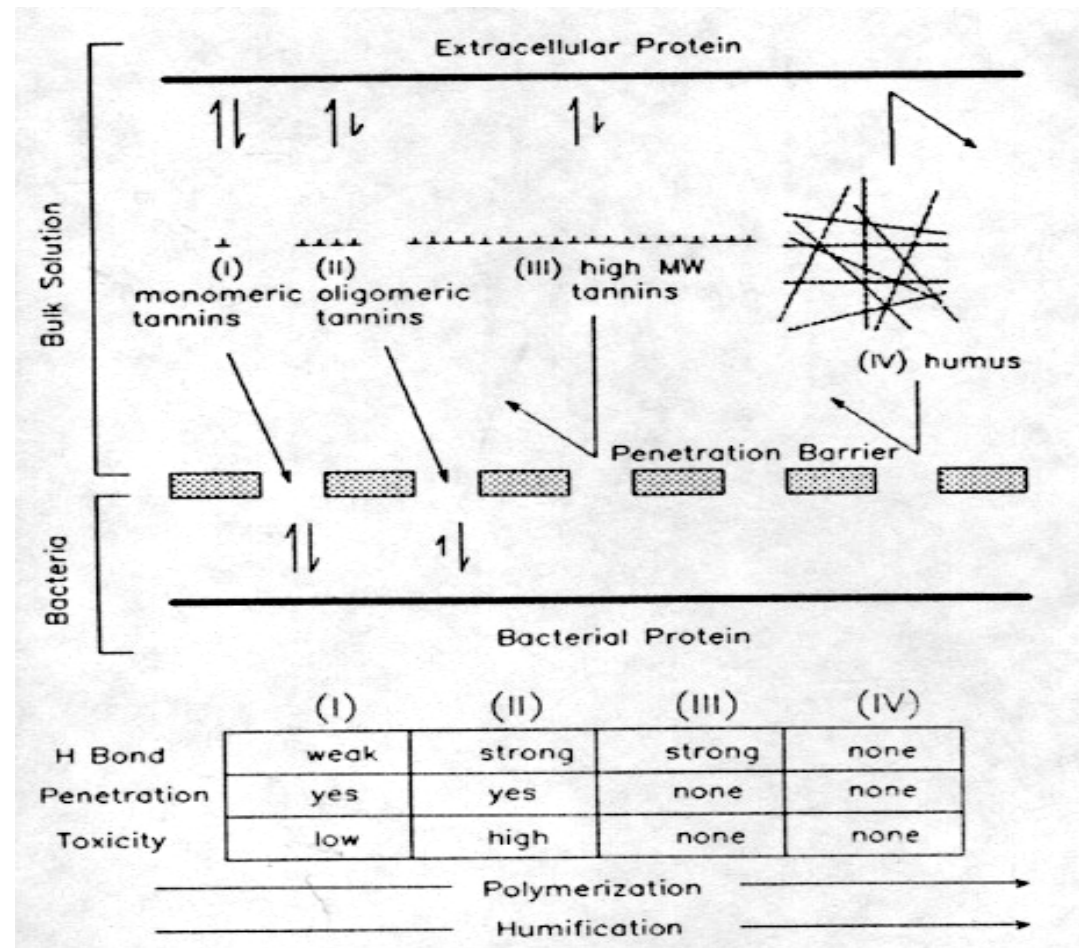


Field und Lettinga, 1992



Mechanisms of tannin toxicity

2. Action on membranes

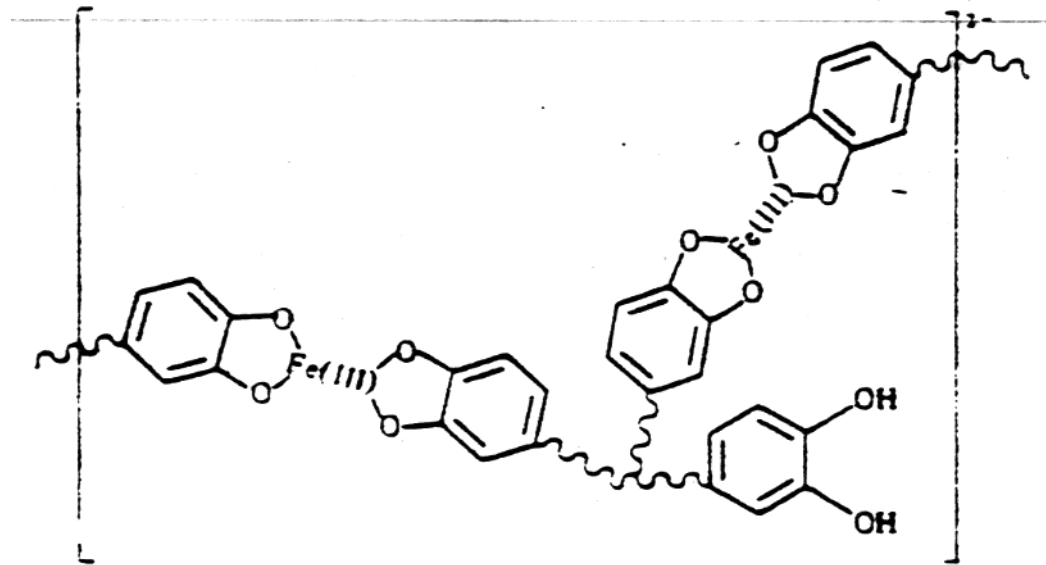


Scalbert, 1991



Mechanisms of tannin toxicity

2. Metal ions deprivation



Scalbert, 1991

Insoluble tannin-ferric complexes

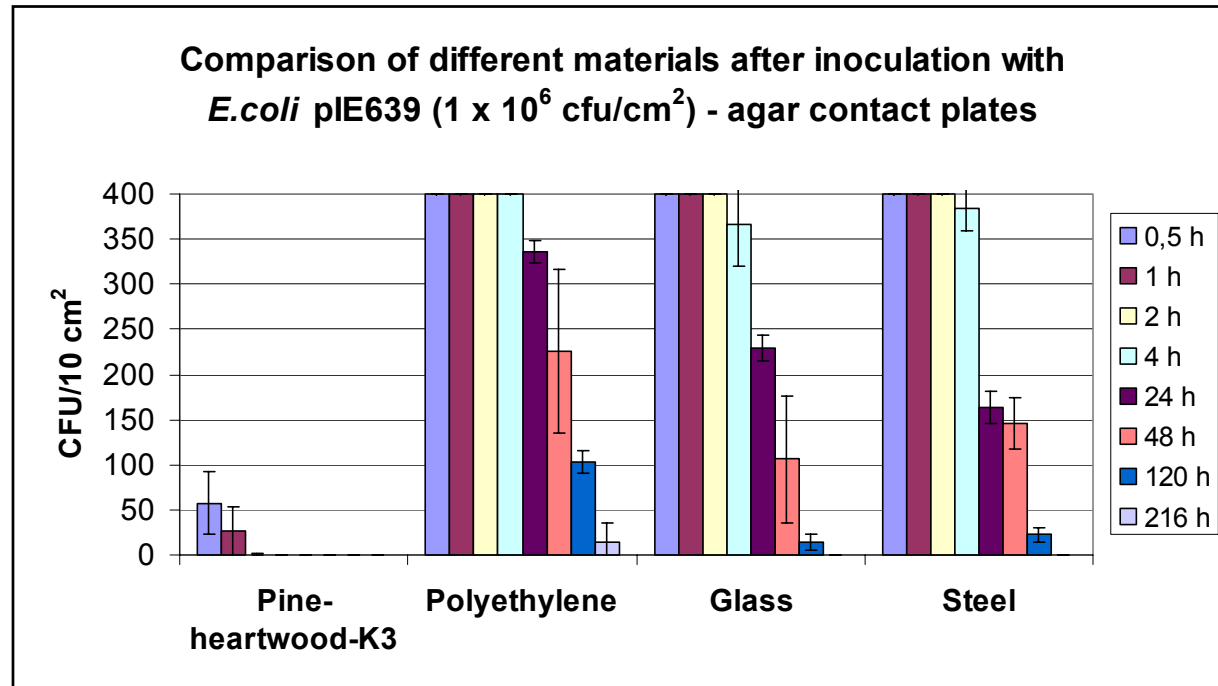


Microbial defences against tannins

1. Secretion of tannin-binding polymers
2. Tannin-resistant enzymes
3. Tannin oxidation
4. Siderophores
5. Tannin biodegradation



Conclusions



- Different wood varieties have different hygienic characteristics!
- Pine wood – a natural raw material with antibacterial properties.
- Pine wood possesses clear hygienic advantages opposite other woods and plastic.



Acknowledgments

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