The impact of DNA sequencing methods on food safety and quality

Over the last decade we have seen huge developments in our ability to analyse Deoxyribonucleic Acid (DNA). DNA defines the structure of all living things, it describes what we are. Every living thing has different DNA. So analysis of DNA can be used to differentiate different species (of plants, animals or micro-organisms), but it can also be used to differential between individuals within a species. Indeed the DNA of every single living thing is slightly different.

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A DNA molecule is a simple structure composed of a sugar (deoxyribose), a phosphate group, and an organic base. There are only four types of base in DNA and it is the sequence of these bases throughout the DNA molecule, that make organisms and indeed individuals different.

Sequencing of DNA

DNA sequencing is simply the discovery, by testing, of what order these bases are in within a single organism. The sequencing of microbial DNA began many years ago. It was a long and complex process, and tended to be done only by university research laboratories.

Now, however, developments in technology and instrumentation mean we can sequence a microbial genome (all of the DNA within a cell) quickly, easily, and at relatively low cost.

Indeed it is now possible to sequence an organism's entire DNA within a day. Within food testing, two DNA sequencing approaches are beginning to be used.

These are:

- Whole Genome Sequencing (ascertaining the complete DNA sequence of a single isolate).
- Metagenomics (use of sequencing to establish the microbial population within a food or environmental sample).

Whole Genome Sequencing (WGS)

Whole Genome Sequencing (WGS) has the potential to render other forms of microbiological identification obsolete.

It is more accurate than a serotype, more discriminatory than a pulsed-field gel electrophoresis assay and it can prove relationships between strains with higher resolution than ever before.

This is the method which is being adopted by regulatory agencies around the world to identify bacterial isolates from foods and those with food poisoning.

The technique has become more prevalent in the last five years due to advances in sequencing technology that have led to dramatic falls in cost. It is now possible to sequence a bacterial genome on a routine basis for a few hundred Pounds (or Euros).

When a genome (all of the DNA within an individual organism) is sequenced, the initial output is hundreds of thousands of short sequences. Each of these sequences is a few hundred bases long and represents a tiny fragment of the total genome.

The next challenge is to assemble these short sequences by comparing them to each other and ordering them according to their overlapping ends. This process is analogous to reconstructing an ancient document from fragments of parchment.

The next step is to give the sequence some meaning by comparing it to other sequences (usually contained within a database). The comparison is reliant on the number of other genomes against which your sequence is compared.

The analysis gets larger as more genomes are added in to the comparison database. Comparing whole genomes against databases of other whole genomes is currently performed by the US Food and Drug Administration (FDA) in the USA using their 'Genome Trakr' service.

This service relies on the vast storage and computing power available to them from the National Centre for Biotechnology Information (NCBI). This is a resource available to anybody, and a genome submitted for analysis will be placed into context via comparison against all other sequences.

The necessary use of a public database for this analysis has led to concern from some in the food industry who fear that doing the right thing and submitting sequences will reflect badly on them in the event their sequence is shown to be related to an outbreak.

The issue is one that still requires adequate resolution. But it is clear that the food industry is showing great caution about submitting such WGS data to public databases.

What is clear is that WGS can link food/environmental isolates to clinical isolates; it can do this over large time ranges, thus enabling outbreaks that give a low level of cases over a long time period to be identified.

This great step forward can only be of

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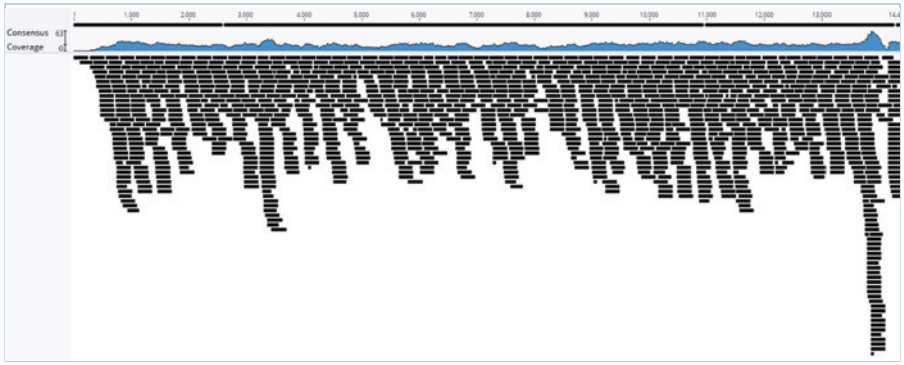
Fig. 1. A small portion of a genome assembled from raw sequence reads. Each of the small black bars represents one sequence approximately 500 bases long.



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benefit to food safety for all concerned. The real issue is that all parties have to come to terms with the new technology, understand its abilities and come to an agreement on if and how such comparison data will be used in the future.

Campden BRI is actively working internationally with industry and regulators to advise and help reach a mutually beneficial result.

Metagenomics

Metagenomics is another sequencing based approach, but it does not go into the depth of detail of WGS. Instead this technique looks at the sequencing of one small area of the genome, but it can define in a single test the complete microbial population within a sample.

How does this work? To undertake a metagenomic analysis, a sample is taken (this may be a food or environmental sample).

Unlike other microbiological tests which require enrichment of a sample in a broth culture for a day or more, metagenomic tests require no enrichment.

They are subjected to a polymerase chain reaction test to amplify a particular part of the bacterial DNA (usually the 16S rRNA gene).

This amplified section is then sequenced and the resulting data compared to a database of strains. As the amount of DNA sequenced is small compared to WGS, the level of identity of bacteria is lower (WGS will define strains, metagenomics will usually define to a level of Genus).

The technique will, however identify most of the major groups of organisms present in a sample in a single test run, with no enrichment, no agar plating, no requirement for growth. This eliminates many of the issues we have when looking for populations within our food or environmental samples.

Traditional population microbiology relies on the growth of bacteria on different selective agars; this allows us to understand which groups are present or growing within the sample.

However, if we do not have a suitable medium for particular bacteria, or if some organisms are present but unable to grow on the media we use (for example stressed or injured bacteria), then we will not see them at all.

These bacteria may be an important part of the sample microflora playing a part in quality or shelf life, but to us they will be invisible. Metagenomic analysis completely overcomes this issue, allowing us to see exactly what bacterial population is present within a sample, and how that population changes over time.

The technique lends itself to more accurate shelf life analysis (i.e. development of populations over the life of the sample), determination of the cause of quality degradation or spoilage, and even to observation of the growth of organisms during fermentation processes (cheese, yoghurt, alcoholic beverages etc).

Conclusions

Only a few years ago, genomic analysis was the preserve of university research laboratories making little intrusion into the world of the industrial microbiologist.

However, we have moved forwards incredibly quickly, industrial laboratories now have access to new genomic tools, which will revolutionise the way microbiology is done.

We will be able to trace contamination routes, link isolates to outbreaks, and analyse microbial populations in new ways.

There are some concerns particularly in the access to, and use of WGS databases to attribute outbreaks to foods, these are being tackled now and will be resolved.

Work being done at Campden BRI is showing that metagenomics can provide an incredible approach to population microbiology that will have a huge impact on shelf life assessment and spoilage investigation, adding a greater level of realism to results and allowing adjustments to shelf life, in some cases potentially extending life and reducing waste. ■