British Journal of Nutrition (2015), **114**(S1), S1–S15 doi:10.1017/S0007114515004043

Probiotics: a proactive approach to health. A symposium report

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(Submitted 31 July 2015 – Final revision received 27 August 2015 – Accepted 10 September 2015)

Abstract

This report summarises talks given at the 8th International Yakult Symposium, held on 23–24 April 2015 in Berlin. Two presentations explored different aspects of probiotic intervention: the small intestine as a probiotic target and inclusion of probiotics into integrative approaches to gastroenterology. Probiotic recommendations in gastroenterology guidelines and current data on probiotic efficacy in paediatric patients were reviewed. Updates were given on probiotic and gut microbiota research in obesity and obesity-related diseases, the gut–brain axis and development of psychobiotics, and the protective effects of equol-producing strains for prostate cancer. Recent studies were presented on probiotic benefit for antibiotic-associated diarrhoea and people with HIV, as well as protection against the adverse effects of a short-term high-fat diet. Aspects of probiotic mechanisms of activity were discussed, including immunomodulatory mechanisms and metabolite effects, the anti-inflammatory properties of *Faecalibacterium prausnitzii*, the relationship between periodontitis, microbial production of butyrate in the oral cavity and ageing, and the pathogenic mechanisms of *Campylobacter*. Finally, an insight was given on a recent expert meeting, which re-examined the probiotic definition, advised on the appropriate use and scope of the term and outlined different probiotic categories and the prevalence of different mechanisms of activity.

Key words: Probiotics: Gut microbiota: Immune system: Irritable bowel syndrome: Diarrhoea: Diabetes: Cancer

The 8th International Yakult Symposium, held on 23–24 April 2015 in Berlin, was entitled 'Probiotics, a proactive approach to health'. The title was chosen for two reasons: to emphasise the importance of taking steps to maintain health throughout life and, because the gut microbiota has a major influence on the whole body (not just on the gut), its modulation by probiotics can be part of a strategy for achieving this. This report summarises the talks given by the panel of international expert speakers, who covered different aspects of microbiology, gastroenterology, immunology as well as metabolic and infectious disease. All speakers approved the manuscript before submission.

Aspects of probiotic intervention

The small intestine: a target for probiotics

The primary message from Professor Michiel Kleerebezem (Wageningen University, The Netherlands) was that the small intestine is pivotal for health[1]. With its distinct community of commensal microbiota and concentration of immune cells, it is a key target for probiotic intervention. Analysis of its microbiota, however, has been hampered by a lack of non-invasive sampling methods for healthy volunteers: sampling has usually been carried out via naso-ileal catheters. Although luminal microbiota samples are easily obtained from the distal ileum of ileostomy subjects, these are usually inflammatory bowel disease (IBD) or cancer patients.

Newly developed radio-controlled capsules (IntelliCap®) are currently being evaluated for the extraction of small volume samples (100–200 µl) from the small intestine of healthy volunteers, to avoid the use of invasive technologies or causing undue discomfort[2].

16S rRNA phylogenetic microarray (The Human Intestinal Tract Chip) analysis of the ileostomal, small intestine and faecal samples has found that the gut microbiota of the small intestine is much simpler than that of the colon, with far fewer species[3–5]. Ileostomy samples can serve as models for the microbiota of the proximal small intestine, as the microbial compositions are reasonably similar[6]. Predominant genera in the small intestine are *Streptococcus*, *Veillonella*, *Clostridium* and *Escherichia*[7]. Metagenomic and metatranscriptomic studies have confirmed that the small intestinal microbiota is strongly focused on the import and fermentation of simple carbohydrates[6]. *Escherichia* and *Streptococcus* spp. seem to be involved in carbohydrate

Abbreviations: AAD, antibiotic-associated diarrhoea; BA, butyric acid; CD, Crohn's disease; FMT, faecal microbiota transplant; GI, gastrointestine; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; LPS, lipopolysaccharide; PC, prostate cancer; RCT, randomised-controlled trials; SCI, spinal cord injury; T2D, type 2 diabetes; TLR, toll-like receptor; UC, ulcerative colitis.

