Practical uses on nanopore long-read sequencing in a high-throughput contract food microbiology laboratory

Andrzej A. Benkowski

Eurofins Microbiology Laboratories
6304 Ronald Reagan, Madison, WI 53704, United States
Corresponding author: Andrzej.Benkowski@ft.eurofinsus.com, Phone +1 (608) 949-3022

Abstract

Nanopore sequencing is a versatile diagnostic and exploratory tool for a commercial laboratory with clients that demand fast turnaround and low cost. Eurofins Microbiology Laboratory based in Madison, WI utilizes the technology to answer a variety of questions posed by their clients including but not limited to food and environmental pathogen confirmation, food spoilage investigations, probiotic multi-strain blend identifications, and microbiome analyses of fermentation-derived food, beverage and dietary supplement products. The robustness of the platform allows for a multitude of different uses in a contract lab setting where the focus is data-driven answers for a diverse client base and an interlaboratory network, as shown by the case studies highlighted in this presentation.

Case Studies

Non-Confirming *Listeria* spp. Investigation

A client recently experienced an unexplained increase in *Listeria* spp. presumptive results at several food production facilities, and a large portion of these presumptive results could not be confirmed by the traditional cultural method. The investigation's main objectives were to: 1) discover the cause of these non-confirming presumptive (NCP) tests, and 2) determine an approach to reduce their frequency.

Potential root cause(s) of the increase in NCP included the rapid platform detecting DNA from dead cells, cross-reactivity with non-target bacteria, and issues with the cultural confirmation method. Along with a large head-to-head study looking at various rapid pathogen detection platforms to measure performance, the confirmatory bacterial growth from the NCP samples were evaluated by Oxford Nanopore Technologies (ONT) GridION long-read sequencing platform using Rapid Sequencing and Barcoding Kits with SpotON Flow Cells R9.4 in conjunction with EPI2ME (Metrichor) Fastq What's in my Pot (WIMP) workflows[1]. All samples contained *Listeria* reads when sequenced, though in many cases the number of *Listeria* reads was quite low, and *Enterococcus* spp. was by far the most prominent genus present.

Table 1. Identification results of NCP *Listeria* isolates and corresponding rapid platform detection qualitative data (D = Detected, ND = Not Detected).

Listeria ID	Prominent Species	BACGene	Cq	24E BAX	MDS	rtBAX	Culture
Listeria innocua	*Enterococcus faecium*	D	33	ND	D	D	ND
Listeria grayi, Listeria monocytogenes	*Enterococcus spp./Paeniclostridium sordellii*	D	27	ND	D	D	ND
Listeria monocytogenes, Listeria innocua	*Enterococcus faecalis*	D	32	ND	D	D	ND
Listeria monocytogenes	*Enterococcus faecalis*	D	31	ND	D	D	ND
Listeria innocua	*Enterococcus faecalis*	D	30	ND	D	D	ND
Listeria monocytogenes, Listeria innocua	*Enterococcus spp./Staphylococcus aureus*	D	28	ND	ND	ND	ND
Listeria monocytogenes, Listeria innocua	*Enterococcus faecalis*	D	30	ND	D	D	ND
Listeria mono, Listeria innoc, Listeria grayi, Listeria welshimeri	*Enterococcus faecalis*	D	29	ND	D	D	ND
Listeria innocua, Listeria monocytogenes	*Enterococcus faecalis, Listera*	D	19	ND	NA	NA	D
Listeria monocytogenes, Listeria innocua	*Vagococcus spp.*	D	35	ND	D	D	ND
Listeria innocua, Listeria monocytogenes	*Enterococcus faecalis*	D	38	ND	ND	D	ND
Listeria grayi, Listeria innocua	*Enterococcus faecalis*	D	28	ND	D	D	ND
Listeria innocua	*Enterococcus spp./Listeria*	D	38	ND	NA	NA	D
Listeria monoc, Listeria innoc, Listeria grayi	*Enterococcus faecium/Enterococcus faecalis*	D	29	ND	ND	ND	ND

This data helped support the conclusion that the NCPs were exclusively the result of issues with the cultural confirmation due to low levels of culturable *Listeria* and high levels of background flora able to proliferate on the prescribed growth media.

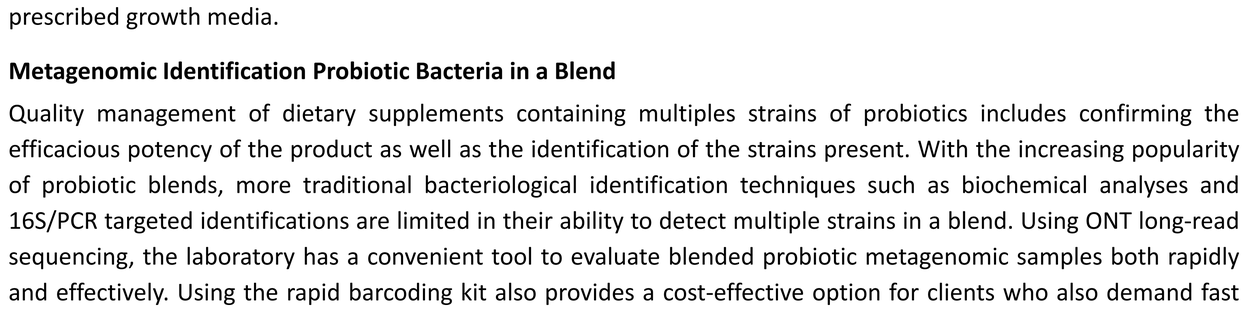
Metagenomic Identification Probiotic Bacteria in a Blend

