



David Reynolds looks at the varying properties of 'good bacteria'

Tenants of the last 1.5 metres

BACTERIA CAN cause disease. Nothing controversial there – this has been accepted since Koch first proposed his postulates in 1876 for bovine anthrax. The 'disease' referred to here is, of course, infectious disease – whooping cough, wound infections, typhoid fever, cholera and the like. These are all readily treatable with antibiotics and fluid replacement but can bacteria do worse? Can they cause other, non-infectious diseases?

Well, yes they can - gut infections by *Salmonella* and *Campylobacter* are known to predispose to joint

Probiotics vs cancer

The development of colorectal cancer takes approximately 40 years in man. The initial cause of a lesion with the potential to develop into a tumour is often the result of damage to the gut lining by toxic compounds produced by the normal bowel flora – a complex interdependent community of some 1300 species of bacteria (Nelson, 2004). Carcinogenic N-nitroso compounds can be synthesised by the gut bacteria from nitrate and nitrite often found as preservatives in foods or generated by the action of the bacteria on bile produced by

TABLE 1. Origins of Probiotics

Probiotics have long been used as a means of preventing disease in both man and other animals. The deliberate use of probiotics to prevent human disease dates back to the beginning of the 20th century.

Elie (Ilya) Metchnikoff working at the Pasteur Institute in Paris believed that the bacteria in fermented milk preparations helped to fight pathogenic bacteria and so extend the normal life span (*The Prolongation of Life*, Metchnikoff, 1907).

inflammation (Reactive Arthritis, Reiter's syndrome) and other autoimmune diseases such as Guillain-Barré syndrome. Streptococcal throat infections can lead to rheumatic fever, Mycobacteria may have a role in Crohn's disease and *Helicobacter pylori* has been shown to cause gastritis. At least there are some bacteria that do us no harm – the normal flora that exists on and within our bodies. Well... no - it is these bacteria that have the most insidious role in causing disease – they can give you bowel cancer and 1 in 20 of us will suffer the consequences by the age of 75. Is there anything we can do to defend ourselves against this enemy within?

the liver. Bacteria can activate pro-carcinogens in the gut and release tumour promoters such as ammonia from protein in the diet (Mallett & Rowland, 1990).

But not all of the bacteria in the normal microflora are a problem and in this lies an answer. Professor Ian Rowland's group at Ulster University has shown that lactic acid bacteria (LAB – the group members include *Lactobacillus*, *Lactococcus*, *Bifidobacterium*, *Enterococcus*, *Pediococcus* & *Leuconostoc*) – part of the normal gut microflora and found in live yoghurts and probiotics (see Table 1) - produce lower amounts of these carcinogenic compounds and less of the enzymes

involved in generating carcinogens from non-carcinogenic complexes (Rowland, 1992). The presence of LAB in increased numbers in the gut helps to suppress the progression to tumours (Bolognani *et al.*, 1997).

Drinking a probiotic every day is not the whole answer though. Diet is also involved – the level of fat in the western diet predisposes to colorectal cancer. Studies have shown that high dietary fat affects the metabolic activity of gut bacteria as well as the levels of secondary bile acids, which may act as tumour promoters in the colon. Animal model studies indicate that it is total dietary fat, rather than the type of fat, that promotes carcinogenesis (Reddy, 1981). However, changes in diet can help – inclusion of non-digestible oligosaccharides from some plants (notably - onions, garlic, leeks, asparagus, chicory and artichoke) into the diet stimulates the growth of LAB and helps to prevent the progression to cancer. These components, collectively termed prebiotics, provide the saccharolytic LAB with nutritional advantages enabling them to out-compete carcinogen-producing proteolytic and lipolytic bacteria in the gut.

Yoghurts and probiotic drinks based on *Lactobacillus* spp., *Enterococcus* spp. or *Streptococcus* spp. are often used by people as part of a slimming diet or to help counteract such problems as acute gastritis, gastrointestinal infections, constipation, ulcerative colitis, food allergies, antibiotic-induced disorders, irritable bowel syndrome and even cardiovascular diseases. In contrast to the data on cancer prevention, the evidence for these beneficial effects has been largely circumstantial.

