HUMAN MICROBIOTA IN HEALTH AND DISEASE

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ABSTRACT

Each human body plays host to a microbial population which is both numerically vast (at around $10^{14}$ microbial cells) and phenomenally diverse (over 1,000 species). The majority of the microbial species in the gut have not been cultured but the application of culture-independent approaches for high throughput diversity and functionality analysis has allowed characterisation of the diverse microbial phylotypes present in health and disease.

Studies in monozygotic twins, showing that these retain highly similar microbiota decades after birth and initial colonisation, are strongly indicative that diversity of the microbiome is host-specific and affected by the genotype. Microbial diversity in the human body is reflected in both richness and evenness. Diversity increases steeply from birth reaching its highest point in early adulthood, before declining in older age. However, in healthy subjects there appears to be a core of microbial phylotypes which remains relatively stable over time.

Studies of individuals from diverse geographies suggest that clusters of intestinal bacterial groups tend to occur together, constituting 'enterotypes'. So variation in intestinal microbiota is stratified rather than continuous and there may be a limited number of host/microbial states which respond differently to environmental influences. Exploration of enterotypes and functional groups may provide biomarkers for disease and insights into the potential for new treatments based on manipulation of the microbiome.
In health, the microbiota interact with host defences and exist in harmonious homeostasis which can then be disturbed by invading organisms or when ‘carpet bombing’ by antibiotics occurs. In a portion of individuals with infections, the disease will resolve itself without the need for antibiotics and microbial homeostasis with the host’s defences is restored. The administration of probiotics (live microorganisms which when administered in adequate amounts confer a health benefit on the host) represents an artificial way to enhance or stimulate these natural processes.

The study of innate mechanisms of antimicrobial defence on the skin, including the production of numerous antimicrobial peptides (AMPs), has shown an important role for skin commensal organisms. These organisms may produce AMPs, and also amplify the innate immune responses to pathogens by activating signalling pathways and processing host produced AMPs. Research continues into how to enhance and manipulate the role of commensal organisms on the skin. The challenges of skin infection (including diseases caused by multiply resistant organisms) and infestations remain considerable. The potential to re-colonise the skin to replace or reduce pathogens, and exploring the relationship between microbiota elsewhere and skin diseases are among a growing list of research targets.

*Lactobacillus* species are among the best known ‘beneficial’ bacterial members of the human microbiota. Of the approximately 120 species known, about 15 are known to occur in the human vagina. These organisms have multiple properties, including the production of lactic acid, hydrogen peroxide and bacteriocins, which render the vagina inhospitable to potential pathogens. Depletion of the of the normal *Lactobacillus* population and overgrowth of vaginal anaerobes, accompanied by the loss of normal vaginal acidity can lead to bacterial vaginosis – the commonest cause of abnormal vaginal discharge in women. Some vaginal anaerobes are associated with the formation of vaginal biofilms which serve to act as a reservoir of organisms which persists after standard antibiotic therapy of bacterial vaginosis and may help to account for the characteristically high relapse rate in the condition. Administration of *Lactobacillus* species both vaginally and orally have shown beneficial effects in the treatment of bacterial vaginosis and such treatments have an excellent overall safety record.

*Candida albicans* is a frequent coloniser of human skin and mucosal membranes, and is a normal part of the microbiota in the mouth, gut and vagina. Nevertheless *Candida albicans* is the most common fungal pathogen worldwide and is a leading cause of serious and often fatal nosocomial infections. What turns this organism from a commensal to a pathogen is a combination of increasing virulence in the organism and predisposing host factors that compromise immunity. There has been considerable research into the use of probiotic *Lactobacillus spp.* in vaginal candidiasis. Studies in reconstituted human epithelium and monolayer cell cultures have shown that *L. rhamnosus GG* can protect mucosa from damage caused by *Candida albicans*, and enhance the immune responses of mucosal surfaces. Such findings offer the promise that the use of such probiotic bacteria could provide new options for antifungal therapy.

Studies of changes of the human intestinal microbiota in health and disease are complicated by its size and diversity. The Alimentary Pharmabiotic Centre in Cork (Republic of Ireland) has the mission to
'mine microbes for mankind’ and its work illustrates the potential benefits of understanding the gut microbiota. Work undertaken at the centre includes: mapping changes in the microbiota with age; studies of the interaction between the microbiota and the gut; potential interactions between the gut microbiota and the central nervous system; the potential for probiotics to act as anti-infectives including through the production of bacteriocins; and the characterisation of interactions between gut microbiota and bile acids which have important roles as signalling molecules and in immunity.

One important disease entity where the role of the gut microbiota appears to be central is the Irritable Bowel Syndrome (IBS). IBS patients show evidence of immune activation, impaired gut barrier function and abnormal gut microbiota. Studies with probiotics have shown that these organisms can exert anti-inflammatory effects in inflammatory bowel disease and may strengthen the gut barrier in IBS of the diarrhoea-predominant type. Formal randomised trials of probiotics in IBS show mixed results with limited benefit for some but not all.

Studies confirm that administered probiotics can survive and temporarily colonise the gut. They can also stimulate the numbers of other lactic acid bacilli in the gut, and reduce the numbers of pathogens. However consuming live organisms is not the only way to influence gut microbiota. Dietary prebiotics are selectively fermented ingredients that can change the composition and/or activity of the gastrointestinal microbiota in beneficial ways. Dietary components that reach the colon, and are available to influence the microbiota include poorly digestible carbohydrates, such as non-starch polysaccharides, resistant starch, non-digestible oligosaccharides (NDOs) and polyphenols. Mixtures of probiotic and prebiotic ingredients that can selectively stimulate growth or activity of health promoting bacteria have been termed ‘synbiotics’. All of these approaches can influence gut microbial ecology, mainly to increase *bifidobacteria* and *lactobacilli*, but metagenomic approaches may reveal wider effects. Characterising how these changes produce physiological benefits may enable broader use of these tactics in health and disease in the future.

The current status of probiotic products commercially available worldwide is less than ideal. Prevalent problems include misidentification of ingredient organisms and poor viability of probiotic microorganisms leading to inadequate shelf life. On occasions these problems mean that some commercially available products cannot be considered to meet the definition of a probiotic product. Given the potential benefits of manipulating the human microbiota for beneficial effects, there is a clear need for improved regulation of probiotics.

The potential importance of the human microbiota cannot be overstated. ‘We feed our microbes, they talk to us and we benefit. We just have to understand and then exploit this.’ (Willem de Vos).

**Key words:** Microbiota, probiotic, Lactobacilli, commensals, microbiome, host immunity, bacterial vaginosis, Candida albicans, prebiotics, synbiotics, antimicrobial peptides, bacteriocins, staphylococcal skin infections, irritable bowel syndrome.
The human body is home to a vast and complex realm of microbes. Each of us is host to around $10^{14}$ microbial cells (weighing about 2kg) representing more than 1,000 species. Understanding the interaction of this microbiome with the host in both health and disease can provide important insights into how we can benefit from its presence.

In order to be tolerated by our bodies our intestinal flora are adapted for their environment and interact with our immune system, binding to intestinal cells and mucus and eliciting specific immune responses.

**FIGURE 1: IMPACT FROM INTESTINAL INTERACTIONS**

Numerically the microbiome dominates our bodies, dwarfing the amount of genetic material derived from our own cells (Figure 2).
The great majority (up to about 80%) of these microbial species have not been cultured and this has led to applications of novel culture-independent approaches to provide a phylogenetic framework for the study of the more than 1000 different intestinal species. In recent years the use of techniques for high throughput diversity and functionality analysis of the gastrointestinal tract microbiota has allowed fundamental questions about about the role of microbes in the gut to be explored6,7 (Figure 3).

The development of the human intestinal tract (HIT) chip, a phylogenetic microarray has allowed much more rapid analysis of the phylotypes represented in the abundant normal microbiota throughout life8. Comparative analysis with other methodologies such as pyrosequencing shows that this technique is robust and highly reproducible9.

Studies in monozygotic twins, showing that these retain highly similar microbiota decades after birth and initial colonisation is strongly indicative that diversity of the microbiome is host-specific and affected by the genotype10.

Microbial diversity in the human body shows both richness and evenness. Diversity increases steeply from birth reaching its highest point in early adulthood, before declining in older age11. Comparing the extremes of age shows marked differences in the composition of the microbiome with lower representation of butyrate producing species and an increased presence of potential pathogens in centenarians (Figure 4).
Diversity may be important and the composition of the microbiota may influence the health of babies from the earliest days of life. Babies crying because of intestinal colic showed significantly lower levels of microbial diversity than controls at 2 and 4 weeks. Representational Difference Analysis (RDA) analysis showed that 28% of this variance was explained by differences in a dozen microbial groups.$^{12}$

It appears that there is a common core of approximately 400 microbial phylotypes in normal healthy subjects. Sequential sampling of individuals suggests that each individual has an individual core of phylotypes which remains stable over time.$^{13}$ Individual variation may explain the bulk of variation in populations with even extreme dietary manipulation (e.g. a very low caloric diet) producing relatively low levels of variation (60% vs 10% respectively).

Studies of sequenced faecal metagenomes of individuals from different countries has identified specific clusters of co-occurring bacterial groups, termed enterotypes. Confirmation of these enterotypes in larger cohorts, indicates that variation in intestinal microbiota is generally stratified rather than continuous.$^{14}$ This, in turn, suggests the existence of a limited number of host–microbial symbiotic states that might respond differently to diet and drug intake. Clustering of phylotypes in enterotypes and functional groups has enabled the search for early biomarkers of disease as well as providing new insights into the potential for novel therapies based on manipulation of the microbiome.
EXPERIMENTAL MICROBIAL THERAPIES

When a clinical condition is clearly caused by disturbance of the normal gut microbiota, such as in *C. difficile* Infections (CDI), one possibility is to restore the normal intestinal microbiota through ‘transplantation’ of normal donor colonic or duodenal infusions. This ‘faecal transplantation’ has been tried in hundreds of cases over the last 50 years and seems to be characterized by a high degree of success.\(^{15}\)

Recent studies have allowed the application of modern analysis to characterise the changes of microbiota on transplantation.\(^{16}\) Deep and high throughput analysis of the microbiota for several months after transplantation using the HIT Chip confirmed the correction of the microbiota:

- Initial low diversity in patients with CDI
- Correction of low diversity by the transplantations
- Stability of the newly established diverse community
- A clear shift in the microbiota of the recipients towards the donor signature, correcting the effect of the patients’ gram-negative pathogens.

IMPACT OF GI TRACT MICROBES ON OBESITY

Faecal transplantation studies in mice show that transferring the microbiota from lean and fat mice to germ-free mice induces greater weight gain in those receiving the ‘fat’ microbiota. The discovery of this obesity-associated microbiome,\(^{18}\) raises the possibility of using transplanted microbiota to influence metabolic processes in humans.

In a controlled study (autologous transplantation) examining the effect of faecal transplantation from lean subjects to those with metabolic syndrome, results showed significant improvement of whole body insulin sensitivity at 6 weeks.\(^{19}\)

SUMMARY AND CONCLUSION

Recent years have witnessed a microbiota revolution as we have utilised methodologies that allow us to study the diversity, enterotypes, and functional signatures of the intestinal microbiota. This has enabled the recognition of early biomarkers, provided new insights into diseases, and helped to develop novel therapies. Importantly it has also allowed us to focus on human rather than model animals.

The potential importance of the human microbiota cannot be overstated. Put simply, we feed our microbes, they talk to us and we benefit. We just have to understand and then exploit this.
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The gastrointestinal tract shows progressive colonization from the mouth to the colon with markedly different proportions of bacterial phyla present depending on the site of sampling. Humans are born sterile, but during birth a microbial population starts to establish in the gut. At first, the composition is very variable, with mainly facultative anaerobic or aerobic bacteria but after approximately 2 years it stabilizes. Composition of the large bowel is then mainly obligate anaerobes, Bacteroides and Firmicutes, such as clostridia.

The gut microbiota is affected by a number of factors including: mode of delivery at birth (vaginal or caesarean section), host genotype and lifestyle factors (including dietary habits). Systemic antibiotics can have long term effects on microbial composition of the gut and can disrupt some bacterial groups for several years.

The characterization of the human microbiota employs many tools and has given rise to entire fields of study (Figure 1).

The next generation of gene sequencing technology will enable enormous increases in the speed of sequencing at greatly reduced cost. Combination of 454-pyrosequencing of a hyper-
variable region of the 16S rRNA gene in combination with sample specific barcode sequences, enables parallel in-depth analysis of hundreds of samples with limited sample processing\(^1\).

**FIGURE 1**

Modeling demonstrates that the method correctly describes microbial communities down to phylotypes below the genus level. Determining the microbial composition in patients and healthy controls using this technology may also provide novel therapeutic targets.

