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Microbial Metagenomics and its applications in the food industry

The problems in attempting to accurately determine the total microbial population in any food matrix by conventional cultural techniques are numerous and well documented.

We look for individual groups of organism using selective agars which often selectively inhibit all but the target organism. Even when we do use non-selective agars in an attempt to obtain a total bacterial count, we use a single technique such as a pour plate and incubate under specific conditions which may allow the growth of only a small number of the total organisms present in the sample.

This is compounded when plates are routinely incubated at 30°C irrespective of whether the samples are normally stored at refrigerated or ambient conditions. We would have a much better chance to recover all the potential spoilage organisms in chilled food (which may be adapted to grow at the lower storage temperatures) if we reduced the incubation temperature and incubated the plates for longer. We may also increase our chances of growing obligate aerobic organisms if we adopted spread plates rather than pour plates for this method.

Traditional cultural techniques also often struggle to allow the growth of stressed or injured cells. As a lot of the food samples which we test have been processed in some way, many of the organisms we are attempting to culture fall into this category.

Not all the organisms present in a sample may share the same atmospheric growth requirements.

All of the above reduces our chances of recovering a large proportion of the organisms which may be present in any given food sample.

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.

Dr Greg Jones from Campden BRI has pioneered work in this area and has quoted a case study in which his team performed a 16S rDNA profile from a spoiled poultry sample. The conventional wisdom is that the dominant microflora of a spoiled poultry sample would be comprised predominantly of Pseudomonas spp. In this case study, the highest proportion of profiles (59%) were in fact assigned to Photobacterium profundum, an organism previously thought to be uniquely associated with fish, which suggests that traditional cultural techniques may in fact fail to detect the predominant microbial populations in many food matrices.

Outbreak caused by Sorbitol fermenting E coli 0157.

An article in the May edition of Eurosurvelience reports an ongoing, protracted and geographically dispersed outbreak of haemolytic uraemic syndrome (HUS) and gastroenteritis in Germany, involving 30 cases since December 2016. The outbreak was caused by the sorbitol-fermenting immotile variant of Shiga toxin-producing (STEC) *Escherichia coli* O157. Molecular typing revealed close similarities between isolates from 14 cases. One HUS patient died, and results of a case–control study suggest packaged minced meat as the most likely food vehicle but food safety investigations are ongoing.

This serves as a reminder for labs to be vigilant when interpreting typical appearances and morphology of

target organisms on selective agars, as the primary selective reaction used to distinguish E coli 0157 from other E coli on the sorbitol MacConkey agar plates is the fact that most E coli 0157 strains do not ferment sorbitol and produce straw coloured colonies on the agar opposed to the pink colonies of the mannitol fermenting organisms.

Although external quality assessment schemes quite often include unusual or atypical strains such as sucrose fermenting Salmonella's to check that labs have the ability to detect these variants, it is worth remembering that these organisms are present in the wider environment and will occasionally occur in routine samples.

Salmonella infections caused by poultry

As a keeper of four hens who live at the end of my garden, I was interested to read an article published by the Centre for Disease Control (CDC) in America on the incidence of Salmonella linked to what they refer to as "Back Yard" poultry. From January to May this year, the CDC had confirmation of 372 people with Salmonella infections caused by eight different serotypes were linked to contact with live poultry in backyard settings. Of those, 71 required hospitalization and thirty-six percent of the infected people were children younger than 5 years old. Officials interviewed 228 of the sick people and 190, (83 percent), reported contact with live poultry in the week before they became ill. From 1990 to 2016, in the US a total of 65 outbreaks of human Salmonella infections have been linked to contact with live poultry.

How Shigella can survive in the Gastrointestinal Tract

Foodborne pathogenic bacteria have to survive in the harsh conditions of the host's gastro-intestinal tract if they are to proliferate and multiply to large enough numbers to cause disease. Firstly they have to survive the journey through the acidic conditions of the stomach, then they have to battle with the established bacterial gut flora and then they have to overcome the toxic effects of the high bile concentrations in the small intestine. Researchers from Massachusetts General Hospital (MGH) have been looking not only at how Shigella survives this journey but also how it takes advantage of substances that would kill many less persistent organisms.

The researchers analysed how the Shigella's gene expression changed in response to exposure to bile salts. Previous work has shown that two hours of exposure to bile salts increases the ability of Shigella to adhere to, and invade epithelial cells lining the gastrointestinal tract. By prolonging the exposure to mimic the time required for Shigella to transit the small intestine, it has been demonstrated that the longer exposure to bile salts also led to the formation of biofilms – communities of bacteria that produce a protective coating to resist harsh environmental conditions. The researchers also found that the reabsorption of bile salts that normally takes place in the lower small intestine causes the biofilm to disperse, releasing the bacteria to infect tissues in the large intestine or colon.

These observations provide a more complete picture of how Shigella transits the small intestine to reach the colon for infection.

Additionally, since many of the mechanisms used to resist bile exposure are the same ones which are used to resist antimicrobials, it is suggested that exposure to bile salts also primes the bacteria to antibiotic resistance.

Each year Shigella is responsible for at least 80 million infections and approximately 700,000 deaths worldwide

The risk of Hepatitis E from undercooked pork

The food standards agency has re-issued advice on the risk of contracting hepatitis E from undercooked pork products following media reports last month.

Press reports claimed that hepatitis E is present in 93 percent of British pig herds with 6 percent producing levels high enough to infect humans. It was claimed that 1:10 of pork sausages purchased in the UK contained the hepatitis E virus.

The FSA advice was to cook pork products until steaming hot throughout and the meat is no longer pink and juices run clear. The FSA communique stated that the risk from acquiring the hepatitis E virus from eating thoroughly cooked pork or pork products is low.