However, recent research



Professor Esko Nurmi (& Doppelganger) — his 1973 paper in *Nature* heralded in 'Competitive Exclusion' (picture by Mari Toivonen, 1998)

work carried out at Dundee University indicates that probiotics based on *Bifidobacterium* spp. can help to counteract the inflammatory bowel disease - ulcerative colitis (Kennedy *et al.*, 2002).

Probiotics vs infectious disease?

There are claims for other roles for probiotics - the use of LAB to prevent infectious disease is gaining popularity. This has been taken up more enthusiastically in the prophylactic treatment of animals than in man and products are currently available that claim to help prevent enteric disease in commercially reared pigs, calves and more particularly – *Salmonella* colonisation of broiler chickens (meat birds) and hens (laying birds). Given the extent of their use, the scientific case for their effectiveness is not as strong as might be expected.

Experimentally, dosing chicks with either single

strains or mixtures of *lactobacilli* have given little protection (Watkins & Miller, 1983; Weinack *et al.*, 1985). Using a mixture of a small number of bacterial species, Barnes *et al.*, (1980) found that although coliform bacteria initially disappeared from the crop and caeca of probiotic-treated birds, following challenge, levels of *Salmonella Typhimurium* were 10-100 times higher than in the untreated controls.

Experience has since shown that relatively complex mixtures of bacteria (50-100 species) are necessary to obtain adequate protection against *Salmonella* (Impey *et al.*, 1982; Nurmi, 1985; Stavric *et al.*, 1985; Stavric, 1987). These complex mixtures of bacteria are thought to 'competitively exclude' *Salmonella* and some other bacteria from the gut.

Competitive Exclusion

During the 1970s several workers studied the problem

of *Salmonella* contamination of chicken meat. The growth in the popularity of chicken meat had led to the industrialisation of chicken meat production. Systems had evolved whereby eggs were laid by hens on one farm, incubated and hatched out in near-sterile conditions in an automated hatchery, before being reared on a second farm. Some of these birds were becoming *Salmonella* positive during the rearing process. This caused little in the way of symptoms in the chickens – there was no discernible effect on growth rates, morbidity or mortality, hence meat producers were not affected by the presence of *Salmonella* in the birds.

However, at slaughter the process of evisceration was known to cause the gut contents of birds to contaminate the slaughter line and thus the meat of other birds processed on the line. If *Salmonella* positive birds were slaughtered on the line, all downstream birds and their meat would potentially be contaminated, with increased risk to the consumer.

One approach used to reduce this risk was to try to prevent *Salmonella* colonising the birds before slaughter. Chickens are increasingly resistant to *Salmonella* colonisation as they get older: 50% of one-day-old chicks can be infected using 10 cfu *S. Typhimurium* but it takes 106 cfu to colonise just 10% of the population by day 14 (Milner & Schaffer 1952). This suggested that maturation of the gut was in some way preventing *Salmonella* from colonising these older birds. One possibility was that the gut microflora was developing over time and increasing the resistance to *Salmonella*. This microflora was normally obtained by chicks from the environment and the mother hen. However, the change

from extensive farming, with eggs hatched by hens, to intensive chicken farming, with eggs hatched in incubators and an absence of adult birds, meant that development of this normal adult gut flora was delayed or prevented. Hence the partially developed gut flora of the intensively farmed chicks was less able to prevent *Salmonella* establishing itself in the guts of the birds.

The key step in what became known as **Competitive Exclusion (CE)** was to show that chicks fed the diluted minced gut contents of mature, free-range hens were more resistant to infection with *Salmonella* than were untreated birds. The contention was that the mature gut flora was a climax community that filled all of the available ecological niches in the gut and thus excluded less well-adapted bacteria such as pathogenic *Salmonella*.

This concept was demonstrated by the Finnish microbiologist Professor Esko Nurmi in a paper in *Nature* in 1973 (Nurmi & Rantala, 1973). The mechanism thought to be involved in CE has since been refined and several components are thought to be involved (see Table 2).