**FIGURE 2: HEATMAP OF BACTERIAL DIVERSITY**

Figure 2 shows a ‘heatmap’ of bacterial diversity illustrating the increase in diversity in samples from infants at different ages compared to their mother.
Figure 3 shows the number of phylotypes sampled from different sites in the gastrointestinal tract as a function of the number of reads. Illustrated here is the impact of *Helicobacter pylori* infection.

FIGURE 4: INFLAMMATORY BOWEL DISEASE IN TWINS (T-RFLP)

Using these techniques we can compare the microbiota of subjects with and without diseases and study e.g. the effects of host genotypes on disease. Figure 4 shows the T-RFLP profiles for
a healthy twin pair (left), a discordant twin pair (middle) and a concordant twin pair (right). The size of the pie slice represent relative abundance value. Black, blue, green, red and purple TRFs are also represented with the same color coding for Bacteroides sp. primers.

We have also been able to show the changes in diversity over time in the microbiota of infants whether they were born vaginally or by caesarean section. The genus abundance over time shows clear differences at early time points which largely disappear at 24 months under the influence of external factors such as feeding.

These techniques have also been used to study the gut bacteria of Ötzi – the iceman – an individual who lived in 3300 BC and was discovered well preserved in ice 5 millenia later. Sampling from various sites in the gastrointestinal tract yielded an impressive list of pathogens and associated virulence factors.

**SUMMARY**

The use of 454-pyrosequencing of a hyper-variable region of the 16S rRNA gene in combination with sample specific barcode sequences has great potential to help in our understanding of the human microbiota in health and disease. Pilot studies are already underway in a number of diseases and areas of interest including:

- Bacterial vaginosis
- Psoriasis
- Paradontitis, neutropenia
- Ulcer and gastritis
- Neonatal necrotizing enterocolitis
- Probiotics, Allergy, Birth

**REFERENCE**


**ADDITIONAL READING**


Probiotics are live microorganisms which, when administered in adequate amounts, confer a health benefit on the host\(^1\).

Probiotics set a number of challenges for microbiologists, namely:

**Terminology**
- Defining the terminology associated with probiotics

**Guidelines**
- Defining the characteristics of probiotics according to guidelines

**Methodology**
- Developing new tools to evaluate preclinical and clinical efficacy or to better understand the mechanisms of action.

**Safety**
- Monitoring of issues (i.e. genetic stability, antibiotic-resistance, local adverse effects) occurring over time.

In the last 2 decades (from June 1989 to March 2011) 6806 studies of probiotics have been published in indexed journals most dealing with efficacy but few addressing the quality of marketed probiotic products. Over approximately the same period there has been an evolution in the therapeutic areas where probiotics have been claimed to offer benefit. In the early 1990s these products were mainly promoted for diarrhoea, but in the last decade this has been generalised to intestinal dysfunction and irritable bowel syndrome with atopic diseases joining the list in 2008.

Probiotics vary greatly in popularity between countries: in 2009 in Italy there were 206 manufacturers of 1609 products of this type. In some cases, the sales of individual products seem inversely proportional to the amount of scientific literature generated about them.

The Guidelines drafted in 2002 by FAO / WHO\(^1\) proposed important guidance for identifying and characterizing organisms to species level, assessing safety and conducting clinical studies of efficacy.

The first of these is important since different strains of the same species may act in different ways and produce different results. For this reason it is absolutely essential to identify the strain of organism to be used as a probiotic and there are well established ways to do this by genotyping. However it has been known for some time that probiotic supplements may not contain what they are promoted to contain, may be of deficient microbiological quality, and may make misleading health claims\(^2,3\).

Some of the more frequent misidentifications of ingredient organisms include:
L. crispatus instead of L. acidophilus

L. gasseri instead of L. acidophilus

L. paracasei instead of L. casei or L. jughurti

and even Streptococcus sanguis instead of L. Acidophilus

Some probiotic organisms have been given made up names, presumably for marketing purposes, including: Bifidus actiregularis, Lactobacillus immunitass, Bifidus attivo essensis.

One important problem for manufacturers to overcome is the need for sufficient viable organisms to be delivered in the product, since a large load may be needed to survive gastric acid exposure. This consideration leads to recommendation to take doses of the order of 10^8 – 10^{11} colony forming units (cfu) per dose, one to several times per day.

Studies of probiotic products commercialised in Italy have consistently revealed deficiencies in their probiotic attributes. Lactobacillus sporogenes, or Bacillus coagulans, as it should be correctly classified, represents the archetypal misidentified probiotic. Bacillus probiotics could offer some advantages, such as low cost of production processes, ease of preparation, resistance to production process and extended shelf life over a wide range of temperatures. Although the use of L. sporogenes spores as a probiotic has increased in recent years, clinical evidence of its benefits are limited to only a few studies involving small patient populations.

More recently we conducted a study to determine if products available in the USA market in 2009 were correctly labeled in terms of quantity of viable bacteria, identification of species and cross contamination by species not on the label. Disturbingly, we found that only 4 of 13 products (31%) were in accordance with label claims. Furthermore 6/13 (46%) contained less viable bacteria than claimed, 5/13 (38%) had at least one species missing, and 7/13 (54%) contained contaminant organisms (Bacillus spp., Staphylococcus spp., Enterococcus faecium, molds).

In a similar, as yet unpublished study with 24 commercial products available in the Italian market in 2010, we found: 15/24 (62.5%) products in accordance with the label; 9/24 (37.5%) containing less viable bacteria than declared (regarding one species at least); 4/24 (17%) with at least one species missing; and 2/24 (8%) containing contaminant Enterococcus faecium.

Nonetheless, and considering their widespread use, probiotics have not been associated with many significant safety issues. There have been no deaths apparently due to probiotics (except for one study with acute pancreatitis patients). With regard to Lactobacillus bacteremias there have been 180 cases in 30 years and 69 cases of endocarditis.

There have been case reports of outbreaks caused by S. cerevesiae and S. Boulardii. One safety question that has arisen is ‘Can probiotics transfer antimicrobial resistance to other bacteria?’. From this several subsidiary questions arise:
Is co-administration of antibiotics and probiotics appropriate?
Can a probiotic develop resistance?
Can resistance become stable?
Can a probiotic transfer resistance?

We have shown resistance to macrolides and/or tetracycline in 14 and 4 isolates respectively of 40 isolates of *Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus crispatus*, and *Lactobacillus casei* isolated from faeces of apparently healthy volunteers. Serial exposure to antibiotics led to selection of resistant mutants. However, acquired resistance was rather unstable and was lost after subcultures in antibiotic-free medium in most mutants. These findings lead us to recommend that since lactobacilli are often used as probiotics, their ability to acquire resistance should be evaluated for isolates that may be candidates to be included in probiotic based products.

In an unpublished study we examined the presence of antibiotic resistance in Italian probiotics. In 13 commercial products, containing 21 isolated strains we found:

- 12/13 (92%) products containing at least one strain showing resistance to at least one antibiotic
- 10/13 (77%) products containing at least one strain harbouring known resistance genes

And among the isolates:

- 5/21 (24%) erythromycin-resistant strains
- 2/21 (9.5%) tetracycline-resistant strains
- 14/21 (67%) gentamicin-resistant strains
- 0/21 (0%) penicillin-resistant strains

**SUMMARY**

Strain identification still remains a worldwide problem. Some products are misnamed and not correctly identified. A combination of proper molecular methods seems the most suitable approach to this problem. Viability of probiotic microorganisms is essential, but some marketed products do not possess a suitable shelf-life. It is possible that resistant genes may be spread via some probiotics. It is apparent that some commercially produces products should not be included on the list of true probiotics. There are many “fancy names” and much “mislabelling” and there is a clear need to improve regulatory issues.

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**MODES OF ACTION OF MICROBIOTA RESTORATION** - Dr Gregor Reid
**Lawson Health Research Institute, University of Western Ontario, London, Canada.**

Inside every human, potentially pathogenic and non-pathogenic organisms can be found. When infection occurs, it can be due to an external source or by propagation of the person’s own organisms. Antibiotics and other chemicals can disturb the microbiota by essentially carpet bombing a niche and killing pathogens as well as so-called beneficial organisms. It takes many weeks for homeostasis to recover. In a portion of patients with infection, such as in the bladder, vagina or respiratory tract, infection can self-resolve without the intervention of antibiotics. This, and the damage caused by antibiotics, has lead to a search for ways to understand and restore microbial homeostasis more quickly.
Probiotics are "live microorganisms which when administered in adequate amounts confer a health benefit on the host". Strains of probiotics have been examined for a role in preventing and managing infections, especially in the gut and vagina.

A key factor in applying probiotic interventions is to understand how the microbiome develops from the moment of birth, and thereby identifying what strains might foster a niche dominated by bacteria that benefit the host.

The assembly of the human infant gut microbiome is characterised by an increase in phylogenetic diversity over time. However the abundance of major taxonomic groups shows abrupt shifts corresponding to life events in the infant such as dietary changes from milk to solid food, with the functional genes present reflecting the foods to which the gut is exposed.

So how might probiotics be used to affect the microbiota of the gut and vagina? McNulty et al. found ingestion of probiotic yogurt did not result in the organisms taking up residence in the human or mouse gut, nor marked alteration in the composition of the host gut microbiota, but it did alter metabolic pathways in the resident microbiota, particularly those related to carbohydrate processing. In other words, it had an effect on the host.

The study involved faecal sampling from adult female monozygotic twin pairs after consumption of a commercially available fermented milk product (FMP) containing a consortium of *Bifidobacterium animalis* subsp. *lactis*, two strains of *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactococcus lactis* subsp. *cremoris*, and *Streptococcus thermophilus*. There were no significant changes in bacterial species composition or in the proportional representation of genes encoding known enzymes observed in the faeces of the humans consuming the FMP. However the metatranscriptome exhibited significant changes, including most prominently those related to plant polysaccharide metabolism.

In other studies, Jacques Ravel’s group characterized the vaginal microbiome of 396 asymptomatic North American reproductive-age women who represented four ethnic groups (white, black, Hispanic, and Asian). Species composition was determined by pyrosequencing of barcoded 16S rRNA genes. The resident bacterial communities clustered into five groups: four were dominated by *Lactobacillus iners*, *L. crispatus*, *L. gasseri*, or *L. jensenii*, whereas the fifth had lower proportions of lactic acid bacteria and higher proportions of strictly anaerobic organisms, indicating that a key ecological function, the production of lactic acid, seems to be conserved in all communities. The proportions of each community group varied among the four ethnic groups, and these differences were statistically significant. This study indicates that the inherent differences within and between women in different ethnic groups may govern their risk of diseases including bacterial vaginosis (BV). But, it remains to be determined why ethnicity per se would cause this, or whether particular dietary intakes or sexual practices have over-riding influence.

Longitudinal studies of women show changes in the vaginal microbiota over time in individuals, largely coincident with menstruation, but there are differences between individuals, albeit generally lactobacilli are dominant in healthy women. In a study by Hummelen et al., four...
different forms of BV were identified based upon abundance of different pathogens. These differences may affect the success of the treatment options that were developed mostly to eradicate *Gardnerella*, not the other infecting strains. Lactobacilli can be present in low numbers during BV, and depending on the species, this can influence recovery of the microbiota.

A study of *Lactobacillus crispatus* CTV-05 administered vaginally after topical antibiotic treatment of BV showed that vaginal concentration of certain BV-associated bacteria (*Gardnerella vaginalis* and *Atopobium* spp.) and the absence of endogenous *L. crispatus* at enrollment predicted colonization with the probiotic lactobacilli.

Placebo controlled clinical trials have confirmed that probiotic *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 improve outcomes when used during and after single dose antimicrobial treatments for both BV and vulvovaginal candidiasis. Why these combinations work is the subject of considerable research. Recent work by Kohler *et al.* indicates that lactobacilli repress *Candida* genes involved in biofilm formation and fluconazole resistance and this may help explain the success of conjoint treatment with the probiotic and the antibiotic in eradicating the fungi.

Lactobacilli have a number of properties, including production of surfactants, hydrogen peroxide and bacteriocins, that are relevant to resisting pathogens (figure 1).

The vagina may be subject to bacterial overgrowth of two distinct types. Aerobic vaginitis (AV) is characterized by inflammation, yellow discharge, and vaginal dyspareunia. In this condition, group B streptococci, *Escherichia coli*, *Staphylococcus aureus* and *Trichomonas vaginalis* are frequently cultured. While BV is associated with an overgrowth of vaginal anaerobes and in some cases an offensive, fishy-smelling discharge, AV is an inflammatory condition that leads to high production of cytokines in the vaginal fluid.

