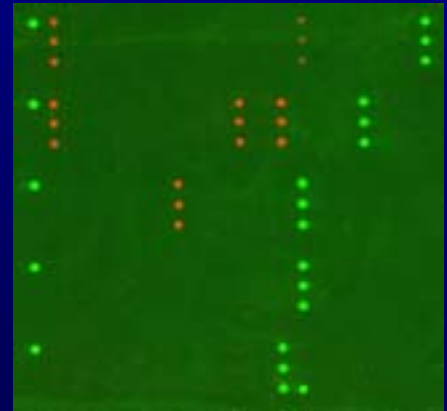


Detection of microbes in compost using an oligonucleotide microarray

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Aims of the project

- Design and development of a compost-microarray for the detection of microorganisms
 - *Pathogens
 - *Degrading organisms

Introduction

- Composting is an aerobic process by which diverse organic materials are degraded by diverse set of microorganisms
- Naturally occurring and environmentally sound process
- Gaining worldwide popularity and is of economic importance



Draft EU Biowaste Directive

"By the end of 2004 a directive on compost and other biowaste will be prepared with the aim to control potential contamination and to encourage the use of certified compost."

Introduction (cont.)

- Different applications of the final product
- A high microbial diversity is essential for an efficient and satisfactory composting process
- Potential survival of pathogens presents a considerable risk
- Composting processes must eliminate pathogens



Introduction (cont.)

- Microbial diversity of compost not well characterised
- Molecular biological methods
 - greater advances
- Molecular techniques based on 16S rRNA sequences used in microbial ecology studies today
 - Discovery of new microorganisms



Microarrays

- Recent advance in molecular technologies
- Allow the parallel detection of up to several thousand microbial strains, species, genera or higher taxonomic groups in a single experiment
- Probe design is critical



Microarray Process

- Selection of probe sequences
- Spotting of DNA-sequences on solid surfaces
- Extraction of sample DNA
- Amplification, labeling and hybridisation
- Detection of bound target DNA via scanning and analysis



Probe Design

- All probes need to be used under same set of hybridisation conditions
- Specificity of probe
- Probe melting temperatures
- Secondary structure



Selection of probe sequences

- Pathogens and important compost organisms
- Design of oligonucleotide probes using ARB
- Screening of public databases
 - GenBank NCBI
 - RDP
- Generunner used for secondary structure determinations



Target organisms for which probes were designed for microarray

Acidovorax avenae avenae/cattleyae/citrulli
Ralstonia solanacearum/Pseudomonas syzygii/Blood disease bacterium

Plant bacteria

Gluconacetobacter diazotrophicus
Gluconacetobacter liquefaciens
Xylella fastidiosa

Plant pathogens

Streptococcus pyogenes
Haemophilus influenzae
Staphylococcus aureus

Human and animal pathogens

Aeromonas hydrophila

Degrading bacteria

Arthrobacter sp.

Thermophilic bacteria

Thermomonospora curvata

Pseudomonas aeruginosa

EUB 338

EUB 338II

EUB 338III

UNIV 1389

Archaea

Spotting of probes

- 16S rDNA oligonucleotide-probes spotted in triplicate (LAMBDA G.m.b.H., Freistadt)
- Aldehyde glass-slides
- 12T spacer used at 5' end of probes



Preparation of labeled target DNA

- Extraction of DNA from cultures or compost
- PCR amplification
 - Cy3 or Cy5-labeled 8f primer
 - Phosphate-labeled 1492r primer
- Digestion of phosphate-labeled strand
 - Single stranded labeled product



Hybridisation

Optimisation of

- Target DNA amount
- Amount of spotted probe
- Presence/absence of T-spacer
- Buffer conditions
- Temperature & time



Scanning and image analysis

- Scanning using GenePix 4000B array scanner (Axon Instruments Inc., USA)
- GenePix Pro 4.0

