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New insights for spoilage, shelf life and contamination of meat and fish products with advanced microbial profiling (AMP)

AMP is a powerful DNA technique that we use at Campden BRI to determine the unique mix of microorganisms in a sample (its microbiome) without needing to culture them in the lab.

AMP can benefit the whole of the food industry, but in this white paper I will focus on the ways we have been using AMP for meat and fish products to reveal:

- What microbes are in a product and what traditional culturing techniques miss
- The effect that super-chilling has on the microbiome of products
- How meat from different cutting plants can be traced to its source

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Issued: April 2018

Do you really know what organism is spoiling your product?

If you have a spoilage issue and we identify the culprit organism using traditional techniques, we place dilutions of the food sample on different selective agar plates. The types of agar, the incubation temperature and duration would be chosen by microbiologists based on what organisms they expect to be in the sample from experience of similar products. Injured organisms, organisms that may affect quality or shelf life of your product, or major spoilage groups which are present in your product – even in large numbers – can remain "invisible" simply because they cannot grow on the chosen agars. Whilst this is currently the method used across the industry, 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.

AMP reduces experimental biases that occur when using selective agar-based approaches

The chart below compares the genus/genera of bacteria and their abundance that were identified in raw beef burgers using AMP, plate count agar (PCA) and violet red bile glucose agar (VG). PCA is a non-selective agar that is used to assess the total viable bacteria in a sample and VG is selective for Enterobacteriaceae.

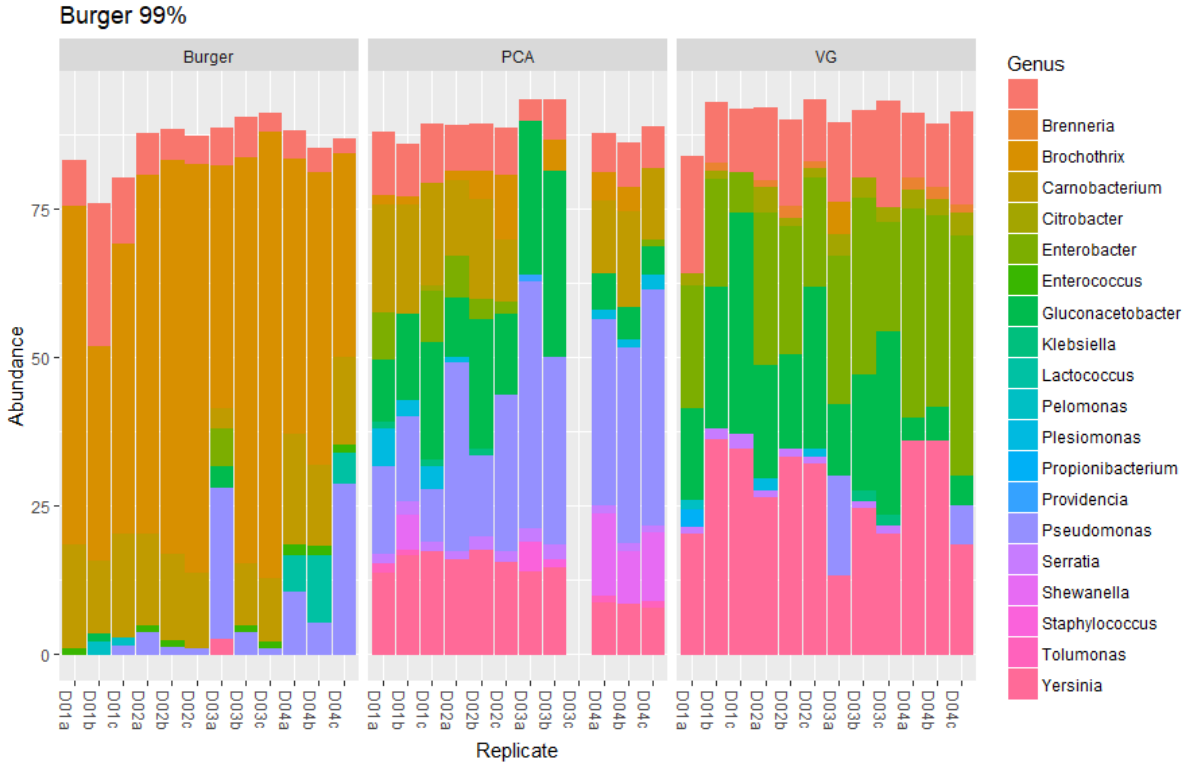

Figure 1 Composition of beef burger microbial flora at taxonomic level 'genus' as identified by AMP (burger), non-selective PCA agar and selective VRGBA agar.

