Diagnostic Microarray in Parallel Detection Of Potato Bacterial Pathogens: Hype or Opportunity

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Disease Diagnostics: dynamic and evolving. (Valid saying after 80 years)

"We need better methods for diagnosis: none of the methods given are to be considered as' ' standardized' to think of them in such a way would put an end to efforts of improvement. They are useful only until better procedures can be developed"

(Riker & Riker, 1936, Introduction to research in plant diseases- a guide to the principles and practices for studying various plant disease problems)

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Diagnostic challenges: One pathogen – one disease in plants is RARE

Mixed infections with different pathogens are common in plants, which challenges pathogen detections due to increased analysis cost and time.

Potato Pathology

Potato is known to suffer from about 160 diseases and disorders

- 50 fungal
- 40 viral
- 10 bacterial and
- disorders of unknown origin

Diagnostic Technology: Demands

- Technical Demands
  - Specificity
  - Sensitivity
  - Detection of only Viable pathogen
  - Multiplexing
  - Quantification
  - Robustness
  - Validation
- Economic Demands
  - Short diagnosis time (Speed)
  - High-throughput capability

Singleplex vs Multiplex

From technological and economic view point methods which allow simultaneous detection of multiple pathogens in a single test are of particular interest for use in end user diagnostic laboratories. The lack of methodology, however, has been the reasons for developing singleplex tests or tests designed for analysis of only few target pathogens

Diagnostic DNA Microaaray

DNA microarray is a technology which, in theory, offers a platform for an unlimited multiplexing capability. It is used in conjunction with bioinformatics and statistical data analysis. Microarray technology is rapidly advancing with increased number of potential applications.

Diagnostic Microarray

Originally developed for investigations of gene expression and analysis of single nucleotide polymorphism (SNP). Recent efforts have advanced beyond these investigations to applications in microbial diagnostics, especially focused to parallel detection of pathogens from plant, food and water. However, the progress along this line has been very slow.

Components of typical working sample

Photo: Degefu, Luke, Oulu

Included are,

- Adhering soil
- Potato tissue
- Target Pathogen

Current knowledge of Detection Microarrrays does not represent the actual working sample

- Most diagnostic microarray reports to date are based on DNA isolated from pure cultures of bacteria.
- Studies involving the evaluation of the method within the context of complex samples, where the target pathogen is often present in limited quantity in the sample matrix against a background of many other microorganisms are very limited.

DNA Microarray analysis of Potato bacterial pathogens

Aim:

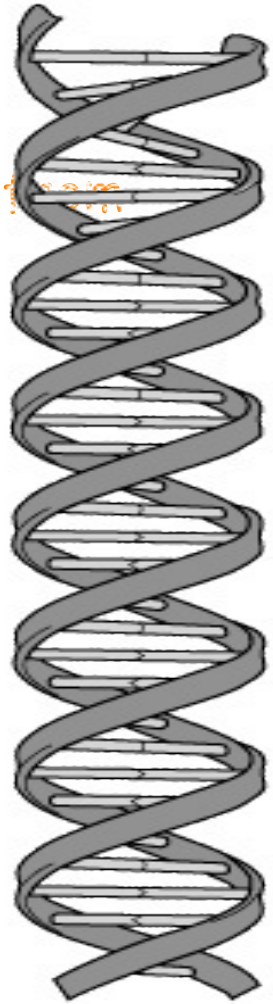
The aim of this study was to investigate the analytical specificity and sensitivity of the microarray technology for detecting bacterial pathogens of potato directly from tubers!!! and/ or conditions emulating (imitating) the natural tuber samples to evaluate its suitability for the detection in routine seed certification scheme.

- NO additional work flow such as pre- hybridization PCR amplification of target is included in the study

Investigations addressing the issues at hand

- Microarray analysis of DNA Cocktail imitating the tuber sample
- Microarray analysis of DNA directly isolated from infected potato tissue
- Microarray analysis of DNA from enriched culture (potato peel extract from latently infected tuber)

Experiment 1:Microarray on DNA cocktail (Purified DNA three sources mixed in 1:1 ratio



- DNA from SOIL (soil microbes)
- DNA from pure culture of the TARGET BACTERIA
- DNA from POTATO