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Publication of this supplement was supported by an educational grant from Yakult Europe B.V. This review has undergone the standard review process and may be cited.

import, using phosphotransferase transport systems to convert carbohydrates to pyruvate by glycolysis, and then converting this intermediate to the fermentation end products such as lactate, acetate, formate and potentially ethanol. The secondary fermentation of lactate and acetate by *Veillonella* and *Clostridium* spp. produces propionate, acetate and butyrate. As lactobacilli are not dominant in the small intestine, probiotics have the potential to overcrowd the endogenous microbiota and cause a dramatic, albeit transient, community shift that cannot be achieved in the densely colonised colon.

A pioneering study on healthy men revealed a significant host mucosal transcription response to ingestion of probiotic lactobacilli in the small intestine, with 300–750 genes affected in a strain-specific manner[8,9]. Network biology approaches for transcriptome data interpretation have now advanced sufficiently to enable a molecular explanation of the clinical outcome of a *Lactobacillus* intervention. Moreover, the *in vivo* data also confirmed specific wound healing-accelerating effects of the probiotic *Lactobacillus rhamnosus* GG (LGG) on mucosal physiology, which are achieved through previously established secretion of the proteins P40 and P75 that modulate epidermal growth factor-receptor signalling[10]. These analyses may also be employed to predict physiological consequences using comparative transcriptomic analyses, which have established significant correlation of responses to probiotics with responses measured for pharmaceutical drugs[11]. It should be noted, however, that most of these pharmaceuticals work systemically, whereas probiotics work locally in the gut. Nevertheless, some probiotic effects and mechanisms may be similar to those achieved by specific drugs and may provide guidance to future probiotic intervention trials.

There is considerable variation in people's responses to any probiotic; probiotic-responsive genes also cluster according to individual and not by intervention[8,12]. Volunteers for a probiotic trial may be considered healthy yet actually vary enormously in the molecular makeup of their mucosa, indicating that differential molecular solutions for health are possible and can influence the responsiveness to a probiotic intervention, which may (in part) explain 'non-responders' in probiotic trials. How this molecular individuality of human subjects is achieved remains unclear and could include many factors such as genotype, epigenetic imprinting, dietary habits, lifestyle and/or endogenous microbiota. Professor Kleerebezem underlined the importance of a crossover trial design, and for probiotic intervention studies exemplified the potential of subject stratification prior to enrolment, on the basis of their predicted susceptibility to the intervention being tested.

Probiotics as part of an integrative approach to gastroenterology

Professor Jost Langhorst (Kliniken Essen-Mitte, Germany) explained that IBD, irritable bowel syndrome (IBS) and other gastrointestinal (GI) diseases are driven by multiple factors, including genetic predisposition, immune dysregulation, gut dysbiosis and barrier dysfunction. Crohn's disease (CD) patients, for example, have a low diverse gut microbiota with reduced *Faecalibacterium prausnitzii* and increased *Escherichia coli*. Relatives of the patients, with the same CD genetic disposition, also showed some degree of dysbiosis and gut barrier abnormality[13]. As several microbial-related factors (e.g. dysbiosis and low microbiota diversity, presence/persistence of pathobionts and pathogenic antigens) link a defective mucosal interface to inflammation[14,15], the gut microbiota should be a treatment target[16].