**FIGURE 1.**

<table>
<thead>
<tr>
<th>Biosurfactant Production</th>
<th>Bacteriocin and Hydrogen Peroxide Production</th>
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<tbody>
<tr>
<td>Biosurfactants are produced by lactobacilli and their presence on the mucosal surface helps prevent adhesion and infection by the pathogenic organisms.</td>
<td>Lactobacilli produce substances that can inhibit the growth or kill pathogens. Illustrated here are hydrogen peroxide and bacteriocins.</td>
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<tr>
<td>Lactobacilli</td>
<td>Non-pathogenic cocci</td>
</tr>
<tr>
<td>Bifidobacteria</td>
<td>Gram negative pathogens</td>
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<td>Gram positive pathogens</td>
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In combatting bacterial overgrowth caused by aerobic or anaerobic bacteria, vaginal lactobacilli may be important in disrupting bacterial biofilms. The best known biofilm former, *Pseudomonas aeruginosa*, employs an intercellular communication system, known as quorum sensing (QS) to coordinate the expression of tissue-damaging factors. The QS system controls the production of different virulence factors. The results of real time quantitative polymerase chain reaction (PCR) have shown that in all *P. aeruginosa* strains grown in the presence of probiotic culture sterile filtrates, the level of QS genes expression was reduced comparatively with those from control cultures\(^1\). In the case of *Lactobacillus iners*, quorum sensing may help it adapt to an infected state, and form a nidus to recovery in some women.

Bacteriocins are molecules that inhibit the growth of like-species, but they can also act as quorum sensing agents. Scientists from the Alimentary Pharmabiotic Centre in Cork have demonstrated that *Lactobacillus salivarius* UCC118, a probiotic strain of human origin, produces a bacteriocin in vivo that can significantly protect mice against infection with the invasive foodborne pathogen *Listeria monocytogenes*\(^1\). This illustrates another mechanism whereby infection can be averted or overcome.

Indigenous or probiotic lactobacilli (Figure 2) may also help maintain the barrier functions of epithelial tight junctions. These linkages can be compromised by inflammation and pathogens\(^1\), inducing recruitment of macrophages and other host defenses to the site, leading to vaginal discharge.

**FIGURE 2**

Modulation of Tight Junctions
Damage to epithelial tight junctions by inflammatory processes (A) or pathogens (B) can lead to infection and sepsis, as well as impaired nutrient uptake. The ability of lactobacilli to up-regulate tight junction proteins helps prevent these adverse events (C).

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**FIGURE 3**

Signaling effects
Bacteria communicate through a number of signaling mechanisms including quorum sensing. In this illustration, the Lactobacillus signaling molecules down-regulate toxin production in the Gram negative pathogen (for example *E. coli* 0157:H7 in the gut) and Gram positive pathogen (for example *S. aureus* on the vaginal surface).

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LACTOBACILLI SIGNALLING SYSTEMS (Figure 3)

Some strains of Lactobacillus have signalling systems involved in downregulating toxin production in pathogenetic bacteria. A recent study shows that human vaginal isolate Lactobacillus reuteri RC-14 produces small signaling molecules that are able to interfere with the staphylococcal quorum-sensing system agr, a key regulator of virulence genes, and repress the expression of TSST-1 in S. aureus MN8. This provides an example of one mechanism by which interspecies cell-to-cell communication between endogenous or probiotic strains and pathogens might attenuate virulence factor production by the latter.

Lactobacillus reuteri RC-14 can also disable a potent S. aureus exotoxin, toxic shock syndrome toxin (TSST-1). Intriguingly, vaginal swabs from women with BV and from normal women appear to suppress toxin production from S. aureus MN8 (manuscript in preparation).

Using atomic force microscopy, Younes et al. have shown that the adhesion force of L. crispatus 33820 with S. aureus MN8 is greater than for the pathogen and the surface, indicating that the staphylococci are more likely to adhere to the lactobacilli than to remain on the surface. Notably, attachment occurs on contact and bond maturation occurs within a mere 120 seconds of physical contact between the lactobacilli and staphylococci. Co-aggregation is now one of the recognized mechanisms through which lactobacilli can exert their probiotic effects to create a hostile micro-environment around a pathogen.

Modulation of host immunity by non-pathogens might maintain immune homeostasis and preserve the microbiota composition and structure. This provides another potential mechanism by which probiotic strains might influence host defences. There is some evidence that lactobacilli also enhance local immunity. Human, animal and in vitro studies have shown that L. rhamnosus GR-1 down-regulates TNFα, PGE and Cox-2 inflammatory mediators and up-regulates IL-10, G-CSF, Treg cells, and antimicrobial IL-8, vaginal defensins α1, β2, SLPI, NOD2, TLR2, TLR9, CD4 and elafin in HIV patients.

FIGURE 4: THE INTERACTOME: THE TOOLBOX IS FILLING UP (adapted from Bisanz and Reid)
New techniques (e.g. meta-transcriptional and metabolomic profiling) will increasingly enable us to answer questions about what happens in the ‘Interactome’ between the human host, the microbiota and probiotic strains21 (figure 4).

**SUMMARY**

There are many cases in which infections resolve without intervention, likely due to host defenses and the microbiome’s ability to restore homeostasis. Probiotics are an artificial way to simulate or enhance these natural processes. We now have the tools and probiotic strains to demonstrate that microbiota restoration is possible on the skin, gut and vagina.

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SKIN MICROBIOTA: A SOURCE OF DISEASE OR DEFENSE? Dr Jürgen Schaubertn 
Department of Dermatology and Allergy, Ludwig-Maximilian University Munich, Germany.

Our skin is a natural defence barrier and elements important in this function include:

- physical barrier
- chemical barrier
- peptide barrier
- recognition system
- communication system
- resident/non-resident cells

The epidermis is in effect an immune organ of considerable complexity. At the same time the skin has a resident microbial flora which is always present and only occasionally causes problems.
ANTIMICROBIAL PEPTIDES

The skin is exposed to many pathogens including bacteria, viruses and fungi that cause familiar diseases. One element of protection against these is the production of antimicrobial peptides, constituting an innate antimicrobial defense mechanism.

Peptides with antimicrobial activity (AMPs) are found throughout nature in plants, insects, amphibians and mammals. Many proteins and peptides with antimicrobial properties (in vitro) have been found in skin. Table 1 gives a partial listing of these.

<table>
<thead>
<tr>
<th>AMP in keratinocytes</th>
<th>AMP first ID as protease inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cathelicidins</td>
<td>Cathelin</td>
</tr>
<tr>
<td>β-defensins</td>
<td>SLPI</td>
</tr>
<tr>
<td>BPI</td>
<td>SKALP</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>P-cystatin α</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>Cystatin C</td>
</tr>
<tr>
<td>Dermcidin</td>
<td></td>
</tr>
<tr>
<td>RNase 7</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>AMPS in infiltrating cells</th>
<th>AMP first ID as chemokines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cathelicidin</td>
<td>Psoriasin</td>
</tr>
<tr>
<td>α-Defensins</td>
<td>CXCL9</td>
</tr>
<tr>
<td>Granulysin</td>
<td>IP 10 (CXCL10)</td>
</tr>
<tr>
<td>Perforin</td>
<td>I-TAC (CXCL11)</td>
</tr>
<tr>
<td>Eosinophil cationic protein (ECP)/RNase 3</td>
<td></td>
</tr>
<tr>
<td>RANTES</td>
<td></td>
</tr>
<tr>
<td>PF4</td>
<td></td>
</tr>
<tr>
<td>CTAP-3</td>
<td></td>
</tr>
<tr>
<td>Platelet basic Protein</td>
<td></td>
</tr>
<tr>
<td>Fibrinopeptide A, B</td>
<td></td>
</tr>
<tr>
<td>Thymosin b-4</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>AMP first ID as neuropeptides</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-MSH</td>
</tr>
<tr>
<td>Bradykinin</td>
</tr>
<tr>
<td>Neurotensin</td>
</tr>
<tr>
<td>Chromogranin A,B</td>
</tr>
<tr>
<td>Proenkephalin A</td>
</tr>
<tr>
<td>Neuropeptide Y</td>
</tr>
<tr>
<td>Polypeptide YY</td>
</tr>
<tr>
<td>Adrenomedullin</td>
</tr>
</tbody>
</table>

There is evidence that these peptides, although they may have other functions, do indeed have a role in antimicrobial defence. For example, studies in transgenic mice that cannot produce cathelicidins suggest that these peptides are an important native component of innate host defence in mice and provide protection against invasive skin infection caused by Group A Streptococcus (GAS)\(^1\). Human keratinocytes secrete the S100 protein psoriasin which appears to have an important role in inhibiting *E. coli* survival on human skin\(^2\).

AMPs are induced in skin in response to injury or infection\(^3\). Cells at wound edges express cell surface AMPS which kill bacteria as part of a nonimmune defense mechanism. These AMPs have been demonstrated to have broad antimicrobial activity against a range of potential...
pathogens. After secretion a single AMP gene product or ‘pro-peptide’ may be processed into multiple peptides with various antimicrobial properties, which may make it more difficult for pathogens to generate resistance.

Cutaneous AMPs such as the cathelicidins act as signaling molecules (figure 1) to promote a wide variety of immune functions. Thus as well as being inducible elements of innate immunity in skin they not only function as endogenous antibiotics but also as ‘alarmins’ to prime other repair and defence processes.

**FIGURE 1: CUTANEOUS AMPs SUCH AS THE CATHELICIDINS ACT AS SIGNALING MOLECULES**

<table>
<thead>
<tr>
<th>Induction of angiogenesis</th>
<th>Antimicrobial activity</th>
<th>Promotion of keratinocyte migration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cathelicidin hCAP18/LL-37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhibition of keratinocyte apoptosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytokine/chemokine release from keratinocytes/leukocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Promotion of wound healing</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Greater understanding of the role of AMPs in cutaneous innate immune function has led to exploration of their role in patients where these functions appear disordered. For example atopic individuals suffer cutaneous inflammation and are susceptible to inflammation leading some workers to question whether expression of AMPs is normal in atopic conditions. Early work in this field supported the hypothesis that there may be decreased expression of AMPs in atopics. However later work in atopic eczema showed enhanced expression of the human cathelicidin LL-37 in lesional skin compared with nonlesional skin, leading the authors to suggest that LL-37 might be associated with the process of re-epithelialization. Recent work suggests that injury downregulates the expression of cathelicidin protein hCAP18/LL-37 in atopic dermatitis (AD). Since itching is a primary symptom of AD and scratching inevitably injures the skin, failure to upregulate AMPs in eczema following injury is likely to affect antimicrobial protection and tissue repair in AD.

**REGULATION OF AMP PRODUCTION IN SKIN**

Toll-like receptors (TLRs), thanks in part to the pioneering work of Jules Hoffmann and Bruce Beutler, are now known to play an important role in alerting the immune system to the presence of a variety of molecules associated with microbial pathogens (figure 2).
Activation of TLRs induce AMP in skin and mediators of inflammation induce AMP expression (figure 3).

The search for cathelicidin AMP inducing factors has revealed an important role for dietary sources of cholecalciferol (Vitamin D3) including fatty fish species (e.g. catfish, salmon, mackerel, sardines, tuna, eel), whole egg, beef liver and fish liver oils, such as cod liver oil. Some foods, notably milk, dairy products and fruit juice are now also fortified with the vitamin.

The metabolism of Vitamin D3 takes place partly in the skin (figure 4) and this substance is now known to have an important role in some key functions of skin:

- VD3 regulates proliferation, differentiation of keratinoytes
- VD3 analogs have been used for treatment of inflammatory skin diseases
VD3 effects adaptive immunity
Keratinocytes produce VD3
VD3 influences innate immunity in skin

Vitamin D3 metabolism may be activated in skin wounds. CYP27B1 – which activates 1,25D3 – is induced in wounds 24 hours after injury and is induced by factors of the wound micromilieu in vitro. Vitamin D3 induces TLR2, CD14 in keratinocytes and enables increased innate immune responses and increased antimicrobial activity. Topical vitamin D induces cathelicidin AMP in human skin and oral vitamin D induces cathelicidin AMP in human skin in atopic patients. The current model of cathelicidin AMP activation in keratinocytes is illustrated in figure 5.
In summary AMPs are regulated by TLR activation, skin inflammation and vitamin D. This opens up some intriguing possibilities for future treatments with possible roles for vitamin D supplementation and TLR activation in the treatment of atopic inflammation and infectious skin disease\textsuperscript{10}.