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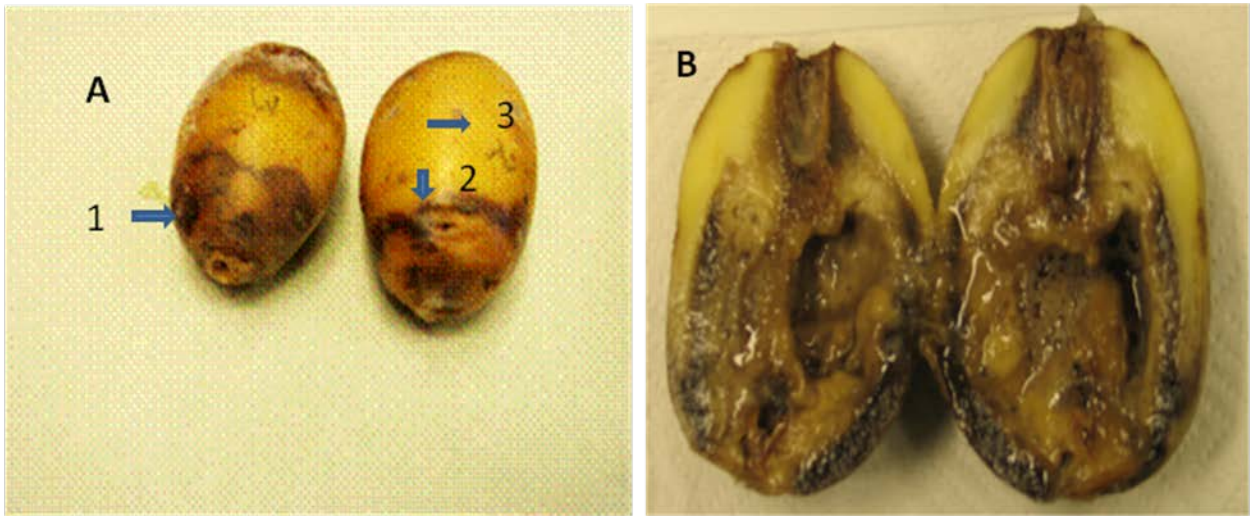
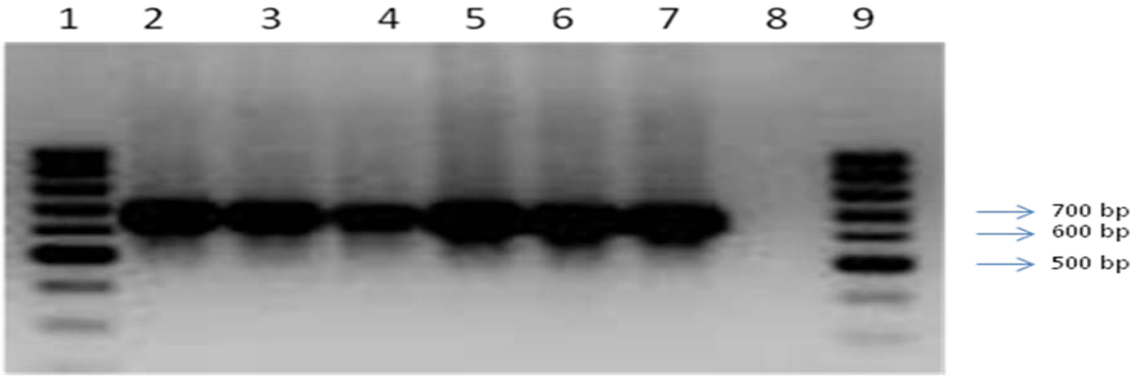
Experiment 2: Microarray of DNA from Soft rot tubers



Degefu et al., 2016. EPPO Bulletin 46:103-111

Experiment 3: Microarray of DNA isolated from bacteria after enrichment

Enrichment in Pectate Medium from tubers confirmed by PCR to carry latent infection of the target bacteria



