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New Technologies from the Microbial World: Alternatives for Biomedical Surrogate Research

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Abstract

A brief description of a few amenable technologies developed for research in biomedical and agricultural diagnostics tested using plant pathogens as microbial surrogates are presented. Plant pathogen surrogate microbes can assist in easing compliance with regulations and reducing costs, ethical and biosafety concerns in biomedical research.

Keywords: Phytopathogens, plant pathogens, sample collection, microbe storage, PCR, NGS

Abbreviations: EDNA: Electronic-probe Diagnostic Nucleic-acid Analysis; NGS: Next Generation Sequencing; ONT: Oxford Nanopore Technologies

Mini Review

Biomedical research relies heavily on model organisms as a surrogate for animal and human biological systems [1,2]. Surrogate research requires a strong level of evidence that relates the technology, the surrogate, and the experimental outcome. To assess such strength of association the new technological contribution has to be subject of meta-analytic approaches including quantification of the relation between the proposed surrogate and the final outcome [3].

Most surrogate models for human medicine are vertebrates, such as rodents and primates, but even simple organisms such as yeast provide valuable insights and feedback by elucidating bioprocesses at the molecular and cellular levels [4, 5]. Similarly, research in food and agricultural microbiology uses surrogate organisms [6]. For example, plant pathogenic viruses [7], bacteria [8], fungi [9], protozoa, insects and nematodes are used as a proxy for zoonotic organisms [10]. The use of animals in biomedical experimentation has attracted negative public attention and the search for 'alternative methods' gave birth to new technology development [11]. We briefly describe a few technologies developed for agricultural purposes that are amenable alternatives for biomedical diagnostics research using plant pathogens as microbial surrogates.

Example 1

Collecting and archiving Nucleic Acids (NA) are key steps in detection and diagnosis when using PCR for health, biosecurity, or microbial forensics applications. The cotton-swipe and paper-based technologies used for sample collection offer advantages, such as storage of NA in the sample at room temperature. However, recovering NA using these collection technologies requires several (wet-lab) steps. In addition, the performance of PCR directly from the sample is hampered by the cotton fibers and residual paper matrix itself, which significantly limits its application in rapid disease diagnostics.

An elution independent collection device (EICD) [12, 13], Patent US 9,423,398 B2, was conceived to streamline sample collection and microbial processing directly into detection assays such as PCR or ELISA. The compact, easy-to-use EICD collects fluid specimens by contact and lateral flow. After samples are collected and aliquoted onto the EICD, minute pieces (1.2 mm diameter) of a built-in soluble element are excised and dissolved directly in commercial PCR or ELISA mixtures without intermediate elution steps, thereby streamlining diagnostic assays. Seventeen plant viruses, fifteen bacteria, one fungus, one insect and one plant gene (used as internal control) were assessed using one-step RT-PCR without an intermediate RNA extraction step. EICD prototypes have been proven ready for PCR processing within 3 minutes, far less time than the 10-30 minutes required using commercially available DNA elution kits. Scanning electron microscopy of pore spaces and crevices of the biomaterials either dry or wet, and with or without bacteria and stable storage of sample DNA and RNA in the EICD has been shown to last a year at room temperature. This

technology has passed the proof of concept stage of development [12, 14].

Example 2

Positive controls of infectious disease can pose biosafety risks during transportation and manipulation, nonetheless, are essential for PCR reliability and are challenging to obtain for rare, exotic, contagious, and/or emerging pathogens. As an alternative to this problem custom synthetic DNA inserts were designed de novo in tandems of forward and reverse complement primer sequences to be inserted in circularized plasmid vectors [15]. To test this new concept an artificial positive control (APCs, 203 bp long) for use in PCR was synthesized using primer sequences targeting four plant viruses infecting wheat (*Barley yellow dwarf, Soil-borne wheat, Wheat streak mosaic* and *Triticum mosaic viruses*) and the internal control plant mitochondrial nad5 gene [15]. The plasmids were maintained dry in EICD to avoid aerosol contamination in the laboratory. Similarly, a second 1126 bp long multi-target APC (GenBank KC555272) including probe sequences in addition to the tandem of primers, was synthetically generated (GenScript USA Inc, Piscataway, NJ) and cloned into pUC57. This APC allowed quantitative PCR of the insects *Liposcelis decolor, L. bostrychophila, L brunnea, L pearmani, L. obscura, L. decolor, Lepinotus reticulatus,* and the plant viruses' High plains wheat mosaic virus (formerly High plains virus), Wheat streak mosaic and *Triticum mosaic viruses, Pythium aphanidermatum* and *Pythium deliense;* [16]. These two arrays of APC priming sequences from different kingdom species demonstrated the advantage of using surrogates from the plant world while developing new technologies to be translated to the biomedical field.