**ROLE OF COMMENSAI FLORA**

Recent work shows that skin commensals affect AMP expression by keratinocytes\textsuperscript{11}. Activation of TLR2 by a small molecule produced by *S. epidermidis* increases antimicrobial defence against bacterial skin infection. Commensals have also been shown to produce AMPs: phenol-soluble modulins (PSM) are secreted by *S. epidermidis* and selectively kill pathogens such as *Streptococci* (GAS)\textsuperscript{12}. Skin commensal staphylococci amplify the innate immune response to pathogens by activation of distinct signaling pathways\textsuperscript{13}. Also *S. epidermidis*-derived delta-toxin cooperates with the host-derived antimicrobial peptides in the innate immune system to reduce survival of pathogenic group A *Streptococcus*\textsuperscript{14}. AMPs produced by epithelial cells are differentially processed and inactivated by commensals\textsuperscript{15} (in this study *Finegoldia magna*) and pathogens (*Streptococcus pyogenes*).

In summary, commensals such as *S. epidermidis*:

- produce AMPs to kill pathogens
- produce toxins to enhance AMP activity
- process AMPs to fight pathogens
- activate TLRs to induce cutaneous AMP production
- activate signaling pathways to induce AMPs

So the commensal bacteria resident on the skin can certainly be added to the important elements that constitute the innate immunity of skin.

The work done so far leads to some stimulating open questions including:

- *Which commensals are the most beneficial?*
- *What factors of commensals regulate AMPs?*
- *How can beneficial commensals be supported?*

When we can answer these questions we will be closer to knowing how we can exploit these mechanisms for the prevention and/or therapy of infectious skin disease or skin diseases associated with barrier dysfunction.

**REFERENCES**


THE SKIN - MICROBES AND INFECTION  Professor Roderick J. Hay  
Kings College Hospital, London and International Foundation for Dermatology, UK.

THE SKIN MICROBIOTA

The human skin is a normal habitat for many microbes including bacteria – *Staph epidermidis*, *Propionibacterium acnes*, micrococci, coryneforms, brevibacteria, and fungi – e.g. *Malassezia* species, but no viruses. Among ectoparasites, only *Demodex folliculorum* is a frequent resident.

As well as the ‘resident’ microbiota, normal skin may be a temporary site of carriage for other bacteria and fungi e.g. *Staph aureus*, *Candida albicans*, and intermittently occurring residence, as with the *Herpes simplex* virus. The presence of diseased or damaged skin (e.g. in eczema) may also change the nature of the resident microbiota.

The commensal microbiota of the skin can, on occasions, cause problems. For example contamination of transdermal medical procedures e.g. with *Staph epidermidis* can be a source of septicaemia in neutropenic patients. In other cases, with e.g. *Propionibacterium acnes*, and *Malassezia*, alteration in local conditions in the skin changes the way the microbe behaves in ways that can cause problems for the host.

The skin has a natural defence system and factors including epidermal growth, antimicrobial peptides (e.g. cecropins and magainins), fatty acids, pH, and immunity (both epidermal/systemic) contribute to the barrier against pathogens.

Skin infections are common and can cause considerable morbidity. Table 1 shows the global burden of illness and cost per disability-adjusted life year (DALY) of treating the three commonest skin diseases caused by infection.

**TABLE 1: COST OF CURE AND IMPACT ON DALY’S- 3 COMMONEST DISEASES**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Estimated Prevalence</th>
<th>Cost of cure $ per 1x10^6</th>
<th>Cost ($) per DALY gained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tinea capitis</td>
<td>78 x 10^6 (SubSah Af)</td>
<td>5,250,000</td>
<td>175</td>
</tr>
<tr>
<td>Scabies</td>
<td>600 x10^6</td>
<td>58,000</td>
<td>1.00-1.50</td>
</tr>
<tr>
<td>Pyoderma</td>
<td>400x10^6</td>
<td>55,000</td>
<td>1.00-1.50</td>
</tr>
</tbody>
</table>

SKIN INFECTIONS

There are 4 stages common to all microbial skin infections illustrated in figure 1, but examining specific infections is instructive.
MALASSEZIA

_Malassezia_ is a commensal yeast on skin – particularly on hair-bearing skin of the scalp and upper trunk. It is a mainly lipophilic microbe with a characteristic cell wall structure and polar bud formation and has been known to cause disease in animals and humans. It is associated with specific enzymes e.g. phospholipases and melanin formation.

The complete _Malassezia globosa_ and _M restricta_ genomes have now been sequenced. That of _M. globosa_ represents the smallest genomic of a free-living eukaryotic cell – 300 times smaller than the human genome. The closest relative to this organism is _Ustilago maydis_ – common crop pathogen (corn smut).

_Malassezia_ spp (including _M.globosa, sympodialis, furfur, restricta, slooffiae, obtusa, dermatitidis, nana_) have been associated with a number of skin diseases:

- Pityriasis versicolor
- _Malassezia_ folliculitis
- Seborrhoeic dermatitis
- Atopic dermatitis affecting the head and neck
- Deep infection (only in neonatal period)

PITYRIASIS VERSICOLOR

This common and harmless skin condition, characterised by a fine scaly rash that gives the skin a variable colour (pink or fawn on pale skin but with pale patches on tanned skin) is usually caused by _M. globosa_. Spontaneous remission is rare. There is no evidence of activation of T lymphocyte responses in Pityriasis versicolor, suggesting ineffective immunity. Suggested
reasons for this include diversion of immune cells, and importantly, inhibition of immune systems by yeast lipid.

SEBORRHOEIC DERMATITIS AND DANDRUFF

The role of *Malassezia* spp in seborrhoeic dermatitis and dandruff has an interesting history. In 1874 Malassez described a fungus associated with dandruff or pilade. In 1976 Leyden and Kligman conducted some experiments and concluded: 'The studies demonstrate that the increased number of scalp microorganisms found in dandruff occurs as a secondary event to increased nutrients and that scalp organisms play no primary role in the pathogenesis of dandruff'. In 1984, Shuster essentially 're-asserted' the central role of this fungus in dandruff, upon which current antifungal treatments for this condition is based.

Seborrhoeic dermatitis and dandruff have typical clinical features of erythema, greasy scales and a typical distribution – involving scalp, eyebrows, behind the ears, and front of the chest. Seborrhoeic dermatitis is a severe form of dandruff but involving other skin sites.

Seborrhoeic dermatitis (SD) is a common condition but also has a number of clinical associations with more serious disease – including tertiary syphilis, chronic neurological disease and AIDS. There appears to be no correlation between species and disease i.e. all *Malassezia* spp have been grown from SD, but three dominate: *M. globosa* (only certain genetic strains), *M. furfur* and *M. restricta*.

In immunological studies no correlation is seen between T or B lymphocyte activity or cell infiltrates and disease. In SD patients, unaffected and affected skin show similar cytokine and immune cells profiles (IL-2 and IL-6 to IFN γ and TNF α). Increased numbers of organisms are observed in AIDS and this is associated with the CD4 count but not the presence of SD.

Sebum has been thought to play a role in dandruff and SD, but sebum composition in patients is highly variable and unrelated to *Malassezia* colonisation or disease severity. However *M. globosa* can produce oleic acid on dandruff-susceptible individuals.

The results of genomics and proteomics show that the *M. globosa* genome encodes for 14 lipases, and 9 phospholipases. Most lipases are expressed on the scalp, but not in culture medium and 8 lipases and 3 phospholipases have been identified. We can conclude that secreted enzymes probably enable survival on the scalp but may damage scalp cells.

*Malassezia* behaves differently in normal and SD skin with higher production of metabolites with immunological activity such as malassezin, and indolcarbazol from *M. furfur* isolates in SD compared to normal skin.

Figure 2 provides a summary of the known immunological interactions in pityriasis versicolor and seborrhoeic dermatitis.
BACTERIAL INFECTIONS OF THE SKIN

Bacteria can cause skin infections of varying severity and the following is a non-exhaustive list:

- Impetigo – *Staph aureus*, streptococci
- Folliculitis/furunculosis (boils)
- Cellulitis and erysipelas
- Necrotizing fasciitis
- Erythrasma
- Gram negative infections
- Cutaneous Tuberculosis
- Rarities eg bacillary angiomatosis
- Secondary bacterial infection eg eczema

STAPHYLOCOCCAL SKIN INFECTION

Impetigo, boils, and folliculitis are caused by *Staphylococcus aureus* (infections can sometimes cause staphylococcal scalded skin syndrome (SSSS) and toxic shock associated with the production of exotoxins). In 1985 in the UK, 90% of *S. aureus* were resistant to penicillin (β-lactam) 32% to erythromycin, and 76% to tetracycline.

Multiple drug resistance in staphylococci is a major and growing problem (figure 3) and is now subdivided into:
Hospital acquired methicillin resistant *Staphylococcus aureus* – HA-MRSA
Community acquired methicillin resistant *Staphylococcus aureus* – CA-MRSA

**Figure 3:** Spread of MRSA associated with skin and soft tissue infection (SSTI) 2001-05 Baltimore

Table 2 summarises the difference between HA and CA MRSA. The latter has a particular propensity to cause skin and soft tissue infections and, in contrast to HA-MRSA, occurs often in immunocompetent individuals.

**Table 2:** Differences between hospital-acquired (HA) versus community-acquired (CA) methicillin resistant *Staph aureus* (MRSA)

<table>
<thead>
<tr>
<th>HA-MRSA</th>
<th>CA-MRSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mainly in hospital</td>
<td>Mainly in community - causes SST infections in hospital</td>
</tr>
<tr>
<td>Often immunosuppressed or chronically ill</td>
<td>Often immunocompetent</td>
</tr>
<tr>
<td>Multiple drug resistance including βlactams</td>
<td>Resistant to β lactams</td>
</tr>
<tr>
<td>Causes all infections</td>
<td>75% in SST infections</td>
</tr>
<tr>
<td>No association with PVL</td>
<td>Associated with PVL</td>
</tr>
<tr>
<td>Associated with normal spectrum of disease severity</td>
<td>Associated with severe infections</td>
</tr>
</tbody>
</table>
Panton Valentine Leukocidin (PVL) in sensitive strains has been identified as a risk factor for severe infection. This cytotoxin causes experimental skin necrosis in animals. It works through synergy between 2 proteins LukS-PV and LukF-PV and may be present even if the bacterial strains are sensitive to antibiotics. Treatment is often ineffective, and the presence of PVL strains may only be recognised by a clinical pattern of poor response, frequent relapse, and severe lesions, often in otherwise healthy patients. There seems to be an association with recent travel.

Del Giudice et al reported a series of *Staph aureus* cases from a dermatology department in France during 2003-2010. They found that 74% of follicular infections compared to 12% of non-follicular infections were caused by PVL positive strains. In a survey of skin and soft tissue infections (SSTI) from two General Practice regions in the Netherlands Mithoe et al found that 11% of patients with Staph aureus were PVL positive. PVL strains were commoner in the North than the South region, perhaps reflecting regional differences e.g. in antibiotic usage.

Practical steps to take in dealing with this problem include:

- Suspect early if relapse is frequent and there are extensive and/or severe lesions.
- Have second line antibiotics available – e.g. rifampicin, clindamycin plus antisepsis e.g. povidone or hibisol.
- Warn the local microbiology laboratory if there is a suspicion of PVL.

**ATOPIC DERMATITIS**

Atopic dermatitis (AD) is common in most countries with a prevalence in 11-13 year olds approaching 12-18%. There are theories that early exposure to antigens may play a protective role in development of the condition. Over 80% of AD shows colonisation with *Staph aureus*. Observation of improvement on antibiotics correlating with reduction of flares raises the question ‘is this infection’? Mechanisms for a role of infection in AD include raised anti-Staph IgE, reduced antimicrobial peptide production and the presence of ‘superantigens’ e.g. α toxin, peptidoglycan and lipoteichoic acid.

**ECTOPARASITES AND THE SKIN**

Demodex is a genus of microscopic parasitic mites that live in or near the hair follicles of mammals. The role of these ectoparasites in human diseases is disputed but there are reports of clinical cases involving Demodex in HIV and immunosuppressed patients. Other occasional human ectoparasites resident in or on the body include scabies and head lice. Cheyletiella, from cats/dogs can survive for hours on human skin, and others e.g. bed bugs and mosquitos, do not live on skin, but visit only to feed. The importance of some of these parasites lies in their role as potential vectors for transmission of disease.
PYODERMA, SCABIES AND RENAL DAMAGE

There is an association between scabies and Group A streptococcal infection in the tropics. In outbreaks of scabies associated with pyoderma, symptomatic nephritis or haematuria/proteinuria can occur in 5-10% of cases. 10 years later 8-21% of affected children still have haematuria and previous scabies is associated with raised urinary albumin : creatinine ratio. This concurrence of infestation and infection has also been associated with rheumatic fever and infant septicaemia.

SUMMARY OF RESEARCH TARGETS FOR THE FUTURE

The challenges of skin infection and infestation remain considerable. As we understand better the mechanisms involved in maintaining the normal microbiota and repelling pathogens, interesting possibilities will undoubtedly emerge.