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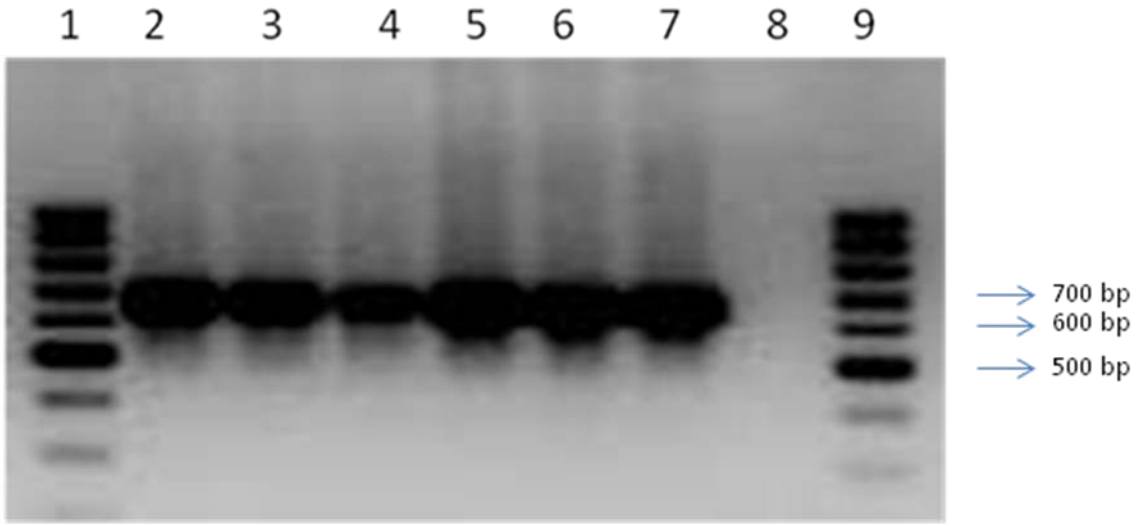
Confirmation of target bacteria in tubers by PCR



Degefu et al., 2016. EPPO Bulletin 46:103-111

Oligonucleotide probes from potato bacterial pathogens (partial list only)

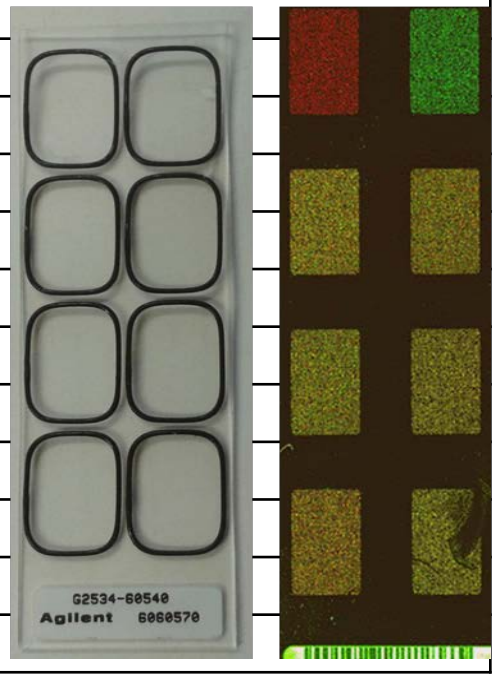
cms_2_30	CCACAACGTGAAGTTCCAAGTCAACAGGGATTTTTTTTTTTTTTTTTTT
cms_5_30	CAAGGAGAGAACATGCACATCGGACTCGTCTTTTTTTTTTTTTTTTTTT
cms_7_30	TTACTACTGCGATAGAATGAGGGGTCGCCCTTTTTTTTTTTTTTTTTTT
eca_449_30	TGGCATGAGTGTGAAAGCAGCATCATAGGTTTTTTTTTTTTTTTTTTT
eca_450_34	CCAATTTCAGCATGAGCAAGGACAAAATGCACAGTTTTTTTTTTTTTTTT
eca_452_46	CCTTATGGTTCATCCGATTCTCCAAGAATAACAGGTAAAGGTAAATTTTT
ecc_072_21_30	TATTGTCAGGGCTAACCTCAAGCGTACCGATTTTTTTTTTTTTTTTTTT
ecc_072_23_37	GGCCTTTGTCATTCAATCACAAGCAAACTTACGCTTTTTTTTTTTTTTTT
ecc_072_24_31	GCCAGTCATCGATATCAACGCGAATCGAGACTTTTTTTTTTTTTTTTTTT
ech_1344_32	GTTTCAGCGACGTGATCAAACCGCAATTGAAGTTTTTTTTTTTTTTTTTT
ech_1345_30	GATACGTTGATCACCCAACTGAACCGCCAGTTTTTTTTTTTTTTTTTTT
ech_1346_30	ACTTCATGGATGTTGGGTTCATGGGTGTCTTTTTTTTTTTTTTTTTTTT
P1_1_707_31_r1	CATGCACGTACGTCTGAATCAGGACTTCGACTTTTTTTTTTTTTTTTTTT
P2_7_242_31_r1	CAAGTATCTGACGGAAGGCTTGTGTCCCTTGTTTTTTTTTTTTTTTTTTT
P3_3_500_33_r2	TCGTTGTACAGGAACTTGTACCAGTGCGTTTTGTTTTTTTTTTTTTTTTT
scab_7_31	GTATCTGACGAGATTCCTGATGCCGTTGGTGTTTTTTTTTTTTTTTTTTT
scab_8_32	GGGTGGTGATGTACTCGAAGTAGGTGTGGATCTTTTTTTTTTTTTTTTTTT
scab_10_32	TCATCCAGATCGTGAGCAAACCAGGTCTGTAGTTTTTTTTTTTTTTTTTT