Example 3

The Electronic-probe Diagnostic Nucleic-acid Analysis (EDNA) was reported in 2013 [17] and provided the framework for a new sequence-based detection system that eliminates the need for assembly of Next Generation Sequencing (NGS) data and eliminates big-data bioinformatic challenges. NGS suffers from a large amount of computational time and power needed to identify a pathogen sequence from the obtained NGS dataset. EDNA allows rapid identification and simultaneous characterization of multiple specific pathogens and changes the roles of NGS data from a query to the queried database relative to other tools. EDNA uses pathogen-specific sequences, known as electronic probes (e-probes), to detect specific viruses or organisms in metagenomic data. E-probes have been validated and generated using either complete or partial pathogen genomes [18].

Although developed with plant pathogens (RNA virus, a DNA virus, bacteria, fungi, and an oomycete), the technology would be also useful in metagenomic sequences from vertebrates [17,19,20]. Recently, to make EDNA easier to operate by non-skilled bioinformatic operators, an online platform named MIFI© (Microbe Finder) was created. The online graphical user interface, MIFI©, was developed upon the concept of EDNA [20] and comprises two parts:

a) MiProbe© which houses all tools needed for building and validating E-probes, and

b) MiDetect© the diagnostic side of the program rapidly identifies the genetic signatures of targeted pathogens in metagenomic datasets, E-probes match of DNA or RNA sequence-data from host tissue associated microbes. MiFi MiProbe© allows the flexibility of using any sequencing platform and users are expected to take advantage of portable sequencing devices like the Oxford Nanopore Technologies (ONT) MinION for diagnostics..

Early trials with ONT have been successful when assessing functional transcripts activation for aflatoxin production in soil [20]. Furthermore, the availability of the MiFi platform has permitted the development and curation of e-probes for pathogens of citrus, grapevine, roses, and blueberry allowing a host tailored diagnostic assay validation [21,22]. MiFi is rapidly evolving to be used in animal and water diagnostics and it is expected to be adopted in the biomedical field potentially using cancer diagnostics as the early proof of concept.

Conclusion

The use of plant pathogens as surrogates during proof of concept stages of new technologies allows procedural flexibility and assists research and development by easing compliance to biosafety regulations, as plant pathogens are not infectious to humans or animals but share equivalent molecular and biophysical properties. We have presented three cases in which technological approaches translatable to biomedical were initially developed using insects, microbes, and viruses from the plant world. EICD, although developed with plant pathogens, is applicable to any field-side DNA collection and storage and molecular-clinical diagnostics for health, veterinary, plant health, biosecurity, forensics, microbial forensics, and food quality. The development of APC arrays of DNA priming sequences from different kingdom species demonstrated that surrogates from the plant world are useful for developing new positive controls. The new APC concept can be translated to new biomedical situations assays improving reliability and biosafety. The development of as APC create opportunity for development and commercialization of non-contagious synthetic positive controls [15,16,23]. EDNA was initially tested with plant pathogens. E-probes, carefully designed unique nucleic acid signatures, used to identify microbes and viruses in NGS generated metagenome databases, can easily be translatable to human, animal and food-borne pathogen genomes. The unique, pathogen-specific sequences (E-probes), are designed for searches of unassembled, unchecked raw base-call read sequence data (i.e. Illumina or MinION) and are validated for sensitivity, and specificity [20,21,24].

Therefore, plant pathogens as proxies would also contribute by reducing costs. In general, the low risk and biosafety level of plant pathogenic viruses, bacteria, and fungal species while testing new technologies has a positive impact speeding up research. Moreover, surrogate plant microbes are easy to maintain, manipulate and

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add high research value as demonstrated during the development of EICD, APCs and EDNA. However, additional corroboration is needed for these new technologies with human biomedical research validation. Research in biomedical and agricultural diagnostics using of plant pathogens as microbial surrogates is creating new development possibilities and products. Scientists in agricultural diagnostics walking into this new research space are seeking partners in biomedical research to further validate these technologies in ways that will be meaningful to them.

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