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Microbial Metagenomics and the Food Industry

Imagine a world in which microbial populations are determined without needing to grow them on agar. Imagine being able to find organisms that we cannot culture in the lab. Imagine being able to pick up not just the healthy members of a community, but those that are injured as well. This is now a reality through a new technique known as 'Metagenomics'. The potential applications for this technique are limited only by the populations we can find, making it ideal for spoilage investigations, shelf life analyses and environmental monitoring (to name but a few!). It will also provide more information to the food manufacturer as the metagenomic "count" will be much more closely related to the mixture of species in the product. The following article aims to introduce the reader to this exciting new field of microbial analysis.

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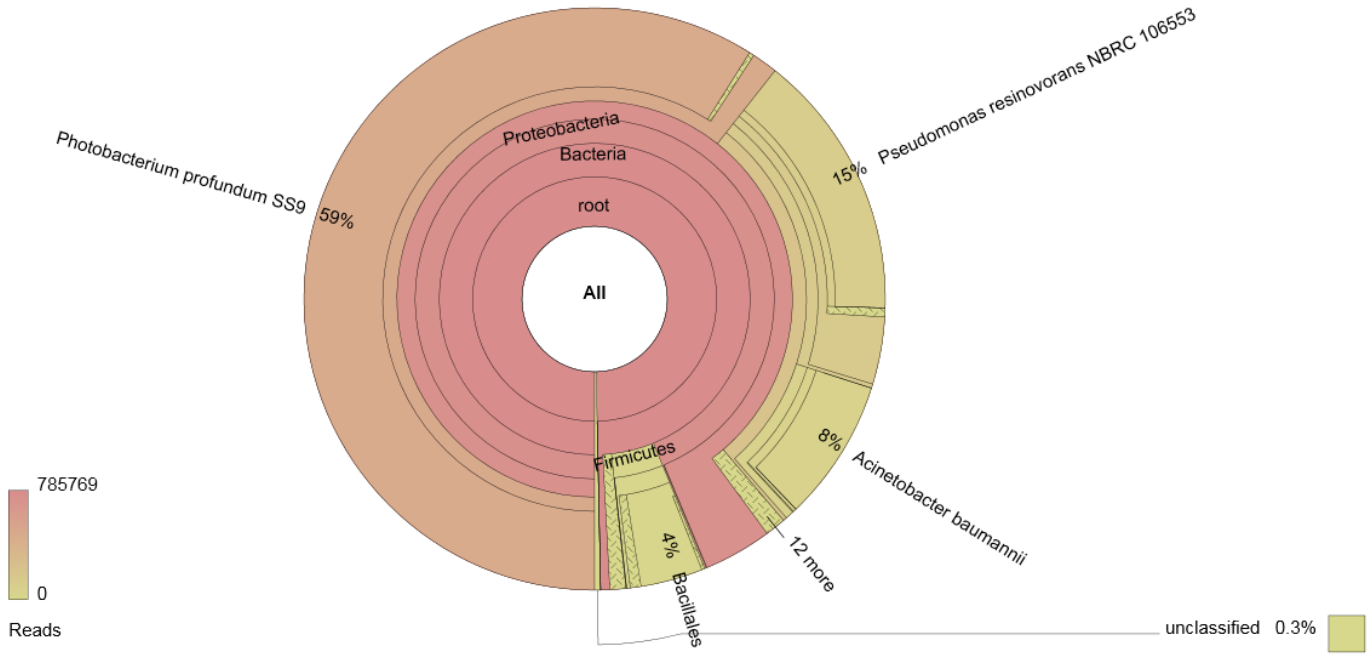
Technology and applications

Metagenomics is a technique that takes advantage of recent advances in DNA sequencing technology allowing huge numbers of different individual DNA sequences to be read at any one time. This means that the individual DNA sequences of a mixed bacterial population can be read directly from a single DNA extract of a food sample. The advantages of this are that previously uncultured organisms can be identified and little manipulation of is needed in order to produce data regarding the bacterial composition of that sample.

Currently if we wish to establish the bacterial types in a population present in a food (the microbiome), we would place dilutions of the food sample on different selective agar plates. The types of agar would be chosen by the microbiologist, according to what organisms they expected to be in the sample. This approach is very unstandardised and will introduce a bias into the results. Basically if an organism, however numerous in the sample, cannot grow on the agars chosen, they will never appear to the analyst and never be considered in any issue related to the food. This could result in an apparent invisibility of injured organisms, organisms that may affect quality or shelf life of products, or major spoilage groups simply because they cannot grow on the chosen agars.

The metagenomics approach reduces experimental biases that occur using the selective agar based approaches. Metagenomics works best for food samples which are expected to contain a mixed population of bacteria. An example of the output of a Metagenomic analysis of the microbial population of a poultry sample is shown below:

Figure 1: Krona chart of the microbial population of a poultry sample as determined via sequencing a pool of 16S ribosome gene amplicons.



Metagenomics is only as good as the database against which the sequences are compared, and this fact presents a dilemma. DNA extracted directly from a product will be more representative of the original sample than if any further manipulation has been carried out. The resulting sequences will comprise a random sub-set of the total population, and it is possible that these will not be held in a database with an accompanying identifier. Therefore, whilst being more representative of the overall population, the chance of much of one's data residing in the 'Unclassified' section of an analysis increases. To increase the chances of identification, PCR can be used to generate a pool of sequences for which there may be better database support. In microbiology, the gene chosen in the overwhelming majority of cases is the gene coding for the 16S sub-unit of the ribosome (16S rDNA). This gene is used for the majority of microbial taxonomy studies and has extensive database support. Running a PCR has the advantage of 'cleaning' the sample of sequences that are not 16S rDNA sequences (e.g. DNA from the host food); however, the choice of primers will exclude some organisms, primarily fungi, from the analysis. This can be addressed by running a separate analysis using fungi-specific primers.