Degefu et al., 2016. EPPO Bulletin 46:103-111

Array Fabrication

Target	Number of probes
Pectobacterium atrosepticum	3545
Pectobacterium carotovorum	3449
Dickeya species	3539
Clavibacter michiganesis sepedonicus	723
Streptomyces scabies	3315
Streptomyces turgidiscabies PAL I	60
Streptomyces turgidiscabies PAI 2	65
Streptomyces turgidiscabies PAI 3	370
Ralstonia solanacearum	137
matK	105
Total	15208

8 x 15 K Format



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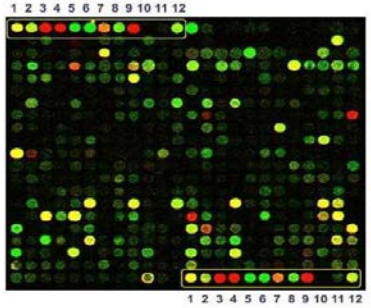
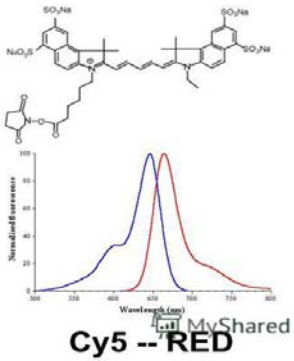
Labeling and Hybridization

Cy3/Cy5 direct labelling of DNA
(for microarrays)





Cy5 -- RED

8 x 15 K Format

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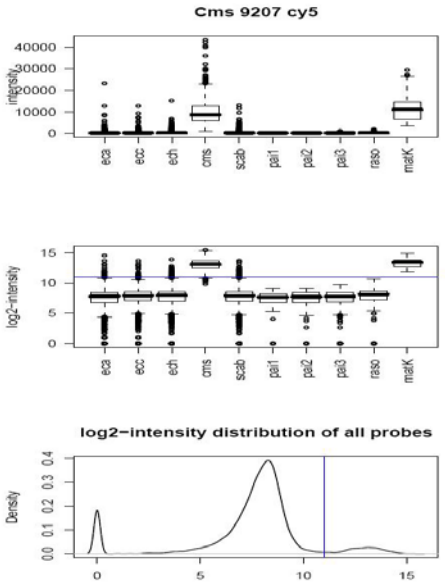
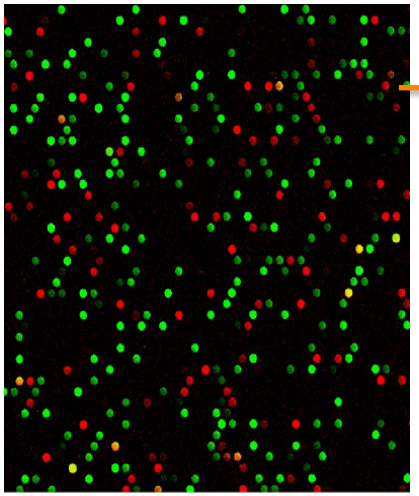
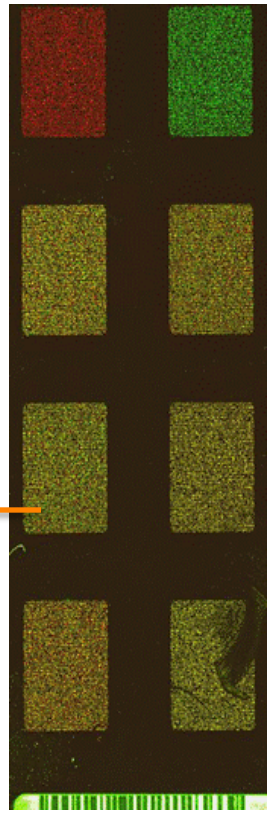
Image and data analysis

Degefu *et al.* (2016). Evaluation of microarray in the detection of major bacterial pathogens of potato from tubers. EPPO Bulletin 46:103-111.