Quality management of dietary supplements containing multiples strains of probiotics includes confirming the efficacious potency of the product as well as the identification of the strains present. With the increasing popularity of probiotic blends, more traditional bacteriological identification techniques such as biochemical analyses and 16S/PCR targeted identifications are limited in their ability to detect multiple strains in a blend. Using ONT long-read sequencing, the laboratory has a convenient tool to evaluate blended probiotic metagenomic samples both rapidly and effectively. Using the rapid barcoding kit also provides a cost-effective option for clients who also demand fast turnaround times. This allows clients to confirm the probiotic species listed on the nutritional label in a single assay, which when coupled with enumeration, allows for better characterization of the product. This, in turn, leads to additional consumer confidence. Eurofins performs hundreds of these types of metagenomic analyses on probiotic materials on a yearly basis.

Pathogen Strain Tracking and Environmental Monitoring

Whole genome sequencing using ONT can allow strain-level identification and tracking of pathogens or other contaminants in a facility. Certain food industries are more susceptible to pathogen contamination due to the materials being handled so the plant environment in thoroughly monitored for pathogens using swabs and qualitative rapid pathogen testing. When pathogens are found in the environment, characterization of the organism and tracking its presence can help identify the source of contamination, improve food safety, comply with regulations, and build consumer confidence. The ONT next-generation sequencing platform in conjunction with SNP calling bioinformatics has allowed pathogen characterization by whole genome sequencing to compete with more traditional strain tracking methods with improved accuracy and sensitivity.

Figure 2. An illustration showing the different types of topological groupings[2]. Monophyletic topology occurs when isolates of interest (A and B) group together to the omission of others. A paraphyletic topology exists when isolates of interest (C and E) group together but not to the exclusion of others (D). A polyphyletic topology is one in which isolates of interest do not form a group (A and E).

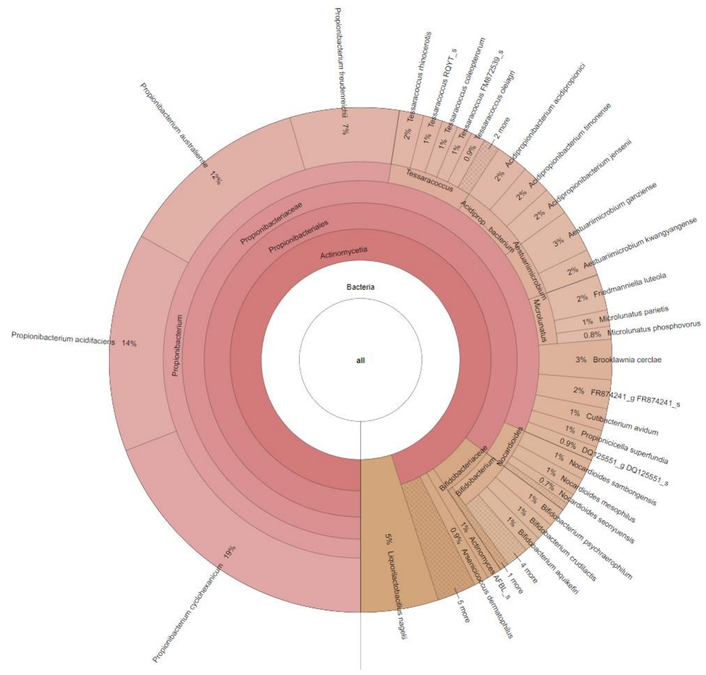


Testing laboratories also utilize ONT long-read sequencing to identify contaminants found in the environment as part of its Environmental Monitoring Program. For example, a laboratory that handles both probiotics with a known high microbial load, while also testing other samples for trace levels of contaminating microorganisms, needs to have a similar HACCP-style plan in place to minimize the risk of cross-contamination between sample types. Thoroughly monitoring the environment and characterizing the organisms present using ONT sequencing helps in the root cause analyses and source tracking of contaminants, curtailing the risk so clients can have the utmost confidence in the data being produced.

Microbiome Characterization of Fermentation-Derived Foods and Beverages

Naturally occurring organisms as part of the fermentation process can include a wide variety of lactic acid bacteria, yeasts, and molds Understanding which organisms are present and their characteristics can assist in flavor characterization, quality and safety evaluations, product marketing, and any potential health benefits. ONT long-read sequencing is an effective tool in characterizing these materials. An example of this evaluation is kefir beverage characterization. Kefir is a fermented milk drink that has been consumed for centuries and poses a number of known health benefits. Kefir manufacturers routinely submit samples for this type of analysis to better understand the properties of the material.

