Next generation sequencing – the future of food microbiology

There is something of a revolution occurring in the way that microbes of relevance to the food industry can be analysed. There is capacity now to sequence large quantities of genomic data allowing an unparalleled level of granularity in various aspects of food microbiology.

by Ed Haynes and Andrew Hudson, Fera Science Ltd, York, UK. www.fera.co.uk

There are times when food companies will need to trace the sources of contamination within their facilities to locate the source of some microbiological contaminant.

Similarly, those involved with protecting the public's health may need to track contaminants back to their source to identify the source of an outbreak.

However to gain the granularity required to complete such tasks it is not sufficient just to know that a species of bacteria is present because any one species is comprised of any number of strains, and in simple terms the harder you look the more strains you will find. Identifying bacteria at the sub-species level is known as sub-typing and this is one area of microbiology that has seen huge advances over the last few years. 'Phenotypic' sub-typing tests include approaches such as serotyping and biotyping, but these are generally fairly crude tools; for example there are very few serotypes of Listeria monocytogenes. More discriminatory methods tend to be based on analysis of the microbe's DNA. There are many such DNA-based tools available and too many to describe here, but two in particular have become used widely.

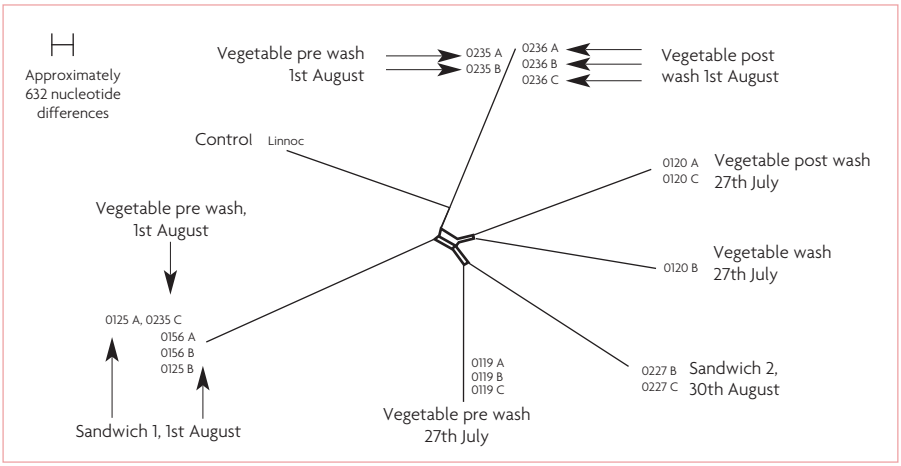
The first, Multi Locus Sequence Typing (MLST), compares the sequences of a small number of genes and assigns them a number to give a code for each isolate. This has the advantage of enabling the easy comparison of results from different laboratories.

The other workhorse is Pulsed Field Gel Electrophoresis (PFGE), which uses restriction enzyme to cut the DNA into fragments that can be separated by electrophoresis. This is a lengthy method that can be a little subjective. Also sometimes the DNA simply will not 'cut' such that there is no pattern to analyse.

However, in recent years, advances in rapid 'Next Generation Sequencing' (NGS) technologies have revolutionised the precision available for the production of data allowing the tracing of microbes in all sorts of environments.

NGS technologies have until quite recently been prohibitively expensive to use but now the costs are reducing and their application to real world problems in food safety and quality is becoming feasible.

Fig. 1. Relationship tree of isolates obtained from a food manufacturing plant, produced as part of Fera's OriGen traceback service.



Applications in the food sector

This article looks at a few examples of such applications which may be of interest to those involved in the food industry.

To date, possibly the area in which NGS technologies have been most frequently

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employed is in food safety. The specific approach is to sequence the whole genome (Whole Genome Sequencing: WGS) of bacterial isolates under investigation and determine how closely related they are. The data can be interpreted to give distance maps which show how closely related your isolates are. As an example, Fig. 1 shows some real data produced by Fera for isolates of bacteria (Listeria innocua) in a facility producing sandwiches.

An obvious first feature is that the washing procedure is not effective as the same strain is being found on the vegetables pre and post wash, which is perhaps not that surprising.