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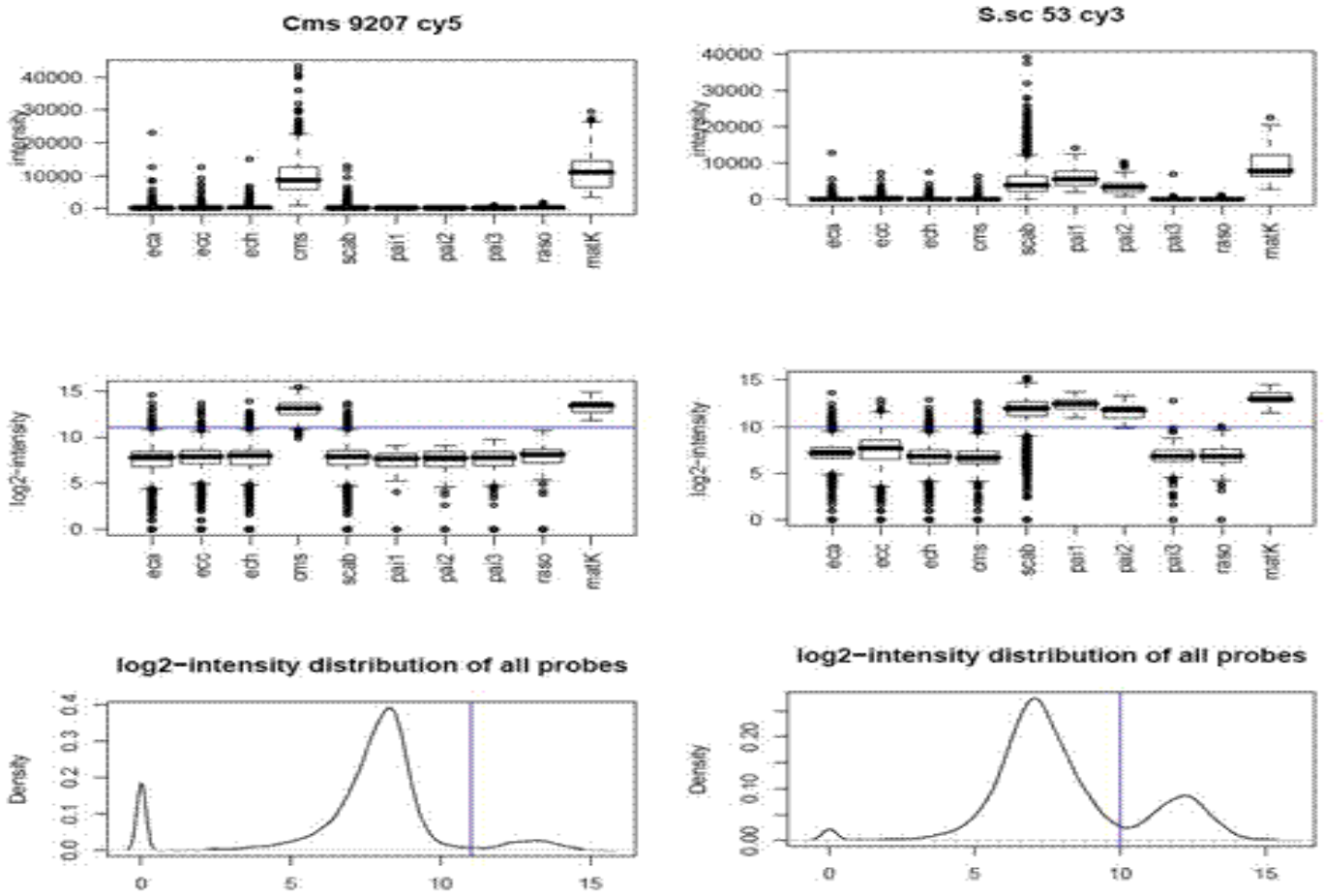
EPPO Bulletin
46:103-111