$SNR = (F532_{Median} - B532) / B532_{SD2}$

$SNR \geq 2$ considered above the threshold

Microarray validation

- Specificity tests with pure culture strains
- Sensitivity tests

dilution series with pure cultures

compost spiking experiments

different ratios of pure culture cells

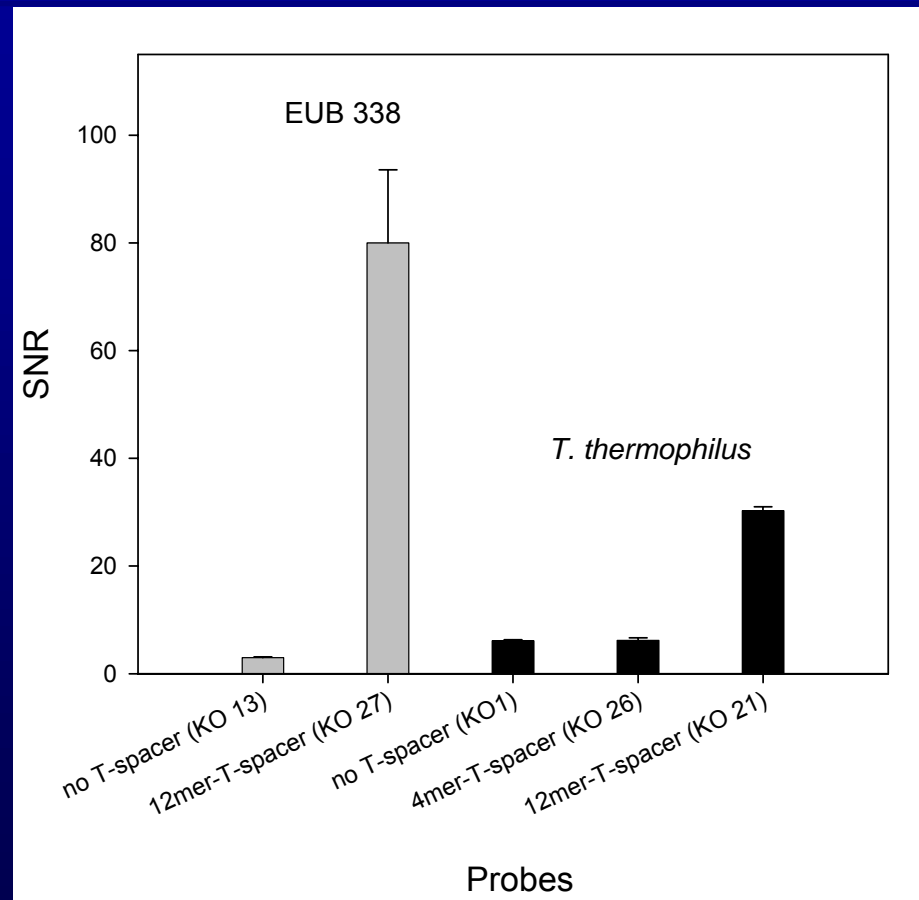


Results

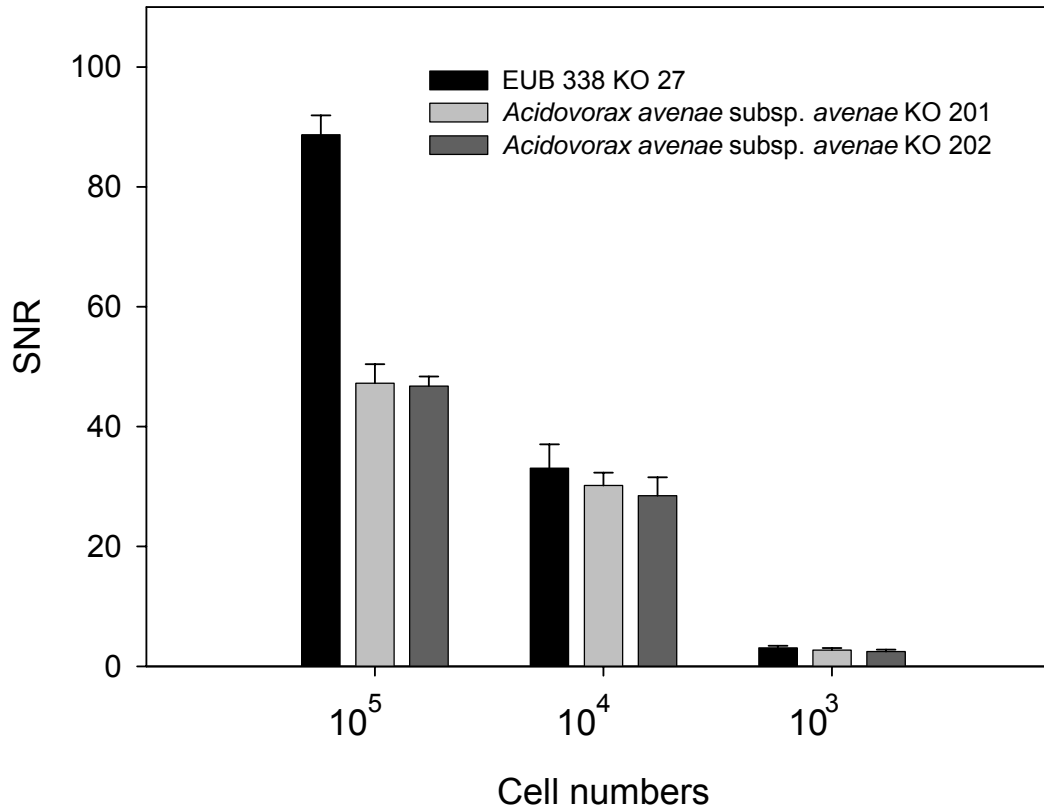