Potential research targets, including targets for manipulation of microbial flora of the skin and/or probiotics in the future might include:

- Recolonising to prevent infection eg Staph aureus, Malassezia – complete replacement or reduction.
- Exploring the relationship between microbiota elsewhere and skin disease e.g. chronic urticaria/Helicobacter
- Other areas unrelated to infection e.g. generation of key nutrients e.g. Vitamin D
- Can one replace and /or use bacteria in other ways e.g. prevention of insect vector biting, changing endozootic bacteria to affect the viability of the ectoparasite.

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LACTOBACILLI AND PROBIOTICS IN GYNECOLOGY AND OBSTETRICS
Professor Werner Mendling
Vivantes - Klinikum im Friedrichshain and Am Urban, Clinics for Obstetrics and Gynecology, Berlin, Germany.

Of the approximately 120 Lactobacillus species known, about 15 are known to occur in the vagina. These bacteria act on glycogen in the vaginal epithelium to produce lactic acid which helps to keep the pH in the vagina in the range of 3.8-4.4 (although some women with no lactobacilli have normal vaginal pH)*. Lactobacilli are now known to produce other substances besides lactic acid including:

- Hydrogen peroxide (H₂O₂)
- Bacteriocins
- Biosurfactants
- Coaggregation molecules
- And other as yet unknown substances

As well as Lactobacillus species, the normal vaginal flora can include transient species including: Gardnerella vaginalis, Atopobium vaginae, Mycoplasmas Bacteroides-, Prevotella- and Porphyromonas species, Peptostreptococci, and Candida albicans among many others.

Bacterial vaginosis is the most common cause of abnormal vaginal discharge in women of childbearing age. This syndrome of unknown cause is characterized by depletion of the normal Lactobacillus population and an overgrowth of vaginal anaerobes, accompanied by loss of the usual vaginal acidity. Women with symptomatic bacterial vaginosis report an offensive, fishy-smelling discharge that is often most noticeable after unprotected intercourse or at the time of menstruation.

When bacterial vaginosis develops the pH rises above 4.5, to levels up to 6.0. The lactobacilli reduce in concentration and may disappear, while there is an increased concentration of anaerobic and facultative anaerobic organisms. This pattern is illustrated in table 1.

There are several species of Lactobacillus present in normal women and the most important include L. crispatus, L. gasseri, L. jensenii, and L. iners but not L. acidophilus.

Diagnosis of BV is confirmed by identifying at least 3 of 4 ‘Amsel criteria’:

- The typical thin homogenous grey vaginal discharge
- Vaginal pH > 4.5

* Recent data show significant differences in the predominance of different lactobacillus species in different ethnic groups with significantly different vaginal pH values up to 513.
Clue cells detected on microscopy of vaginal fluid. Epithelial cells covered with so many small bacteria that the border is fuzzy are termed ‘clue cells’, because their presence is a clue to the diagnosis.

Release of a strong fishy odour when vaginal fluid is mixed with alkali (usually 10% potassium hydroxide) – the whiff test.

**TABLE 1:**

<table>
<thead>
<tr>
<th>Bacteria in the vagina of women with BV (n = 67) and a healthy control group (n = 28) (Eschenbach1)</th>
<th>prevalence (%)</th>
<th>concentration (ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BV</td>
<td>controls</td>
</tr>
<tr>
<td>G. vaginalis</td>
<td>100</td>
<td>50*</td>
</tr>
<tr>
<td>Anaerobes</td>
<td>100</td>
<td>50*</td>
</tr>
<tr>
<td>Bacteroides spp.</td>
<td>100</td>
<td>40*</td>
</tr>
<tr>
<td>Peptococci</td>
<td>50</td>
<td>5*</td>
</tr>
<tr>
<td>Peptostreptococci</td>
<td>50</td>
<td>10*</td>
</tr>
<tr>
<td>M. hominis</td>
<td>90</td>
<td>15*</td>
</tr>
<tr>
<td>Corynebacteria</td>
<td>60</td>
<td>25**</td>
</tr>
<tr>
<td>Streptococci</td>
<td>50</td>
<td>15*</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>40</td>
<td>100*</td>
</tr>
</tbody>
</table>

*p<0,001, **p<0,01

Generally in research studies the Nugent scoring system is used to interpret Gram-stained vaginal smears. A score of between 0 and 10 is generated from counting bacterial morphotypes with 0-3 being normal, 4-6 intermediate and 7-10 indicating BV.

The prevalence of BV varies markedly by population and geography:

- Germany, pregnant women: 20 %
- Black women in USA: > 30 %
- Women in central Africa: > 50 %

Although many women with BV are asymptomatic, presence of BV markedly increases the relative risk of gynaecological infection (pelvic inflammatory disease and infection after hysterectomy) and obstetric complications (pre-term birth and post-partum endometritis). Several studies²,³ have confirmed that treatment of BV significantly reduces the rate of pre-term birth and late miscarriages.

**BIOFILMS IN BV**

In a paper published in 2005⁴ we investigated the composition and spatial organization of
bacteria associated with the vaginal epithelium in biopsy specimens from 20 patients with bacterial vaginosis and 40 normal premenopausal and postmenopausal controls using a broad range of fluorescent bacterial group-specific rRNA-targeted oligonucleotide probes. Bacterial vaginosis was associated with greater occurrence and higher concentrations of a variety of bacterial groups. However, only *Gardnerella vaginalis* together with *Atopobium vaginae* developed a characteristic adherent biofilm that was specific for bacterial vaginosis (figure 1).

**FIGURE 1: GARDNERELLA VAGINALIS BIOFILM**

We concluded that a biofilm comprised of confluent *G. vaginalis* with other bacterial groups incorporated in the adherent layer is a prominent feature of bacterial vaginosis.

Subsequently we showed that an adherent *Gardnerella vaginalis* biofilm persists on the vaginal epithelium following standard therapy of bacterial vaginosis with oral metronidazole. This seems to serve as a reservoir of the core pathogens for BV. Although the biofilm primarily consisted of *Gardnerella vaginalis* and *Atopobium vaginae*, *Lactobacilli* and *Bacterioides/Prevotella* (7/16) were also present but accounted for only < 10% of all bacteria.

BV therapeutic options include:

- antibiotics (metronidazole, clindamycin)
- lactic acid
- lactobacilli
- Dis-infective agents
- Preventative lactobacillus vaccination

Numerous products aimed at *Lactobacillus* substitution exist on the market in Germany. Well investigated strains for vaginal or oral treatment include *L. rhamnosus* GR-1 and *L. reuteri* RC-14 (formerly *L. fermentum*) or *L. rhamnosus* GG and others.

**VAGINAL ADMINISTRATION**

A study of *L. rhamnosus* GG, administered vaginally, in 10 subjects, showed that the bacteria survived only 5 days *in situ*, but *L. rhamnosus* GR-1 and *L. reuteri* RC-14 were detectable for 19 days. This highlights the importance of selection of strains for urogenital probiotic applications.
A double-blind, randomized, placebo-controlled study of vaginal probiotics for recurrent BV investigated the administration of *L. rhamnosus*, *L. acidophilus*, *Strept. thermophilus* for 7 days on, 7 days off, and then 7 days on. The study showed a significant reduction of recurrences of BV in the active vs placebo group (15.8 vs. 45.0 %) and reduction of *G. vaginalis* incidence (3.5 vs. 18.3 %) after 2 and 11 months.

A phase 2a study assessed colonization efficiency, safety, tolerability, and acceptability of *Lactobacillus crispatus CTV-05* administered by a vaginal applicator. Twenty-four women with BV were randomized in a 3:1 ratio of active product to placebo. Participants used *Lactobacillus crispatus CTV-05* at $10^2$ colony-forming units (cfu)/dose or placebo for 5 initial consecutive days, followed by a weekly application over 2 weeks. Sixty-one percent of the 18 women randomized to the active group were colonized with *L. crispatus CTV-05* at Day 10 or Day 28. The treatment was well tolerated and accepted.

Antonio et al. investigated two potencies of gelatin capsules containing *Lactobacillus crispatus CTV-05* for safety and vaginal colonization in 90 young women. Of 40 participants who lacked *L. crispatus* colonization at enrollment, 36 (90%) were successfully colonized by CTV-05 at 1 or more follow-up visits, whereas only 24 (51%) of 47 participants colonized by *L. crispatus* at enrollment were positive for CTV-05 at follow-up (P < .001). The authors concluded that that the factors that predict failure to become colonized by probiotic lactobacilli include exposure to semen, vaginal intercourse, and the presence of lactobacilli of the same species at enrollment.

Supplemental vaginal treatment with two vaginal lactobacilli strains for 3 months did not improve clindamycin therapy of BV, but reduced the relapse rate of initially cured women significantly after 6 months. A double-blind, placebo-controlled study showed that use of tampons with *L. fermentum*, *L. casei var. rhamnosus* and *L. gasseri* for 4 weeks after vaginal clindamycin in BV patients did not improve therapy results of BV after two months, but improved significantly results of Nugent-Score 4 – 6 patients.

In a study in Nigeria, 40 women diagnosed with BV were randomized to receive either two dried capsules containing *Lactobacillus rhamnosus GR-1* and *Lactobacillus reuteri RC-14* each night for 5 days, or 0.75% metronidazole gel, applied vaginally twice a day (in the morning and evening). Follow-up at day 6, 15 and 30 showed cure of BV in significantly more probiotic treated subjects (16, 17 and 18/20, respectively) compared to metronidazole treatment (9, 9 and 11/20: P=0.016 at day 6, P=0.002 at day 15 and P=0.056 at day 30).

**ORAL TREATMENT**

In a study by Reid et al., forty-two healthy women were randomized to receive one of three encapsulated *Lactobacillus rhamnosus GR-1* plus *Lactobacillus fermentum RC-14* probiotic dosage regimens or *L. rhamnosus GG* by mouth each day for 28 days. However, the vaginal flora, assessed by Nugent scoring, was only normal in 40% of the cases, and 14 patients had asymptomatic bacterial vaginosis. Treatment with *L. rhamnosus GR-1/L. fermentum RC-14* once and twice daily correlated with a healthy vaginal flora in up to 90% of patients, and 7/11
patients with bacterial vaginosis converted to normal or intermediate scores within 1 month. Ingestion of L. rhamnosus GG failed to have an effect. This study confirms the potential efficacy of orally administered lactobacilli and showed that over $10^8$ viable organisms per day is the required dose.

Another study by the same group\(^\text{15}\) explored the efficacy of oral lactobacilli. A randomized, placebo-controlled trial of 64 healthy women given daily oral capsules of Lactobacillus rhamnosus GR-1 and Lactobacillus fermentum RC-14 for 60 days showed restoration from asymptomatic bacterial vaginosis microflora to a normal lactobacilli colonized microflora in 37% women during lactobacilli treatment compared to 13% on placebo (P=0.02). Lactobacilli were detected in more women in the lactobacilli-treated group than in the placebo group at 28 day (P=0.08) and 60 day (P=0.05). Culture findings confirmed a significant increase in vaginal lactobacilli at day 28 and 60, a significant depletion in yeast at day 28 and a significant reduction in coliforms at day 28, 60 and 90 for lactobacilli-treated subjects versus controls.

A study from Vienna\(^\text{16}\) examined the efficacy of orally administered probiotic strains Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 on the quality of the vaginal flora in postmenopausal women in a randomized, double-blind, placebo-controlled study. Twenty-one of the 35 subjects (60%) in the intervention group and 6 of the 37 subjects (16%) in the control group showed a reduction in the Nugent score by at least two grades (p=0.0001). The median difference in Nugent scores between baseline and the end of the study was 3 in the intervention group and 0 in the control group (p=0.0001).

**SAFETY**

In a review of the safety probiotics, Boyle et al.\(^\text{17}\) concluded that probiotics have an excellent overall safety record but that they should be used with caution in special risk groups such as patients with immunosuppression or in very small preterm babies with short bowel-syndrome. Sepsis with oral lactobacilli appears to be extremely rare with n = 7 cases in 1999-2005 with L. rhamnosus, mostly – GG.

**REFERENCES**


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**LACTOBACILLI IN MUCOSAL CANDIDA ALBICANS INFECTIONS** - Professor Martin Schaller
Department of Dermatology, Eberhard Karls University Tübingen, Germany.

**CANDIDA ALBICANS**

*Candida albicans* is facultative pathogenic yeast which is a frequent colonizer of human skin and mucous membranes. It is present on mucosal surfaces in 40-80% of normal human beings and is part of the microbiota in the mouth, gut, and vagina. This organism is normally a harmless colonizer and infections with *Candida* usually occur only when a patient has some alteration in cellular immunity, normal flora or normal physiology. Nevertheless *Candida albicans* is the
most common fungal pathogen worldwide and systemic candidosis is the 4th leading cause of nosocomial infections and accounts for 40% of the mortality.