Brochothrix was identified as the dominant organism in the samples identified using AMP. In the samples grown on PCA, *Yersinia*, *Pseudomonas*, *Gluconacetobacter* and *Brochothrix* were the most abundant. The samples grown on VG agar showed a similar microflora to the PCA media except with a greater proportion of *Enterobacter* and fewer *Pseudomonas*.

A microbiologist would draw very different conclusions about the source of a spoilage problem when presented with the data from these three different methods.

Despite increasing knowledge and advances in food preservation techniques, microbial spoilage of foods continues to cause substantial financial losses. A better understanding of key spoilage bacteria is crucial to better control contamination and microbial spoilage of foods. AMP enables us to provide our clients with a much more accurate picture of what is causing spoilage or contamination issues in their products.

AMP reveals that a product's microbiome changes during superchilling

Tracking individual species over a product's shelf life using AMP can provide unexpected insights into the microbial ecology of foods. We got some surprising results when we used AMP during a study into how super-chilling could extend the shelf life of various products, including sausages, prawns and beef burgers. Samples were super-chilled to -2°C and then stored at chill temperature (5°C) to mimic the scenario of the product leaving a manufacturer and being released into a chilled distribution and retail system. We then tested the products using AMP throughout their shelf lives.

Our AMP analysis revealed that the microbiome of super-chilled products is not static. The bacterial populations changed despite the products being held below the organisms' minimum growth temperatures. This raises the intriguing question of whether microbiologists must now consider a minimum temperature for adaptation as well as a minimum temperature for growth.

Tracing meat to where it was processed

Food factories inevitably build up a microflora over time despite the best efforts of hygiene teams. Monitoring the microbial population in detail could confirm 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.

We've recently shown that the chicken from different cutting plants can be traced to its source by studying its microflora using AMP.

A poultry company provided us with 20 packs of pre-cut poultry portions from two of its processing plants. We used AMP to analyse the poultry samples.

Figure 2 shows the abundance of the different genus of bacteria identified in the chicken samples taken from the two plants. Samples taken from Site 1 have a higher proportion of *Bifidobacterium* and *Faecalibacterium* species whereas samples taken from Site 2 have a higher proportion of *Acinetobacter* and *Arthrobacter* species. The differences observed can also be displayed as a dendrogram (Figure 3).