Computing and data analysis



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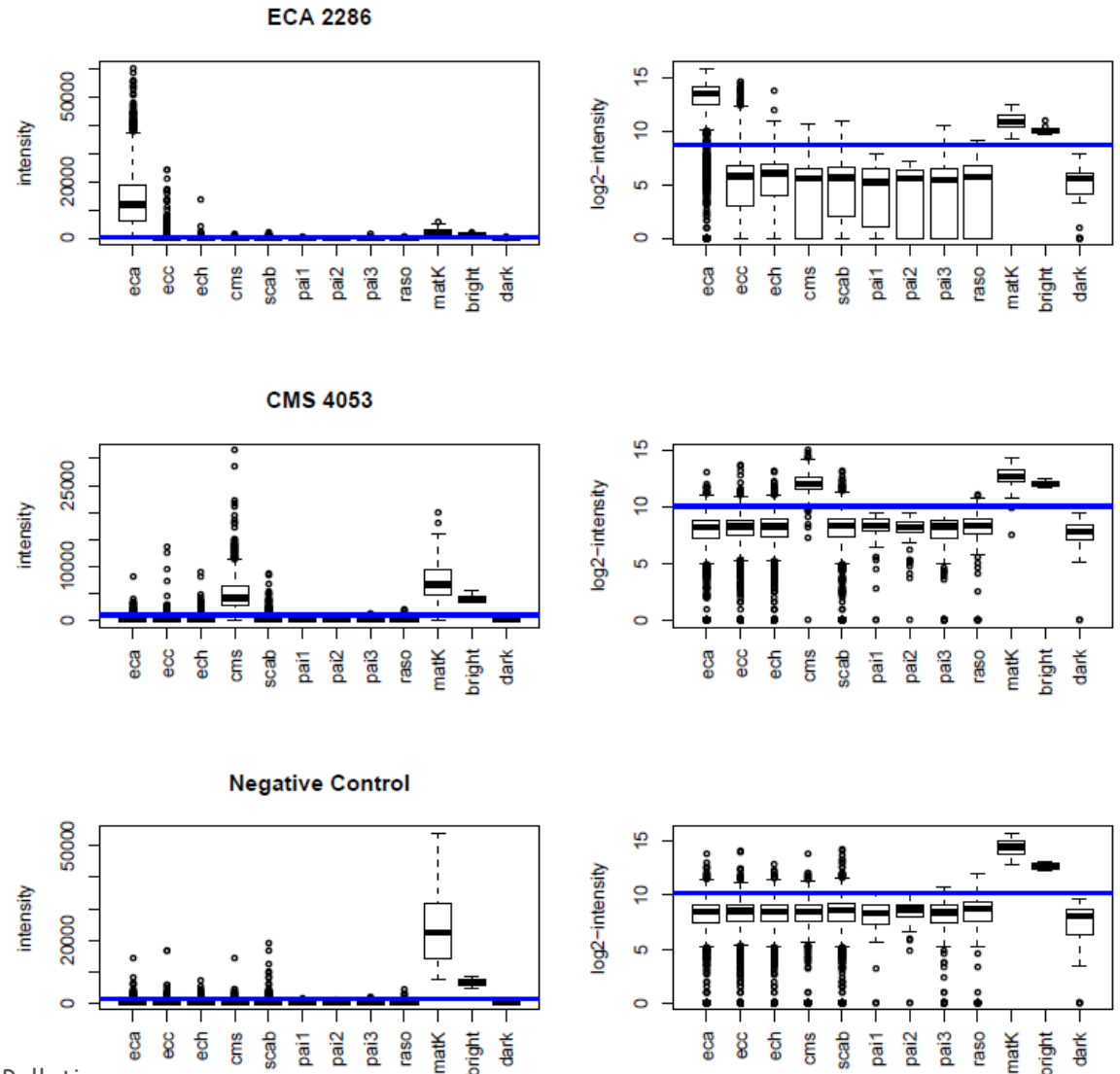