The impracticality of using minced adult hen gut to treat millions of chicks was not lost on Professor Nurmi and in a later paper anaerobically fermented gut contents were shown to give the same protective effects (Rantala & Nurmi, 1973). Later it was shown that even after sequential fermentation of the gut microflora, the protective effect was retained (Snoeyenbos *et al.*, 1978, Mead & Impey, 1984). Also, fermenting the bacteria meant there was little chance of propagating any pathogenic viruses or protozoa that may have been present (Mead & Impey 1984), opening up the



Normal microflora - bacterial barrier at the mucosal surface of chicken caecum

TABLE 2. Possible competitive exclusion mechanisms

Direct attack	production of bacteriocins and organic acids that can damage pathogenic bacteria.
Nutrient competition	competition for the nutrients available in the gut.
Receptor competition	competition for attachment sites on the gut enterocytes.
Immune stimulation	of the non-specific immune system of the gut by the normal microflora.
Physical barrier	a bacterial mat blocking access to the mucosa



Fermentation hall — Aviguard, Microbial Developments Limited

possibility of a commercial product.

Professor Nurmi's concept has been commercialised both in Finland (Orion Corp – Broilact) and the UK (MDL – Aviguard) with several other products available worldwide (Tecto CE, Avifree, PreEmpt & Avian Pac). These CE products contain the entire normal mature avian gut flora fermented, freeze-dried and sealed in foil sachets. They are used widely in the poultry industry and are either sprayed onto birds (method used for recently hatched birds) or given via the drinking water.

In addition to prevention of *Salmonella* infection, some of the commercial CE products have been shown to prevent gut colonisation by pathogenic, antibiotic resistant *Escherichia coli* bacteria (Weinack *et al.*, 1981, Hofacre *et al.*, 2002). Furthermore, work in the USA by Dr Charles Hofacre's group in the University of Georgia (Athens) has shown that one CE product helps prevent necrotic enteritis (caused by toxigenic *Clostridium perfringens*) in chickens (Hofacre *et al.*, 1998a, 1998b).

Defined CE products

All of the early work on *Salmonella* prevention using CE was done with undefined or only partially defined mixtures of bacteria. This was unavoidable as many of the bacteria were uncultivable in isolation using the techniques of the time. However, it became clear that when selected bacteria were used as CE preparations they were much less effective than the mixtures derived from the entire gut flora. Single strains or mixtures of *lactobacilli* (Watkins & Miller, 1983; Weinack *et al.*, 1985), or other bacterial species (Barnes *et al.*, 1980) have not performed well. ▢

Indeed, a defined product available in the USA (PreEmpt) was recently withdrawn from the market.

Later work demonstrated that relatively complex mixtures of bacteria (50-100 species) were necessary to obtain adequate protection and that they did so by establishing a balanced microflora in the chicken gut (Impey *et. al.*, 1982; Nurmi, 1985; Stavric *et. al.*, 1985; Stavric, 1987).

Attempts to produce a defined culture to prevent necrotic enteritis have met with only limited success. The Institute of Food Research (Norwich) and Veterinary Laboratories Agency (Weybridge) have worked together on a *Lactobacillus johnsonii* probiotic to exclude *Clostridium perfringens* which shows some positive effect in a colonisation model (La Ragione *et. al.*, 2004). However, there is no evidence yet that this probiotic will work to prevent infection in a necrotic enteritis disease model such as that used by Hofacre (Hofacre *et. al.*, 1998a, 1998b). *Bacillus subtilis* spores have also been used to attempt competitive exclusion of *Salmonella* and *Clostridium perfringens* (LaRagione & Woodward, 2003) but the results have been disappointing in comparison with the performance of commercial undefined CE products. To date there is no defined bacterium or group of bacteria capable of effective competitive exclusion of gut pathogens to the degree seen with undefined products.

So where does this leave us? The risk of bowel tumours is reduced if the normal bowel flora is skewed by supplementation with bacteria that produce fewer carcinogens from our food. Prevention of gut infections (at least in birds) is enhanced by the presence of a broad-

spectrum bowel flora, which includes those bacteria known to generate carcinogens. This is, of course, not an issue for poultry (the usual recipient of competitive exclusion therapy), which rarely attain an age when bowel cancer becomes a significant risk to

their longevity. Also, CE products are not so far available for human use and although it is unlikely that an undefined product would be allowed by the regulatory authorities, defined products remain a real possibility. However, this does rather beg

the question as to who would be the source of any **Competitive Exclusion** product developed for people..?

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