- Optimised hybridisation conditions of 52 °C, 3-5 h, 5 X SSC
- Most probes specific
- Spotting of higher concentrations of probe
 - Better signals not obtained
- Higher concentrations of target DNA
 - Better signals not obtained
- T-spacer use
 - Better signals obtained



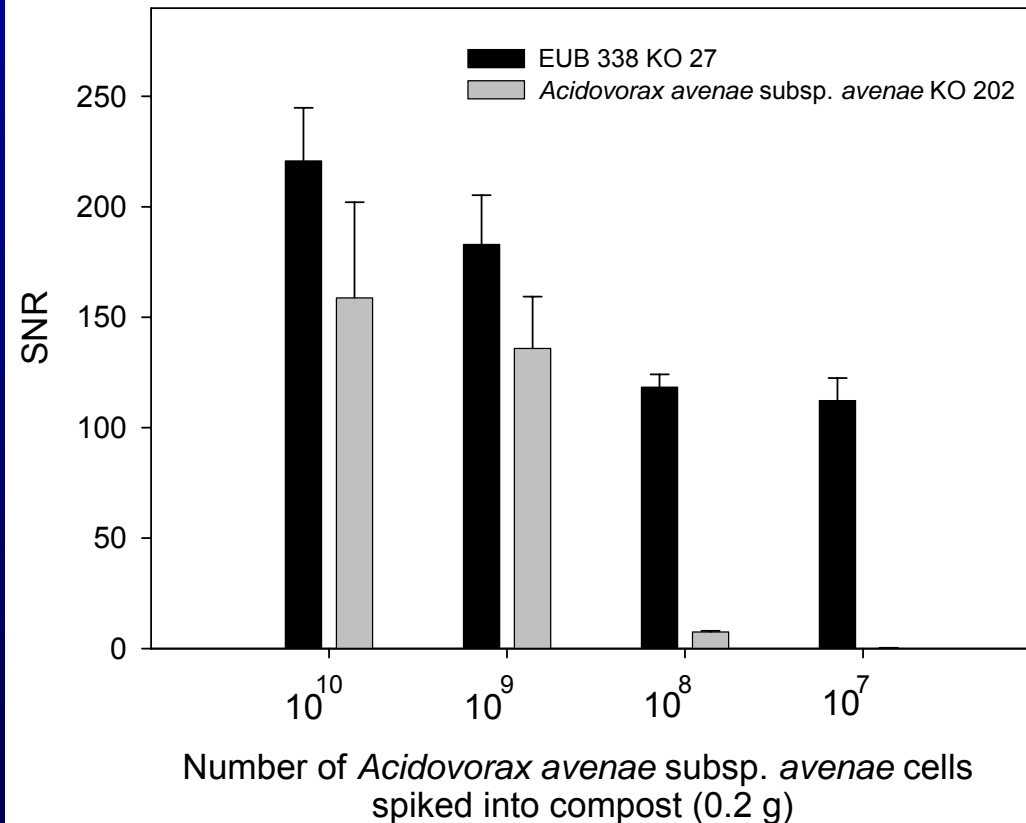
Impact of poly T-spacers on the hybridisation signals