Professor Langhorst recommended an integrative approach to chronic GI disorders, combining evidence-based complementary and alternative medicine (CAM) with mainstream medicine and lifestyle modifications. Health-care professionals also need to keep up to date with CAM as patients with chronic GI disorders explore and ask about all available treatments[17]. As an example, botanical therapies used in IBD were reviewed by Professor Langhorst. Psyllium, for example, may be as effective as mesalamine in prolonging remission in ulcerative colitis (UC)[18] and also showed effectiveness for active CD when used in combination with probiotics[19]. Other plants and plant-derived substances investigated are the following: curcumin; frankincense; a combination of myrrh, chamomile and charcoal; bilberries; tormentil; and wormwood[20]. Results on psyllium and curcumin are now positive enough to warrant mention in German UC guidelines[21]. Cannabis, a strongly regulated and controlled substance, is also of interest with IBD, but side effects are frequent, and there may be a higher risk of surgery in CD[22]. Traditional Chinese medicine is also of interest in IBD: acupuncture has shown benefit[23].

In industrialised countries, lifestyle and environmental factors have a strong influence on IBD[24], and these factors (e.g. smoking and lack of exercise) may also influence the gut microbiota[25,26]. Mind–body interventions are also used in treatment of chronic GI disorders because of the evidence that stress exacerbates IBD symptoms[27,28]. Stress reduction strategies and mind–body therapy have shown benefit in UC[29,30], and stress can also change the composition of the commensal microbiota[31]. Such observations are part of the rationale for exploring probiotic benefit for IBD. Certain strains have shown benefit (e.g. *E. coli* Nissle, lactobacilli, bifidobacteria and streptococci) in maintaining remission in UC and acute pouchitis, but there is no reliable evidence for CD. Patients are interested in another strategy for gut microbiota modulation – faecal microbiota transplant (FMT) – and may even attempt it themselves. FMT capsules or microbial consortium have now been developed, and a stool bank facility has been established in the USA (http://www.openbiome.org/). Although FMT has shown positive effects with recurrent *Clostridium difficile* infection[32], results have been more uneven in IBD[33,34]. Two recent randomised studies of patients with active UC have been conducted: one had no effect, and one was effective in inducing remission[35,36].

Guidelines and recommendations for probiotic usage

Gastroenterology guidelines for irritable bowel syndrome

Professor Viola Andresen (Israelitic Hospital Hamburg, Germany) explained that guidelines are a combination of medical science, clinical practice and education intended to help promote good clinical practice and to inform the public. Guidelines also need

to ensure cost-effectiveness and support clinical decisions and may even be used in legal disputes. The guidelines in Germany are decided by expert opinion alone or by consensus following the Delphi process[37], as well as by evaluation of evidence found by a systematic search and review of the literature, which considers risk of bias, inconsistency, indirectness and imprecision. Recommendations are based on the Grading of Recommendations Assessment, Development and Evaluation approach[38]. Even if no evidence is available, an expert opinion may still be needed.

The rationale for probiotic use in IBS is based on the role of the gut microbiota in many GI functions and the observation of a disturbed microbiota in patients. The difficulty in evaluating efficacy, however, was illustrated by studies with *Bifidobacterium longum* subsp. *infantis* 35624. A 2005 trial reported alleviation of symptoms[39], yet a later study did not find benefit at this test level (10^{10} colony-forming units (CFU)) but did with a lower dosage (10^{8} CFU)[40]. Despite conflicting results, meta-analyses do suggest probiotic benefit, but the data raise several questions[41,42]. More research is needed to determine mechanisms of actions, which probiotics are effective and at what dosage and duration.

Several countries now mention probiotics in their clinical guidance on IBS. Although the American Gastroenterological Association IBS management guidelines do not cover probiotics, the accompanying technical review indicates that probiotics may be beneficial and can be considered on an individual basis[43]. A related and more comprehensive review concluded that, as a whole, probiotics improve global symptoms, bloating and flatulence, but this was a weak recommendation based on low quality of evidence[42]. The UK's National Institute for Health and Care Excellence does not directly recommend probiotics but does offer advice to people who choose to take them – to take probiotics for at least 4 weeks at the manufacturer's recommended dose while monitoring the effect[44]. German guidelines recommend that selected probiotics can be used for treatment, with the strain selection based on symptoms[45].