In Figure 1, a 16S rDNA profile has been generated for a spoiled poultry sample. If a culture-based analysis had been used for this sample, the investigator would have chosen the media to be used to isolate various groups of organisms *a priori*. In this case, it is likely that *Pseudomonas* spp., Enterobacteriaceae and a Total Viable Count (TVC) would feature in the analysis. The results in Figure 1 give an indication of the influence these choices can have on understanding the microflora of a product. Instead of the accepted wisdom that the dominant microflora would be comprised of *Pseudomonas* spp., the highest proportion of reads (59%) is assigned to *Photobacterium profundum*, an organism currently associated with fish. Enterobacteriaceae are not shown to be significant. TVC and *Pseudomonas* spp. counts potentially offer less information about the microflora of the sample than this 16S rDNA amplicon analysis.

Areas in which Metagenomics will benefit the food industry

Metagenomics has been used for a variety of applications in the food industry already, and a recent selection of these is presented in Table 1:

Table 1: Recent examples of Metagenomics used to analyse food:

Use	Summary	Reference
Monitor effect of antibiotics on mastitic cows.	Antibiotic treatment shown to have no significant effect on *E. coli* related mastitis.	(Ganda et al., 2016)
Track antibiotic resistance genes in response to diet in chickens.	Conventional antibiotic supplemented diet proven to encourage microflora with higher proportion of antimicrobial resistance genes.	(Hegde et al., 2016)
Check label claims on probiotics	Several product label claims found to contain errors when compared to the Metagenomic analysis.	(Morovic et al., 2016)
Profile of pork spoilage organisms	Psychrotrophic spoilers found to be more prevalent, *E. coli* identified as an indicator organism.	(Mann et al., 2016)
Comparison of factory and product spoilage flora	Sausage factory microflora and product flora found to be different as sausage progressed through shelf life.	(Hultman et al., 2015)
Check of enrichment methodology	*Salmonella* enrichment in fact favoured growth of *Clostridia.*	(Jarvis et al., 2015)
Comparison of environmental and product flora	Equilibrium is established between environmental and product flora in a butchery environment	(Stellato et al., 2016)

Fermented foods

All fermented foods share the common characteristic of using a microbial consortium to change starting ingredients into a final product. The makeup of this consortium is often unknown and dependent on environmental organisms, e.g. sourdoughs. Fermentations can also be very tightly controlled, with a single organism used to produce the final product. The organisms most often used in fermented products are Lactic Acid Bacteria (LAB), yeasts and moulds. Identification of most of these organisms to the level of genus or species is not currently possible with culture-based methods. Profiling of fermented foods using Metagenomics offers the food industry a greater depth of knowledge of the progression and composition of a fermentation. There is also the possibility of using these profiles as tools to check authenticity of products such as artisan cheeses. Metagenomics will also aid the development of new fermented foods, as microbial consortia can be accurately chosen based on the flavour profiles they achieve in other products.

Spoilage investigations

Currently, a spoilage investigation has many in-built biases that can limit its efficacy. First, the investigator must make a decision based on experience of similar products as to the suite of media they are going to use to isolate the most abundant organisms. Next, the microflora examined by the investigator will be those that are able to grow on the media selected and during the time and temperature combination chosen. Finally, usually due to cost constraints, the most populous organism is chosen for identification and a name produced. Whilst this is currently the best method we have, there is a chance that the organism identified as the spoiler is not to blame and has merely been selected for by the growth conditions chosen by the investigator. Metagenomics offers a less biased approach to find spoilage microbes. For example, cheese that showed a pink defect was analysed using Metagenomics (Quigley et al., 2016). The organism found to be the causative agent of the pink discoloration was *Thermus thermophilus.* This organism has an optimal growth temperature of 65°C, which is not a temperature used for growth of microbes in standard testing. Metagenomics also has the potential to identify consortia of microorganisms spoiling products.

Shelf life analysis

Shelf life analysis currently depends on culture based methods, and relies on similar assumptions made by the investigator as for spoilage investigations. The potential to track individual species over shelf life in a less subjective manner will lead to insights into the microbial ecology of foods that we never previously expected. This ability will also allow much more nuanced preservative approaches to be taken as the effects of different hurdles can be monitored in more detail.

Environmental Monitoring

Food factories inevitably build up a microflora over time despite the best efforts of hygiene teams. Monitoring the microbial population in detail could show investigators the efficacy of cleaning regimes, and potentially show the effects of zoning different areas of a factory (e.g. High Care vs. Low Care in a chilled food factory). It also has the potential to trace routes of cross-contamination.

Conclusion

The ‘Holy Grail’ of microbial Metagenomics is to replace culture-based methods for enumeration. 16S rDNA amplification currently represents the most accepted way of generating a microbial profile, despite the risk of PCR amplification introducing bias. The relationship between the profile and the number of cells in the sample will take much more study; however, initial results show that similar general trends can be observed. In January 2017 Campden BRI started a member-funded project designed to use microbial profiling to better investigate the microflora of a range of food types over their shelf lives. This data will be compared against culture based methods to highlight discrepancies and compare the trends observed. If you are interested in being a part of this project, please get in touch.

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