Vulvovaginal candidosis (VVC) is very common and affects 75% of women at some time, with 5% experiencing recurrence. Recurrent VVC, defined as at least four episodes per year, often requires long-term antifungal therapy. In contrast, oral candidosis is most commonly manifested in patients with leukemia or other immunosuppressed conditions and is often a clue to acute primary HIV infection.

*Candida albicans* has become a cause for concern because it is increasingly resistant to drug therapies. It is also the source of considerable health economic cost, with diagnosis and treatment of VVC and the concomitant loss of productivity estimated to have cost US$1.8 billion in 1995.

What turns *Candida albicans* from a commensal to a pathogen is a combination of increasing virulence factors in the organism and the interaction of these with predisposing host factors that compromise immunity (figure 1).

To become a pathogen, the normal commensal *Candida albicans* must adhere to mucosal surfaces, cause damage to superficial cells and then invade the deeper cell layers (figure 2).

Keratinocytes on mucosal surfaces are the first cells that are in contact with *C. albicans* and are also the first cells that get damaged by *C. albicans*. These cells are part of the innate mucosal immune system.

Reconstituted Human Epithelium (RHE) is a valuable research model for the study of Candidal infections¹. Infection experiments using these models are highly reproducible and can be used for the direct analysis of pathogen-epithelial cell interactions. To study the impact of innate immunity or the antifungal activity of natural and non-natural compounds, the mucosal
infection models can be supplemented with immune cells, antimicrobial agents or probiotic bacteria.

**FIGURE 2: STAGES OF MUCOSAL CANDIDOSIS**

Probiotic *Lactobacillus* spp. bacteria are widely used in the treatment of vaginal candidiasis. These organisms are suggested to have a number of effects that maintain or regenerate balance between tolerance and defense, including:

- Up-regulation of mucus production
- Improvement of epithelial barrier function
- Increase in IgA production
- Competition for adhesion sites
- Production of organic acids, ammonia, H$_2$O$_2$ and bacteriocins
- Up-regulation of antimicrobial peptides

**EFFECTS OF PROBIOTICS ON *C. ALBICANS***

*Lactobacillus rhamnosus* GG (LGG) is one of the most important and most investigated probiotic strains and shows protective effects against *C. albicans* infections$^2$. Vaginal *Lactobacillus*-strains have been shown to inhibit the viability of *Prevotella bivia* and *Gardnerella vaginalis* in cell culture and co-culture experiments$^3$. The mechanism of this inhibition is not attributable to low pH and lactic acid alone, but to hydrogen peroxide and proteolytic enzyme-resistant compound(s) present in the cell-free culture supernatant.

Oral supplementation with *L. casei* ssp. *rhamnosus* has also been shown to prevent enteric colonization by *Candida* species in preterm neonates$^4$. Enhanced clearance of *Candida*
**albicans** from the oral cavities of mice has been demonstrated following oral administration of *Lactobacillus acidophilus*.  

In our research programme we set out to answer the following questions:

- Do *Lactobacillus* spp. protect against mucosal *Candida albicans* infection?
- What are the mechanisms of protection?
- Is the immune response of the keratinocytes influenced by the probiotic bacteria?
- Is there interaction of probiotic bacteria with virulence of factors of the pathogen?

In culture experiments, we showed only slight inhibition of *C. albicans* growth by *L. rhamnosus* GG (LGG) after 72hrs. We also observed that LGG does not block hyphal induction of *C. albicans*. However in Reconstituted Human Epithelium (RHE) experiments when *L. rhamnosus* GG is added (at 12-24hrs) to an infection of *C. albicans* established for 24 hours – a therapeutic model - *L. rhamnosus* GG mediates protection against *C. albicans*.

However LGG reduces the mucosal immune response. There are two possible explanations for this, either LGG down-regulates inflammatory response of the keratinocytes or reduced cytokine levels occur as result of reduced cell damage. Experiments at earlier time points without cell damage (3h/6h) confirmed that cytokine regulation is independent of mucosal damage.

Monolayer experiments are cheaper than RHE and allow quantitative evaluation of adhesion and invasion. We showed that LGG protects against *C. albicans* also in monolayer experiments with increased protection after 12h pre-incubation with LGG before addition of *Candida albicans* and confirmed that LGG reduces the mucosal immune response in this model. We were also able to show that the presence of LGG strongly reduced the adhesion and invasion ability of *C. albicans* after 3h. Direct visualisation shows that hyphae of *C. albicans* are shorter in the presence of LGG and the probiotic reduces hyphal growth of *C. albicans* by about 40% (figure 3).

**Figure 3: LGG influences hyphal growth**

Similar protective effects, but immune modulation of different degrees, can be seen with other probiotic *Lactobacilli* e.g. *L. casei shirota* (LCS) but this strain does not influence adhesion of *C. albicans*. 
SUMMARY

Using RHE and monolayer cell culture techniques we have shown that *L. rhamnosus GG*

- Protects mucosa from *C. albicans* damage in both preventive and therapeutic approaches
- Modulates the immune response
- Induces immune conditioning of mucosal surfaces

Furthermore LGG reduces the adhesion and invasion capacity of *C. albicans* and inhibits the linear growth of *C. albicans* hyphae. The ability of such probiotic bacteria to inhibit the growth of pathogens such as *C. albicans* and to modulate human immune responses to these organisms, offer the promise that these bacteria could provide new options in antifungal therapy.

REFERENCES


PROBIOTICS AND IBS: RATIONALE, PUTATIVE MECHANISM, AND EVIDENCE OF CLINICAL EFFICACY

Professor Robin Spiller

NIHR Nottingham Digestive Diseases Biomedical Research Centre, Nottingham Digestive Diseases Centre, UK.

WHAT IS IRRITABLE BOWEL SYNDROME (IBS)?

Irritable Bowel Syndrome is characterised by recurrent abdominal pain or discomfort associated with disturbed bowel habit. The Rome criteria I, II & III (1992, 1999 & 2006) were developed to allow uniform entry criteria for randomised clinical trials (RCTs) and to facilitate comparison between studies. The Rome III criteria are summarised in (table 1)\(^1\).
TABLE 1: ROME III CRITERIA FOR DIAGNOSIS OF IBS

- Recurrent abdominal pain or discomfort ≥3 days per month in the last three months associated with two or more of the following:
  - Improvement with defecation; and/or
  - Onset associated with a change in frequency of stool; and/or
  - Onset associated with a change in form (appearance) of stool

- Supportive symptoms
  - Bloating
  - Sense of incomplete evacuation
  - Passage of mucus
  - Urgency and/or Straining

- Criteria fulfilled for the last 3 months with symptom onset ≥ 6 months prior to diagnosis

Absence of structural or biochemical cause of symptoms

Longstreth et al. Gastroenterology 2006;130:1480-91

The time limit for onset of symptoms before diagnosis was designed to avoid giving a chronic disease label to transient symptoms due to e.g. infection. This has been set to 6 months since it is unlikely that a new diagnosis will emerge or that symptoms will disappear if they have already lasted 6 months. Better and faster investigations mean that other diagnoses are now more rapidly eliminated. The threshold of ≥3 days per month of symptoms is empirical but designed to exclude trivial complaints that might occur at lower frequencies.

IBS is common throughout the world, affecting 10-15% of the adult population (Figure 1).

FIGURE 1: WORLD WIDE INCIDENCE OF IBS

Common throughout the world affecting 10-15% of adult population
Predictors for the development of IBS include age, gender, infection and stress. Figure 2 shows the effect of age and gender on the onset of IBS, and incidentally shows the well known preponderance in females. In both sexes the peak time of onset in the early 20’s corresponds to a common time of stress in people’s lives e.g. associated with leaving home for the first time or starting a first job. This pattern of onset is almost an inverse of the ‘U shaped curve of happiness’ derived from studies of reported wellbeing at different ages.

**FIGURE 2: EFFECT OF AGE AND GENDER ON ONSET OF IBS**

![Graph showing the effect of age and gender on the onset of IBS.](image)

A prospective study in General Practice of 5,250 people free from IBS found 86 new cases in the 2,456 followed up at 15 months. Gender was the most prominent risk factor with females outnumbering males (4.6% versus 2.1% respectively OR=2.2). Adjusted for age and sex, other predictors for development of IBS were:

- High levels of illness behaviour: Odds Ratio (OR) = 5.2 (95% CI 2.5-11.0)
- Health anxiety: OR = 2.0 (0.98-4.1)
- Sleep problems: OR = 1.6 (0.8-3.2)
- Somatic symptoms: OR = 1.6 (0.8-2.9)

Infectious gastroenteritis (IGE) may also be an important predictor of IBS. A meta-analysis of studies found a sevenfold increase (pooled risk estimate) in the odds of developing IBS following IGE. This post-infectious IBS (PI-IBS) may be susceptible to primary prevention in treating the initial episode of IGE.

Genetic factors are also important. The Virginia twin study of 6020 twins found approximately twice the concordance for IBS in monozygotic compared to dizygotic twins (17% v 8.4%). There is some evidence suggesting that Crohn’s associated TNFSF15 polymorphisms are increased in IBS. This genetic effect should be more obvious in gene/environment interaction and therefore should be most obvious in PI-IBS.

In May 2000, over 2300 residents of Walkerton, Ontario, developed gastroenteritis from
microbial contamination of the municipal water supply. A longitudinal study found that 36.2% of these developed IBS. This allowed researchers to compare genetic data from residents who developed gastroenteritis and reported PI-IBS 2 to 3 years after the outbreak (n = 228, cases), with data from residents who developed gastroenteritis but did not develop PI-IBS (n = 581, controls).

Multivariate analysis after fine mapping of 4 gene areas found significant differences in genes that encode proteins involved in epithelial cell barrier function and the innate immune response to enteric bacteria. These results illustrate the probable importance of host response to bacteria, cytokine response and permeability in PI-IBS.

**FIGURE 3: RISK FACTORS FOR DEVELOPING POST INFECTION IBS AFTER C. JEJUNI ENTERITIS**

Figure 3 illustrates that most of the known risk factors for PI-IBS reflect local gut damage, but that predisposing psychological factors are also important.

An important role for serotonin or 5-hydroxytryptamine (5HT) in PI-IBS is suggested by evidence that 5-HT containing enterochromaffin (EC) cells increased in PI-IBS but not in those who were infected but recovered fully, and by data showing that there is increased postprandial plasma 5-HT in PI-IBS.

Serotonin transporter (SERT) which regulates levels of 5HT may be important in the gut and SERT mRNA appears to be reduced in duodenal biopsies from diarrhoea - predominant IBS (IBS-D). There is evidence of low grade ‘immune activation’ in the mucosa of patients with IBS and this may reduce levels of SERT.

Studies show increased gut permeability in PI-IBS & other IBS subtypes (e.g. IBS-D). Small bowel permeability increased 3 months following Campylobacter enteritis and for up to 4 years in PI-IBS. This was also seen in PI-IBS 2 years after the Walkerton outbreak. There is evidence that small bowel permeability is increased in both PI-IBS and IBS-D.

Increased colonic paracellular permeability has also been seen in IBS patients. Increased permeability resulting from application of the supernatant from IBS colonic biopsies onto cultured cell layers appears to correlate with the severity of abdominal pain.
Mast cells may provide a link between this increased permeability and pain. Mast cells are known to be potential mediators of the response to stress. Animal studies show psychological stress increases mast cell numbers, and the number of mast cells is increased in IBS. Moreover IBS colonic biopsies release increased amounts of mast cell products which activate human enteric nerves (histamine, 5-HT & tryptase).

ROLE OF ALTERED MICROBIOTA IN DEVELOPING PI-IBS

Acute gastroenteritis leads to depletion of anaerobes, reduction in short chain fatty acids and an increase in pH. There appears to be an increased risk of developing PI-IBS in Traveller’s Diarrhoea in those treated with antibiotics RR= 4.1(1.1-15.3).

SUMMARY

Irritable bowel syndrome is common and ubiquitous.

Risk factors for developing IBS include: female gender; age 20-40 years; infection; and psychological stress possibly acting via mast cells. Abnormal colonic and small bowel permeability is common in IBS. There is evidence of altered microbiota but it is unclear whether this is cause or effect.

RATIONALE FOR USING PROBIOTICS IN IBS

A rationale for the use of probiotics in IBS is given in table 2.