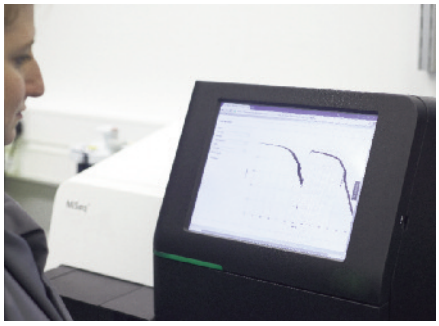
The isolate in sandwich two was not derived from the vegetables as it is only distantly related to the vegetable isolates.

Sandwich one contains an isolate of L. innocua which is indistinguishable from a vegetable isolate, which happens in itself to be different to the other vegetable isolates.

The conclusion there is that sandwich two might have been contaminated through the use of the vegetable as an ingredient (and hence more control of the production of the vegetable is required), cross contamination from vegetable processing to the sandwich or, possibly, that they were both contaminated from the same source.

These data would be greatly enriched by the accumulation of further data on isolates from different sources and over a period of time. You may then be able to establish, for example, whether the facility was 'colonised' by a particular strain, indicating that there may be, for example, long-term problems with cleaning and sanitising a particular piece of equipment or environment. WGS has also been applied to outbreak investigation and was famously involved in determining the causal organism in the German Escherichia coli O104 outbreak. Thus, use in protecting public health is proven and in practice.

This kind of sequence information is also



completely comparable between laboratories and countries. If the sequences from the isolates from the scenario above were to be uploaded to publicly accessible databases (anonymously) they can be compared to data for other isolates obtained all around the world. This may enable close matches to isolates from other foods to be established and this may help to identify ingredients that may be introducing contamination into the facility.

Food spoilage

An intriguing application is to use NGS in another mode – 'metagenomics'. In these analyses, either total DNA from a food sample can be sequenced, or a specific gene common to all taxa of interest (bacteria) can be amplified and sequenced.

By either method, the composition of the microbiota can be investigated. This could be considered as a 'non-targeted' method as you are looking at everything, not just an organism that you might think is the problem.

NGS should help greatly in determining the causes of spoilage by taking samples at time points in the shelf life and seeing which organisms are growing and eventually dominating the microflora. A nice example of this is given where metagenomics were used to show that Pseudomonas dominated the flora of aerobically-stored steaks. Perhaps that is not very surprising, but it does show that the strategy works.

The application to the investigation of spoilage of foods in new packaging atmospheres are obvious and would indicate which organisms would need to be controlled to extend shelf life. The same methodology could be applied to the detection of pathogens in foods as you would be able to detect all (known) pathogens in one test, although the sensitivity which might be expected is an open question.

Food fermentations

Foods that are produced by fermentation are microbiologically complex involving many species of microbe.

Their analysis by conventional means is therefore difficult and involves a huge amount of conventional microbiology requiring extensive resources. NGS has been applied to many such foods including wine, cheese and salami to study the succession of bacteria occurring during the respective fermentations.

Food authenticity

Food authenticity is an increasing area of interest to many of those involved in the food industry. At a recent workshop held by the FSA, issues around authenticity were prominent among priorities for surveillance.

Fera manages the EU Food Integrity Project and was a major player in the detection of horse meat substitution for beef during 'horsegate'.

DNA sequences of particular genes can be used to identify the animal species of single origin meats, and this can also be achieved with multi-species mixtures. The use of NGS allows the detection of multiple meat species in a mixed sample, but also allows for the detection of species which were not expected to be present. Similar methods can be applied to other foods, for example herbs and spices where substitution for oregano has been noted.

It might also be possible to use NGS to identify the origin of foods. For example the brine used in cheese production will vary from plant to plant because the brine contains a characteristic salt-tolerant microbiota. Since cheese is in direct contact with the brine then brine bacteria will become established on the cheese, so providing a fingerprint which might be used to establish provenance.

Conclusions

The new WGS/NGS technology has a range of possible uses within the food industry. By keeping track of the bacteria colonising production facilities there is scope to keep food safer and to avoid costly recalls through a better understanding of the ecology of the production plant. Isolates to be used for this purpose may be picked up from environmental testing programmes making use of material which would otherwise be discarded.

We may get a better understanding of spoilage process so prolonging shelf life and reducing food waste, which is a huge problem for the UK, and we may be able to detect microbiological signatures of high value food products.

The cost of doing this is reducing, but the analysis of the data still requires expert evaluation; it is not a simple task. In addition, the sheer volume of data available for comparison means that high end computing power is needed. But over time the technological constraints will lessen. ■

References are available from the author on request