Sensitivity tests

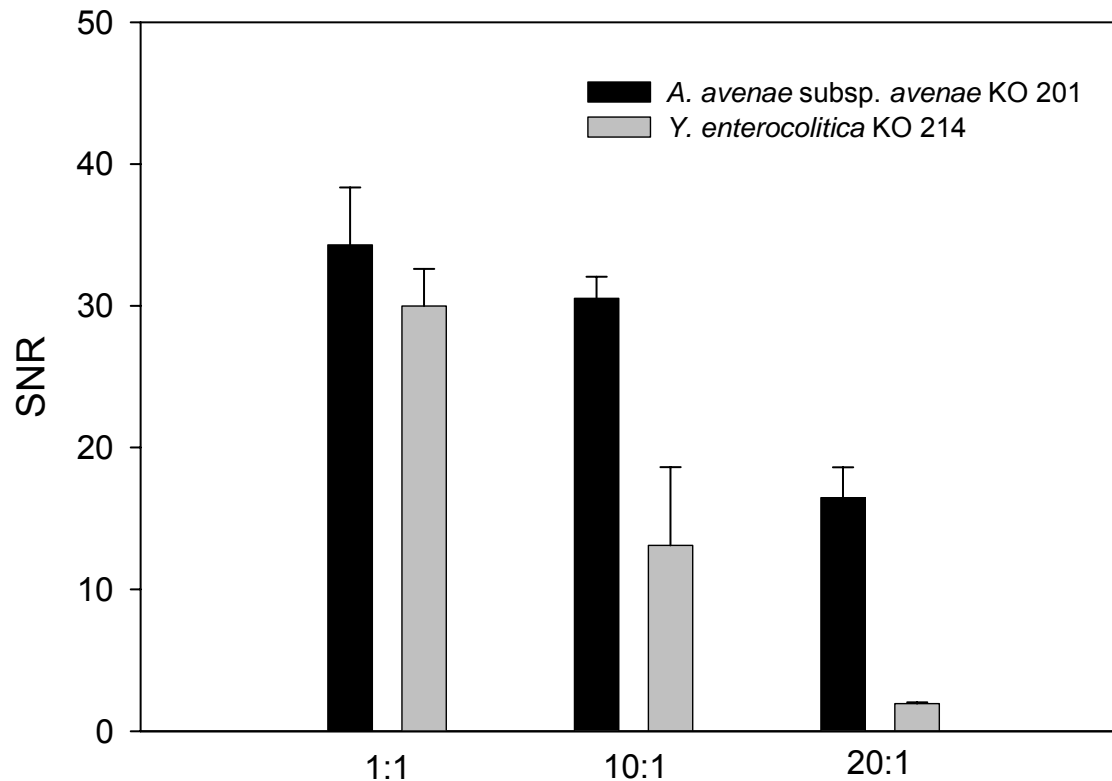


Sensitivity tests



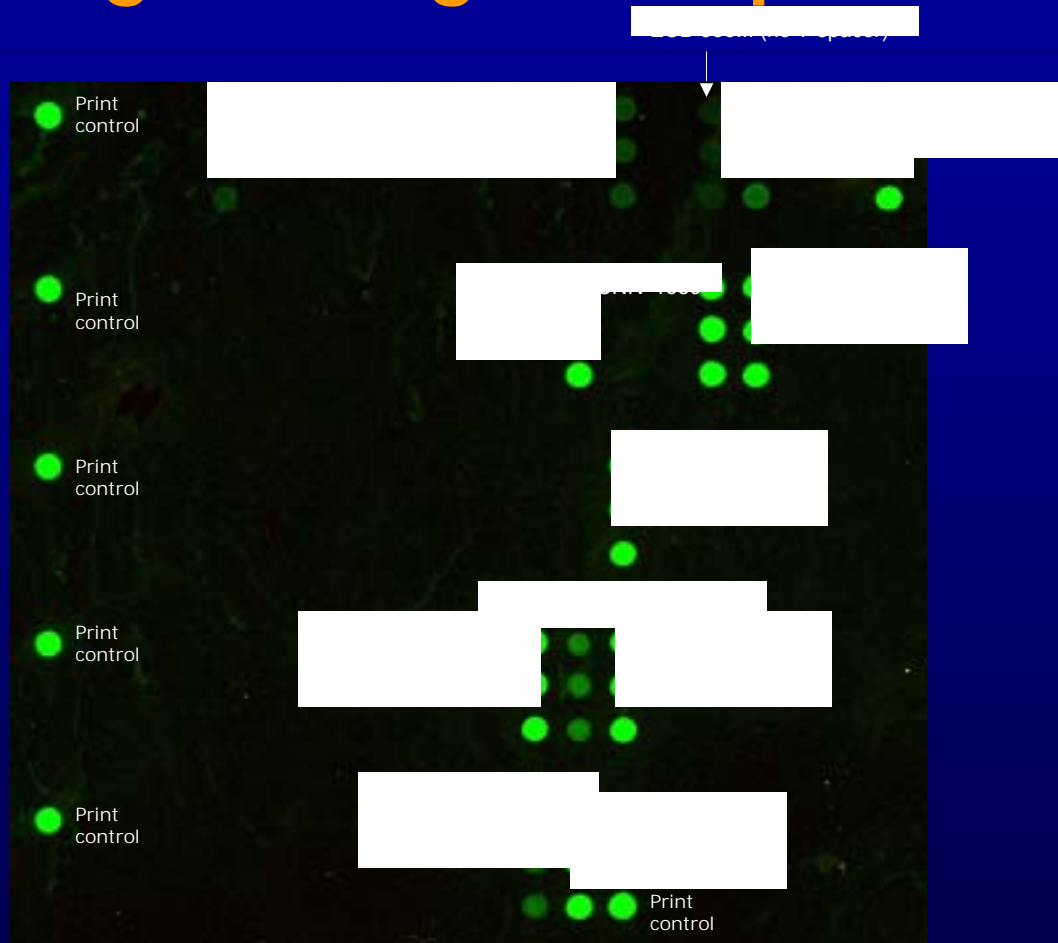
1 μ l of a 1/10 dilution of extracted DNA (100 μ l) used subjected to PCR

Sensitivity tests



Ratios of *Acidovorax avenae* subsp. *avenae* to *Yersinia enterocolitica*

Microarray application with sewage sludge compost



Future work

- Design and spotting of further probes, and validation of new probes
- PCR confirmation of organisms detected in composts
- Application of array to different compost samples



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FWF Der Wissenschaftsfonds.