TABLE 2: RATIONALE FOR USING PROBIOTICS IN IBS

<table>
<thead>
<tr>
<th>Abnormality in IBS</th>
<th>Probiotic effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune activation</td>
<td>Immunomodulation</td>
</tr>
<tr>
<td>Impaired barrier function</td>
<td>Enhance tight junctions</td>
</tr>
<tr>
<td>Unstable microbiota</td>
<td>Stabilises microbiota?</td>
</tr>
<tr>
<td>Slow transit in IBS-C</td>
<td>Laxative effect of SCFAs?</td>
</tr>
</tbody>
</table>

ABNORMALITIES OF MICROBIOTA IN IBS

Table 3 shows variable differences in IBS microbiota versus controls from the many studies that have been conducted. There is evidence of increased instability of the microbiota in IBS, and reduced diversity in IBS-D with reduced Firmicutes/ Bacteroides.
There are limitations with the available studies including:

- Small numbers of subjects
- With 500 assays we should expect 25 to be different by chance
- Symptoms in IBS are erratic hence analysis at one time point may not be representative – there is a need to assess patients when they are symptomatic.
Varying methods make comparisons difficult
- Selective cultivation
- Specific targeted qPCR and FISH
- DGGE
- G+C - and microarray profiling

 Patients not well characterized
- Diet, bowel pattern

 >50% of studies come from Finland, and it is uncertain how well these results will generalize to other countries

 Variability a common finding

 Decreased diversity in several diseases with fast transit

 May be more important to link enterotypes or functional characteristics (metabolome) to symptoms rather than individual species

There are clearly many factors that influence the microbiota in IBS (figure 4).

**FIGURE 4: WHY MIGHT FLORA BE ABNORMAL/UNSTABLE IN IBS?**

Studies examining whether the gut microbiota are disturbed in IBS have shown a variety of changes which are difficult to interpret. However reasons why microbiota may be disturbed in IBS include:

- Iatrogenic achlorhydria during treatment with proton pump inhibitors (PPIs). Some 44% of IBS sufferers in US tertiary care receive PPIs\(^2\)\(^2\).
- Disturbed motility (fast / slow transit). Approximately 1/3 of IBS sufferers have abnormally fast or slow gut transit\(^2\)\(^3\).
- Mucosal immune activation in IBS-D\(^2\)\(^4\).
- Depleted diet in attempt to reduce symptoms
- Infectious gastroenteritis leading to post-infectious IBS
- Somatization leading to frequent doctor visits and increased antibiotic use.

WHICH PROBIOTICS WORK IN IBS?

Tables 4 & 5, and figure 5 summarise the many studies of probiotics in IBS. Those with the most consistent data are circled in red.

**TABLE 4: STUDIES OF PROBIOTICS IN IBS**

<table>
<thead>
<tr>
<th>Probiotic</th>
<th>n</th>
<th>weeks</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. plantarum</td>
<td>60</td>
<td>4</td>
<td>Flatulence ↓</td>
<td>Nobaek 2000</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>4</td>
<td>Global symptoms ↓</td>
<td>Niedzielin 2001</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>4</td>
<td>NS</td>
<td>Sen 2002</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>4</td>
<td>Pain ↓</td>
<td>Saggioro 2004</td>
</tr>
<tr>
<td>L. rhamnosus</td>
<td>64</td>
<td>6</td>
<td>Distension ↓ Pain NSig</td>
<td>Bausserman 2005</td>
</tr>
<tr>
<td></td>
<td>104</td>
<td>4</td>
<td>Pain ↓</td>
<td>Gawronska et al 2007</td>
</tr>
<tr>
<td></td>
<td>141</td>
<td>8</td>
<td>Pain ↓</td>
<td>Francavilla 2008</td>
</tr>
<tr>
<td>L. reuteri</td>
<td>54</td>
<td>24</td>
<td>NS</td>
<td>Niv 2005</td>
</tr>
<tr>
<td>L. acidophilus</td>
<td>40</td>
<td>4</td>
<td>75% &quot;improved&quot; versus 35% control</td>
<td>Sinn 2008</td>
</tr>
<tr>
<td>B. infantis</td>
<td>75</td>
<td>4</td>
<td>Pain, Bloating, BM difficulty</td>
<td>O’Mahony 2005</td>
</tr>
<tr>
<td></td>
<td>362</td>
<td>4</td>
<td></td>
<td>Whorwell 2006</td>
</tr>
</tbody>
</table>

**Lactobacillus plantarum versus placebo**

<table>
<thead>
<tr>
<th>Dose</th>
<th>Patients</th>
<th>Duration weeks</th>
<th>Outcome</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>2X10⁹ cfu/ day</td>
<td>60 Rome IBS</td>
<td>4</td>
<td>Flatulence at 5, 6 &amp; 52 week p &lt; 0.05</td>
<td>Nobaek et al 2000</td>
</tr>
<tr>
<td>1X10⁹ cfu b.i.d</td>
<td>40 pain + 2 Manning</td>
<td>4</td>
<td>9/20 on active “complete improvement” v 3/20 on placebo p &lt; 0.001.</td>
<td>Niedzielin et al 2001</td>
</tr>
<tr>
<td>0.6X10⁹ cfu /day</td>
<td>12 Rome IBS</td>
<td>4</td>
<td>24hour gas / fecal weight NSig</td>
<td>Sen et al 2002</td>
</tr>
<tr>
<td>A= L. plantarum+ Bifidobacterium breve</td>
<td>70 Rome IBS</td>
<td>4</td>
<td>↓ pain &amp; global IBS score P &lt; 0.001</td>
<td>Saggioro et al 2004</td>
</tr>
</tbody>
</table>
### TABLE 5: STUDIES OF PROBIOTICS IN IBS

#### RCTs of Lactobacillus rhamnosus GG versus placebo in IBS

<table>
<thead>
<tr>
<th>Probiotic</th>
<th>cfu/day</th>
<th>n</th>
<th>weeks</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lactobacillus rhamnosus GG</strong></td>
<td></td>
<td>50</td>
<td>4</td>
<td>↓ abdominal distension</td>
<td>Bausserman et al 2005</td>
</tr>
<tr>
<td><strong>Lactobacillus rhamnosus GG</strong></td>
<td>3x10^9 b.d.</td>
<td>104</td>
<td>4</td>
<td>Pain score ↓ 3.9(1.3) → 2.5(1.9) active 4.2(1.3) → 2.9(1.3) placebo</td>
<td>Gawronska et al 2007</td>
</tr>
<tr>
<td><strong>Lactobacillus rhamnosus GG</strong></td>
<td></td>
<td>141</td>
<td>8</td>
<td>Significant reduction in pain 72% active versus 53% placebo</td>
<td>Francavilla 2010</td>
</tr>
</tbody>
</table>

#### Bifidobacterium infantis

<table>
<thead>
<tr>
<th>Dose</th>
<th>N</th>
<th>Duration weeks</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. salivarius versus Bifidobacterium infantis</td>
<td>25 per group Rome II</td>
<td>4</td>
<td>Pain, bloating and bowel movement difficulty ↓ v placebo with B. infantis only p &lt; 0.05</td>
<td>O’Mahony et al 2005</td>
</tr>
<tr>
<td>Bifidobacterium infantis 1x10^6, 10^8 &amp; 10^10 cfu/day</td>
<td>75 per group Rome II</td>
<td>4</td>
<td>↓ Pain / discomfort at week 4 ↓ Symptom score and bloating / distension at week 4 &amp; 6 only for 10^8 cfu/day dose NS effect on QOL scores</td>
<td>Whorwell et al 2006</td>
</tr>
</tbody>
</table>

**FIGURE 5: LACTOBACILLUS GG NORMALISES GUT PERMEABILITY IN CHILDREN WITH IBS & FAP**

59% abnormal permeability at entry → 19% on active versus 38% on placebo

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However there are problems in comparing probiotic studies, including:

1. Variable quality of reporting
2. Variable unvalidated endpoints
3. Use of arbitrary scores rather than numbers of responders
4. Wide range of different species used both single and multiple
5. Meta-analyses abound but are probably invalid except for studies involving the same species.

**SUMMARY**

IBS patients show evidence of immune activation, impaired gut barrier function and abnormal gut microbiota.

Probiotics can exert anti-inflammatory effects in inflammatory bowel disease and may strengthen the gut barrier in IBS-D. Randomised Controlled Trials show limited benefit for some, but not all probiotics, with Numbers Needed to Treat varying from 4.8 – infinity, compared to results from conventional pharmacologic agents in IBS of 8 - 14. The best evidence of efficacy seems to be for improving pain in children. Defining who will respond remains a challenge.

**REFERENCES**


Intestinal bacteria can be thought of as a hidden organ. The intestines contain more bacteria (representing approximately 2000 phylotypes) than cells in the human body, with total metabolic activity rivalling that of the liver. It is clear that the gut microbiota can be both an asset and a liability:

<table>
<thead>
<tr>
<th>Asset</th>
<th>Liability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Defence - bacterial antagonism</td>
<td>Pro-carcinogens → carcinogens</td>
</tr>
<tr>
<td>Priming of mucosal immunity</td>
<td>• Overgrowth syndromes</td>
</tr>
<tr>
<td>Peristalsis</td>
<td>• Essential factor for IBD</td>
</tr>
<tr>
<td>Elimination of dietary carcinogens</td>
<td>• Metabolic effects</td>
</tr>
<tr>
<td>Synthesis of B &amp; K vitamins</td>
<td></td>
</tr>
<tr>
<td>Epithelial nutrients (e.g. short chain fatty acids - SCFAs)</td>
<td></td>
</tr>
<tr>
<td>Conversion of pro-drugs</td>
<td></td>
</tr>
<tr>
<td>Metabolic effects</td>
<td></td>
</tr>
</tbody>
</table>

The Alimentary Pharmabiotic Centre in Cork has a multidisciplinary team working in state of the art facilities to ‘mine microbes for mankind’. Figure 1 outlines the research approach.

**FIGURE 1: APC RESEARCH OUTLINE**
Recent Highlights of APC Research include:

**MAPPING THE GUT MICROBIOTA IN THE ELDERLY**

Alterations in the human intestinal microbiota are linked to conditions including inflammatory bowel disease, irritable bowel syndrome, and obesity. The microbiota also undergoes substantial changes at the extremes of life, in infants and older people, the ramifications of which are still being explored.

Claesson and co-workers applied pyrosequencing to characterize the faecal microbiota in 161 subjects aged 65y and older and 9 younger control subjects. The core microbiota of elderly subjects was distinct from that previously established for younger adults, with a greater proportion of *Bacteroides* spp. and distinct abundance patterns of *Clostridium* groups. Analyses of 26 faecal microbiota datasets from 3-month follow-up samples indicated that in 85% of the subjects, the microbiota composition was more like the corresponding time-0 sample than any other dataset. The group concluded that the faecal microbiota of the elderly shows temporal stability over limited time in the majority of subjects but is characterized by unusual phylum proportions and extreme variability (Figure 2).

**STUDIES ON MECHANISMS OF COLONISATION BY BIFIDOBACTERIUM BREVE**

Development of the human gut microbiota commences at birth, with bifidobacteria being among the first colonizers of the sterile newborn gastrointestinal tract. To date, the genetic basis of *Bifidobacterium* colonization and persistence remains poorly understood.

Mutational analysis demonstrated that the *tad(2003)* gene cluster is essential for efficient *in vivo* murine gut colonization, and immunogold transmission electron microscopy confirmed the presence of Tad pili at the poles of *B. breve UCC2003* cells (Figure 3).
Conservation of the Tad pilus-encoding locus among other *B. breve* strains and among sequenced Bifidobacterium genomes supports the notion of a ubiquitous pili-mediated host colonization and persistence mechanism for bifidobacteria.

**WORK SHOWING THAT L. RHAMNOSUS (JB-1) REDUCES STRESS-INDUCED ANXIETY- AND DEPRESSION-RELATED BEHAVIOR IN MICE**

There is increasing, but largely indirect, evidence pointing to an effect of commensal gut microbiota on the central nervous system (CNS). However, it is unknown whether lactic acid bacteria such as *Lactobacillus rhamnosus* could have a direct effect on neurotransmitter receptors in the CNS in normal, healthy animals.

In this work, Bravo *et al.* showed that chronic treatment with *L. rhamnosus* (JB-1) induced region-dependent alterations in GABA mRNA expression in the brain, in comparison with control-fed mice. Importantly, *L. rhamnosus* (JB-1) reduced stress-induced corticosterone and anxiety- and depression-related behavior. Moreover, the neurochemical and behavioral effects were not found in vagotomized mice, identifying the vagus as a major modulatory constitutive communication pathway between the bacteria exposed to the gut and the brain.

**PROBIOTICS AS ANTI-INFECTIVES – MECHANISMS**

In recent years, there has been a growing interest in the use of probiotic bacteria for the maintenance of general gastrointestinal health and the prevention or treatment of intestinal infections. Whilst probiotics are documented to reduce or prevent specific infectious diseases of the GI tract, the mechanistic basis of this effect remains unclear.