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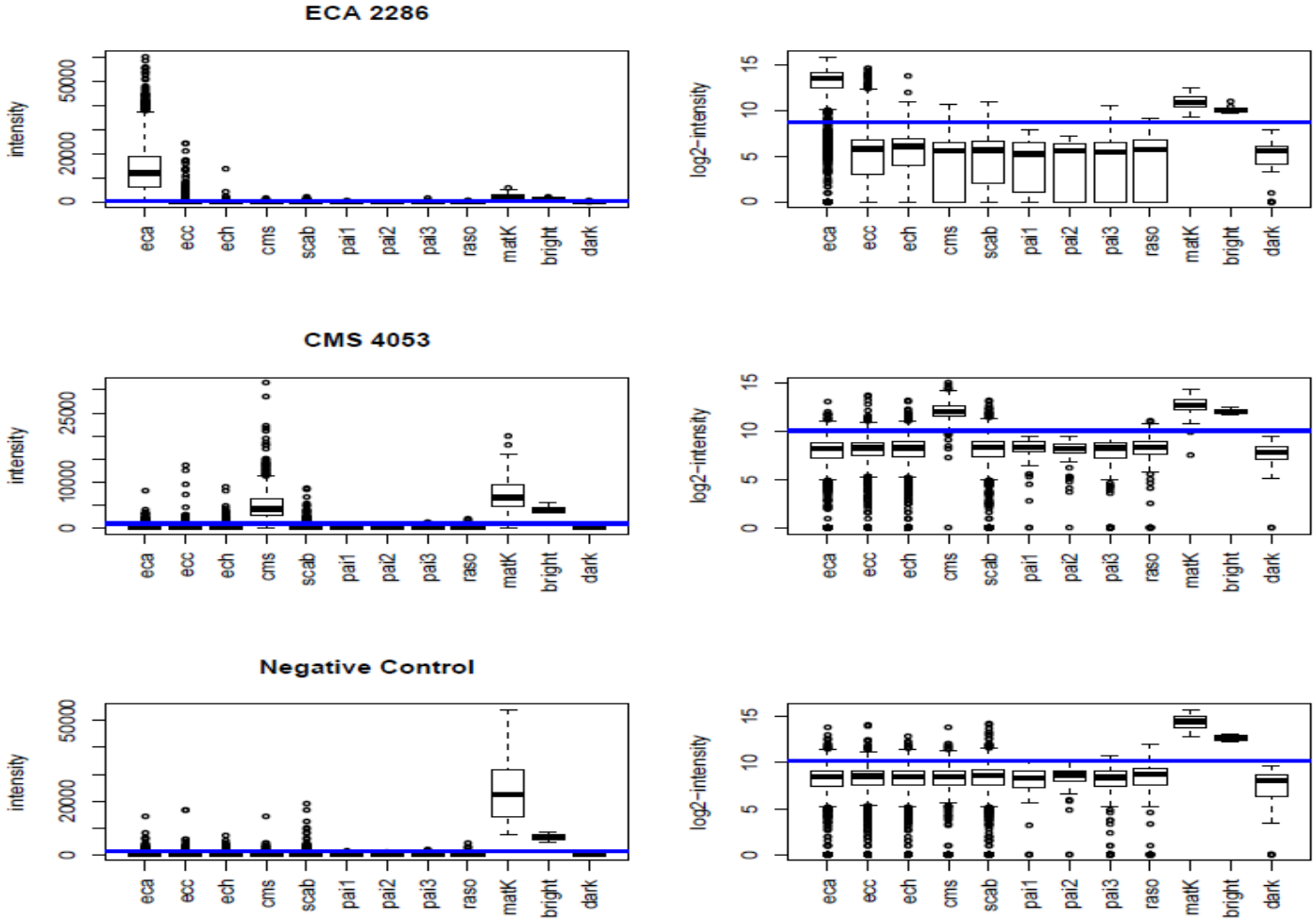
Detection Threshold (cut off point)



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Concluding Remark

- While the high specificity of the probes could be confirmed from the results of the DNA cocktail experiment used as a control, the study demonstrated that the level of analytical sensitivity of the microarray under the tested conditions was not sufficient to detect bacteria directly from tubers even after enrichment culturing. Therefore, in addition to the cost and organizational complexities, the low analytical sensitivity and limited reproducibility of the microarray are constraints for establishing the platform for routine detection of potato bacterial pathogens.

- While detection DNA microarray should not be considered off-limits, it does not quite live up to the HYPE either. Thus, it can not substitute Diagnostic PCR detection system.

Aknowledgements

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