Figure 3. Metagenomic analysis of kefir as shown by a Krona chart showing the taxonomic abundance of a kefir beverage. Microbiome profiles such as this help clients make business decisions related to their formulations in order to make the highest quality product.

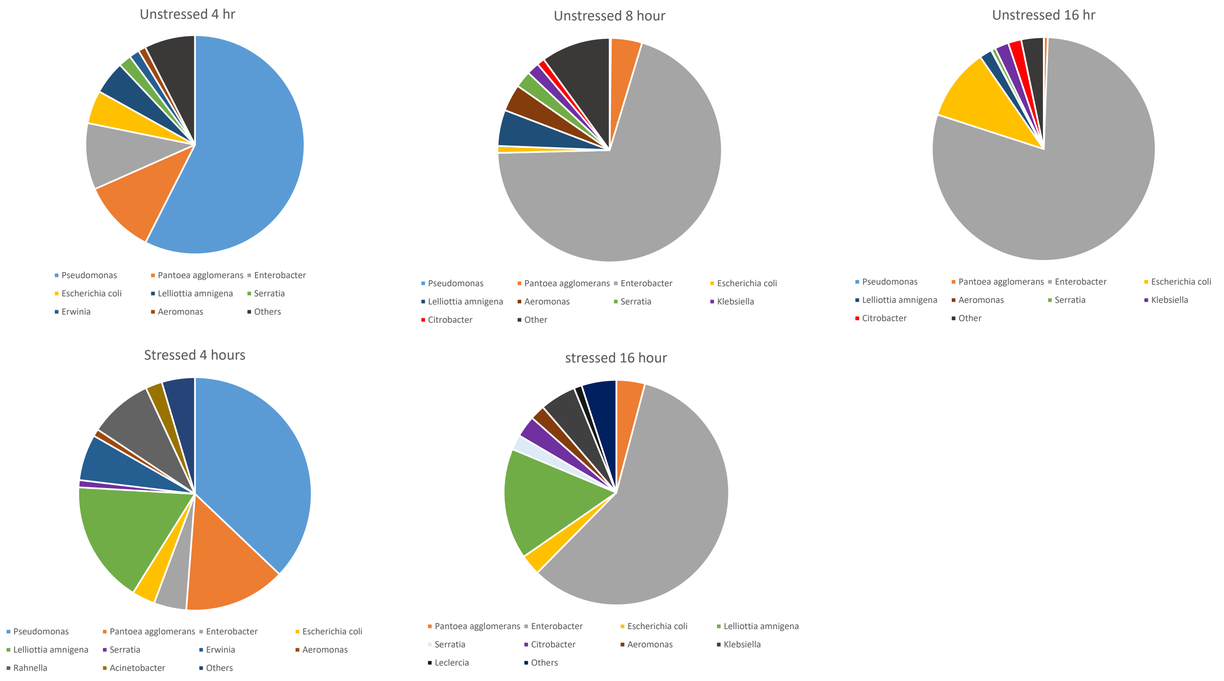


Spoilage Investigations

For decades, classic microbiological techniques have been employed to determine the cause of spoilage often times involving a significant amount of testing for different targets organisms. These assays typically involve attempting to grow the culprit organism on a preferred medium, then identifying the growth to determine the source. These techniques have a number of drawbacks including a limited scope of detection, the requirement that the organism has the ability to be cultured, a lack of sensitivity, and a long lead-time to a root cause. Using ONT Rapid Barcoding and Sequencing Kits to produce accurate long reads sequenced directly off an extraction of the spoiled product allows the laboratory to bypass the labor-intensive and incomplete classic approach to spoilage investigations.

An example of an internal spoilage investigation to learn more about produce spoilage was performed using ONT sequencing of lettuce enrichment microbiomes in order to determine the effect of cold-stress on the composition of enrichment cultures in conjunction with the detection of putative slow growing Shiga-Toxin Producing *Escherichia coli* using real-time PCR[3]. Lettuce matrices were spiked with varying concentrations of pathogens and run through the sample enrichment workflow using samples with and without a cold-stress of 4°C for 48 hours. Aliquots of the enrichment were pulled at various time points and the microbiome evaluated using the ONT GridION and Rapid Sequencing Kit. The results showed differences between the enrichments of cold-stressed and unstressed samples suggesting that cold stress has an affect on the diversity of the lettuce microbiome.

Figure 4. Pie charts showing the population dynamics of enrichment samples for cold-stressed and unstressed leafy green samples.



Conclusions

The use of ONT long-read sequencing is a powerful investigatory tool that can be utilized in a high-throughput contract third-party microbiology testing laboratory environment due to its ease-of-use, price competitiveness, reliable data, and rapid time to results as demonstrated by the case studies presented. Using basic ONT workflows and EPI2ME bioinformatics, the ONT platform can answer a variety of questions about product composition, pathogen detection confirmation, environmental monitoring, spoilage investigations, and microbiome analyses.

References

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