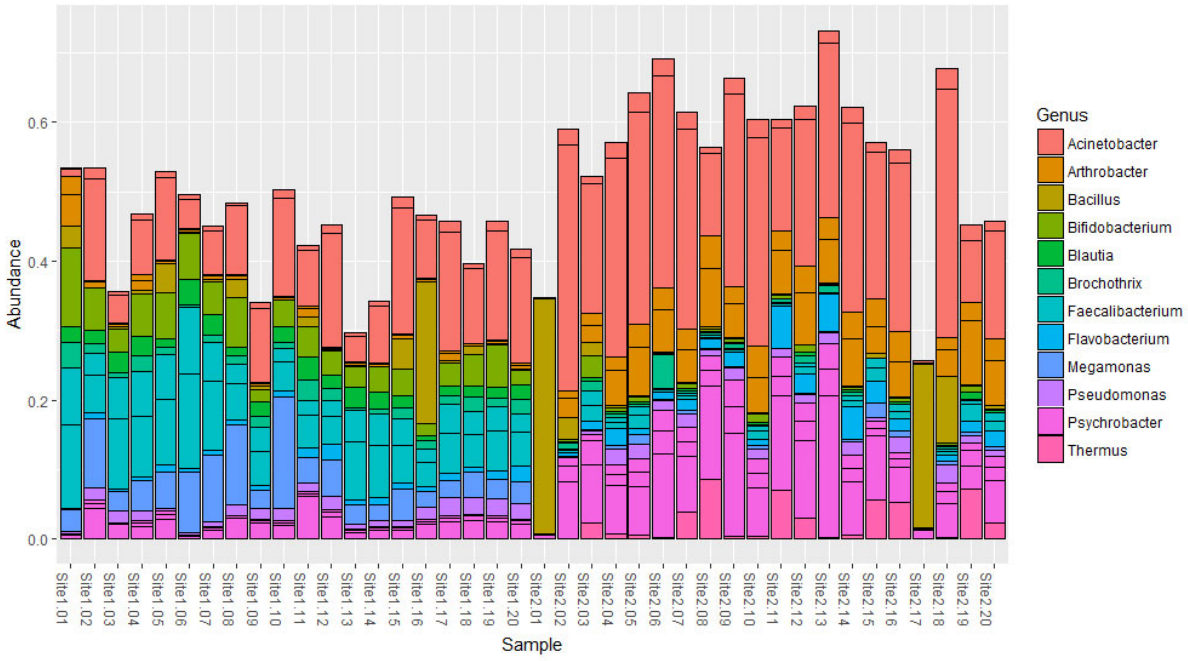

Figure 2: Sample composition at taxonomic level 'genus' (top 99% of genera across all samples).

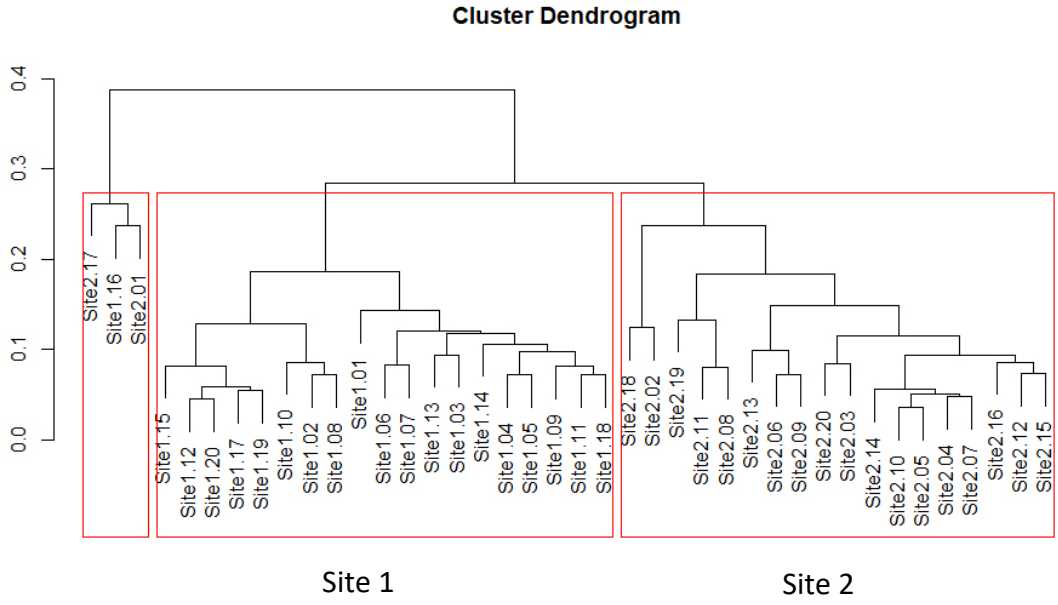

Figure 3: Shows the genetic similarity of samples taken at the two chicken processing plants. Samples that are clustered contain more genetically similar microbial populations.

Figures 2 and 3 show there is a clear difference between the sample sets. The results suggest that the microflora from Site 1 originates from the chicken, whereas the microflora at Site 2 is more likely to originate from soil. By comparing the populations, you can conclude that each site has produced chicken batches with very different microflora, and this can be used to identify the origin of a sample.

AMP could be used to investigate the effects of changes in manufacturing practice. For example, if the soil-derived flora is found in final product, controls and hygiene practices can be changed to prevent this happening. AMP could then be used to monitor the effects of those changes.

Conclusion

The power of AMP is only starting to be exploited. This white paper shows it is ideal for many areas of food manufacture where tracking the source of microbial populations is important, such as shelf life analysis, spoilage investigations or environmental monitoring. It provides food manufacturers with much more accurate information as the results are much more closely related to the mixture of species in the product than the results derived from traditional culturing techniques.

Advantages of AMP over traditional culturing techniques:

- identifies organisms that are difficult or impossible to culture in the lab
- identifies both healthy and injured organisms
- can analyse dozens of samples simultaneously
- can analyse thousands of microbial marker genes simultaneously in a single sample

Potential uses of AMP:

- spoilage investigations
- shelf life analysis
- hygiene monitoring
- authenticity testing

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