It is likely that diverse modes-of-action contribute to inhibition of pathogens in the gut environment and proposed mechanisms include (i) direct antimicrobial activity through production of bacteriocins or inhibitors of virulence gene expression; (ii) competitive exclusion...
by competition for binding sites or stimulation of epithelial barrier function; (iii) stimulation of immune responses via increases of sIgA and anti-inflammatory cytokines and regulation of proinflammatory cytokines; and (iv) inhibition of virulence gene or protein expression in gastrointestinal pathogens.

There is evidence for a role of probiotics both in prevention of infection (e.g. diarrhoea in children) and in prevention of antibiotic associated diarrhoea (Figure 4).

**FIGURE 4: PROPOSED MECHANISMS**

*In vitro* studies, support the hypothesis that the ability of probiotic agents to inhibit adherence of organisms to intestinal epithelial cells is mediated through their ability to increase expression of MUC2 and MUC3 intestinal mucins.

The microbiota fosters development, aids digestion and protects host cells from pathogens - a function referred to as colonization resistance. Gut inflammation changes microbiota composition, disrupts colonization resistance and enhances pathogen growth. Thus, some pathogens can benefit from inflammatory defenses.

**FOCUS UPON BACTERIOCIN PRODUCTION**

Workers from the APC have showed that *Lactobacillus salivarius UCC118*, a recently sequenced and genetically tractable probiotic strain of human origin, produces a bacteriocin in vivo that can significantly protect mice against infection with the invasive foodborne pathogen *Listeria monocytogenes*. This raises the intriguing possibility of probiotics specifically designed to protect against infection in particular circumstances such as pregnancy.
CHARACTERISATION OF THURICIN

Another bacteriocin of interest in the APC is Thuricin CD a bacteriocin produced by *Bacillus thuringiensis*. Thuricin CD is a two component bacteriocin that is in the drug discovery pipeline as a candidate against the target pathogen *C. difficile* (Figure 5).

**FIGURE 5: COLONY OF B. THURINGIENSIS DPC 6431 ON THE INITIAL ISOLATION PLATE SHOWING INHIBITION IN SEEDED OVERLAY OF C. DIFFICILE ATCC 43593**

This bacteriocin offers the promise of a narrow-spectrum antimicrobial with specific anti-*C. difficile* activity. Thuricin CD was effective at killing *C. difficile* in the distal colon model but had no significant impact on the composition of the microbiota. This offers the possibility of developing a targeted approach to eliminating *C. difficile* in the colon, without collateral damage.

**FIGURE 6: MINIMAL ALTERATIONS TO BACKGROUND MICROBIOTA FOLLOWING THURICIN TREATMENT**

Reproduced with permission from PNAS. Rea M C et al. PNAS 2010;107:9352-9357

Reproduced with permission from PNAS. Rea M C et al. PNAS 2011;108:4639-4644
STUDIES OF BILE SALT HYDROLASE (BSH) AS A COMMON GUT-SPECIFIC BACTERIAL FUNCTION

BSH activity is a conserved microbial adaptation to the human gut environment with a high level of redundancy in this ecosystem. Through metagenomic analyses we have identified functional BSH in all major bacterial divisions and archaeal species in the gut and demonstrated that BSH is enriched in the human gut microbiome.

Phylogenetic analysis illustrates that selective pressure in the form of conjugated bile acid has driven the evolution of members of the Ntn_CGH-like family of proteins toward BSH activity in gut-associated species. Furthermore, we have demonstrated that BSH mediates bile tolerance in vitro and enhances survival in the murine gut in vivo.

Reabsorbed bile salts act as signalling molecules regulating systemic endocrine functions and bile acids act as local signalling molecules regulating innate immunity. Regulatory networks recognising bile acids are affected by the microbiota.

We can conclude that functional metagenomic analysis has helped to define the Bile Salt Hydrolase complement in humans. Functional BSH is distributed across gut Bacteria and Archaea. Bile Salt Hydrolase is a widespread gut specific trait and may have implications for overall health in the host. Overall the microbiota alters bile acid recirculation and regulatory networks recognising bile acids are therefore affected by the microbiota. Since bile acids have important roles as signalling molecules and in regulating innate immunity these findings have considerable potential for eventual therapeutic applications.

SUMMARY

I have given a snapshot of some of the important work going on at the APC. The creation of a state of the art facility, and a multidisciplinary team with extensive international collaborations and the exciting work ongoing, sends a clear message that the alimentary microbiota is a source of great potential as we ‘mine microbes for mankind’.

REFERENCES


Probiotics, often in foods such as yogurt or yogurt drinks, are now promoted and consumed to promote intestinal health. Important to assessing whether this is an effective strategy is knowing whether these organisms:

- Survive in the gut
- Stimulate other lactic acid bacteria (LAB)
- Inhibit pathogens

There are some data to support each of these factors influencing the activity of such agents. In most studies probiotic consumption usually results in temporary ‘colonization’ of the gut by the administered strain. A study in 20 healthy volunteers showed that daily consumption of a fermented milk drink (~$10^{11}$ cfu L. casei per day for 21 days) enabled the probiotic Lact. casei strain to be maintained in the gastrointestinal tract (recovered from faecal samples) at a stable relatively high population level during the probiotic feeding period.

Studies, including in infants and elderly subjects also confirm stimulation of other LAB species in the gut – consumption of lactobacilli stimulates bifids and vice versa. Some studies have also shown a decrease in potential pathogens. One randomised double blind placebo controlled study with L. casei taken during and one week after a course of antibiotics, showed that it provided significant protection against antibiotic associated diarrhea and significantly reduced the presence of C. difficile toxin.

More complex and sensitive molecular methodologies reveal extensive changes in LAB and also unrelated bacterial phylotypes after probiotic consumption.

**PREBIOTIC DEFINITION**

The International Scientific Association for Probiotics and Prebiotics (2008, London, Ontario) agreed the following definition for a prebiotic:

‘A dietary prebiotic is a selectively fermented ingredient that results in specific changes, in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health.’

Dietary components that reach the colon, and are available to influence the microbiota include poorly digestible carbohydrates, such as non-starch polysaccharides, resistant starch, non-digestible oligosaccharides (NDOs) and polyphenols.

Resistant Starch is classified in the following way (with examples of each type):
RS1: Physically inaccessible starch
• partly milled grains and seeds

RS2: Native starch granule
• raw potato and green banana

RS3: Retrograded starch (mainly amylose)
• cooled-cooked potato, bread, corn flakes

RS4: Chemically modified (cross-linking, esterification)

RS1, in the form of whole grain cereal has been shown to have more impact in increasing lactobacilli compared to wheat bran alone. RS3 has been shown to modify gut microbiota in rats, greatly increasing populations of bifidobacteria and lactobacilli and reducing populations of potentially pathogenic bacterial groups.

NON-DIGESTIBLE OLIGOSACCHARIDES (NDOS)

Non-digestible oligosaccharides occur in plants (chicory, asparagus, artichoke, onions, garlic, leeks, soy beans) and human breast milk. NDOs have an osidic bond resistant to hydrolysis by intestinal digestive enzymes and this means that they are poorly digested in the upper gastrointestinal tract and reach the colon intact. There they are fermented by colonic microflora to produce short chain fatty acids (SCFA) and gas. NDO substances are widely used in many food and drink products, including yoghurts. Categories of NDO are given in Table 1:

<table>
<thead>
<tr>
<th>TABLE 1: CATEGORIES OF NON-DIGESTIBLE OLIGOSACCHARIDES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inulin and derivatives</td>
</tr>
<tr>
<td>Fructo-oligosaccharides</td>
</tr>
<tr>
<td>Galacto-oligosaccharides</td>
</tr>
<tr>
<td>Soybean oligosaccharides (stachyose/raffinose)</td>
</tr>
<tr>
<td>Xylo-oligosaccharides</td>
</tr>
<tr>
<td>Lactulose</td>
</tr>
<tr>
<td>Isomalto-oligosaccharides</td>
</tr>
<tr>
<td>Arabinoxylan</td>
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</tbody>
</table>

A number of oligosaccharides have been reported to be prebiotic, but the quality of the data is variable. Those with the best evidence include: inulin, fructo-oligosaccharides, galacto-oligosaccharides and lactulose, which have all been shown to have ‘bifidogenic’ effects. The effects of NDO on gut microbiota are summarised in Table 2.
TABLE 2: EFFECTS OF NDOS ON GUT MICROBIOTA:

<table>
<thead>
<tr>
<th>Description</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bifidobacteria counts and proportions are usually increased by ~10x</td>
<td></td>
</tr>
<tr>
<td>Bifidobacteria Increase is usually greater if original count is low</td>
<td></td>
</tr>
<tr>
<td>Lactobacilli are sometimes increased</td>
<td></td>
</tr>
<tr>
<td>Clostridia and enterobacteria are sometimes reduced</td>
<td></td>
</tr>
<tr>
<td>Changes may be specific to particular types:</td>
<td></td>
</tr>
<tr>
<td>sometimes increases in <em>Roseburia, Ruminococcus, Eubacterium</em> have been seen.</td>
<td></td>
</tr>
<tr>
<td>Effects are seen in adults, elderly and infants</td>
<td></td>
</tr>
<tr>
<td>The biological significance of the changes is uncertain.</td>
<td></td>
</tr>
</tbody>
</table>

Beyond the usual categories of prebiotic substances a range of other plant derived substances such as certain flavonoids notably catechins have the potential to influence gut microbiota\(^1\). There is some evidence that consumption of cocoa flavonoids influences the composition of gut bacteria *in vivo*\(^2\).

**SYNBIOTICS**

Synbiotics have been defined as:

‘A mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and/or activating the metabolism of one or a limited number of health-promoting bacteria, and thus improving welfare.’\(^3\)

Combining probiotics and prebiotics is potentially a very effective way of influencing gut microbiota and health\(^4\) but as yet there are few *in vivo* studies in humans; and none that compare pro, pre and synbiotics directly.

**GUT MICROBIOTA – WHAT SHOULD WE MEASURE?**

The majority of studies that have examined the effects of probiotics and prebiotics on the gut microflora have focused on changes in bifidobacteria, but this raises the question: Can increased bifidobacteria alone be considered as a health benefit?

This question gives rise to a number of subsidiary questions including:

- Are bifidogenic probiotics associated with health benefits?
- Are high bifidobacteria associated with positive health?
Are low faecal bifidobacteria associated with poorer health?
Do prebiotics always increase bifidobacteria in all subjects?
Are prebiotic health benefits always associated with increased bifidobacteria?
How big an increase in bifidobacteria is needed for a health benefit?
Is bifidogenic effect a biomarker of other changes in the microflora?

Figure 1 summarises some of the health benefits claimed for prebiotics. An enormous amount of work has been done and continues to be done to link prebiotic health benefits and changes in bifidobacteria.

The current evidence that claimed health benefits for prebiotics are associated with increased bifidobacteria is summarized in Table 3:

**TABLE 3: STATUS OF EVIDENCE FOR PREBIOTIC HEALTH BENEFITS BEING ASSOCIATED WITH CHANGES IN BIFIDOBACTERIA NUMBERS**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atopy and infections in infants</td>
<td>Good evidence from infant formulas15,16,17</td>
</tr>
<tr>
<td>Travellers Diarrhoea</td>
<td>No evidence18,19</td>
</tr>
<tr>
<td>Antibiotic Associated Diarrhoea</td>
<td>Conflicting evidence19,21</td>
</tr>
<tr>
<td>Calcium absorption</td>
<td>No evidence22</td>
</tr>
<tr>
<td>Inflammatory Bowel Disease</td>
<td>Some evidence, but small studies, conflicting results23</td>
</tr>
<tr>
<td>Irritable Bowel Syndrome</td>
<td>Some evidence from 1 study24</td>
</tr>
</tbody>
</table>

**CONCLUSIONS**

We can show that probiotics, prebiotics and synbiotics influence gut microbial ecology. Their main effect is to increase bifidobacteria and lactobacilli, but metagenomic approaches
may reveal wider effects. Changes in the microbiota are often associated with health or physiological benefits. It is clear that some plant polyphenols may act as prebiotics, providing a partial explanation for some of the health benefits associated with high dietary consumption of vegetables.

Research questions for the future include:

- Should we be searching for probiotics other than bifidobacteria and lactobacilli?
- Should we develop more species-targeted prebiotics/synbiotics?
- Should we be less focused on LAB changes as indicators of prebiotic effects and include other possible indicators e.g. *Roseburia, F.prausnitzii, C.butyricum*?
- Is it important to consider more holistic changes in microbiota – e.g. changes in diversity?

**REFERENCES**