Professor Andresen finished by noting the strain-specific nature of probiotics and the heterogeneous nature of available studies. She also discussed whether probiotics should be classified as food or medicine, and their acceptance by experts. Finally, she underlined that the potential success of gut microbiota modulation for IBS was underlined by a case report of a patient who had suffered post-infectious IBS for 2 years, which was refractory to any conventional treatment. The patient became symptom-free within hours of undergoing FMT and remained healthy 14 months later.

Probiotic efficacy in paediatrics: a review of the evidence

After noting the problem of the strain-specific nature of probiotic benefit for meta-analyses and systematic analyses, Professor Hania Szajewska (The Medical University of Warsaw, Poland) gave a comprehensive update of this area.

- Treatment of acute gastroenteritis: in 2014, the European Society for Paediatric Gastroenterology Hepatology and Nutrition published an evidence-based position paper, concluding that use of probiotics with documented efficacy may be considered[46]. The use of the following probiotics may be considered as adjuncts to standard oral rehydration therapy for reducing diarrhoea duration: LGG or the yeast species *Saccharomyces boulardii* (low quality of evidence for both strains; strong recommendation); and *Lactobacillus reuteri* DSM 17938 (low quality of evidence; weak recommendation). There was insufficient evidence to recommend any of the many other probiotics that have been studied.
- Prevention of nosocomial diarrhoea: some, but not all, probiotics have shown efficacy[47,48], particularly for GI infections. A recent study with *Bifidobacterium animalis* subsp. *lactis* failed to show prevention of common infections in hospitalised children[49].
- Prevention of antibiotic-associated diarrhoea (AAD): although there was no evidence to support probiotics as a treatment, a recent meta-analysis that identified sixteen randomised-controlled trials (RCT) in children concluded an overall relative risk (RR) of 0·55 (95 % CI 0·38, 0·8) for AAD prevention, with a number needed to treat (NNT) of 12[50].
- Prevention of necrotising enterocolitis (NEC): a recent Cochrane review calculated an RR of 0·43 (95 % CI 0·33, 0·56) for probiotics in preventing NEC, and an RR of 0·65 (95 % CI 0·52, 0·81) for death[51]. The NNT was approximately 33. The evidence is sufficiently strong enough to support a change in practice. Probiotics with documented efficacy may be considered for prevention of NEC, particularly where incidence is high. More information is needed to establish as to which products are effective, as well as their recommended dosages and duration of use.
- Infantile colic: there is evidence that *L. reuteri* DSM 17938 has benefit for infantile colic[52,53]. (One study reported no benefit, possibly due to participants' heterogeneity[54].) A recent meta-analysis of three RCT concluded that the probiotic is likely to reduce crying time by about 43 min (a welcome benefit for parents), especially in exclusively or predominantly exclusively breast-fed infants[55]. A recent RCT suggested that *L. reuteri* DSM 17938 is also effective in preventing infantile colic; the probiotic reduced crying time from 71 to 38 min[56].
- Prevention of allergic disease: in 2015, the World Allergy Organization published new evidence-based guidelines relating to probiotic use[57]. Current evidence does not indicate that probiotics reduce the risk of children developing allergy, but, despite very poor quality of evidence, it was recommended that there is a likely net benefit from probiotic use in pregnant women who are at high risk of having an allergic child, in women who breast-feed infants that are at high risk of allergy and in infants who are at high risk of developing allergy. However, it remains unclear which probiotic(s) should be used.

Professor Szajewska concluded that the composition of the gut microbiota plays a significant role in the development of a number of disorders affecting children. Although there is huge potential for probiotics, many questions still need to be answered.

Probiotic benefits for other conditions: an update on the evidence

Obesity and obesity-related disease

Professor Mauro Serafini (CRA-NUT, Italy) explained how a Western high-fat diet induces a sustained postprandial state of hyperlipidaemia and hyperglycaemia. This triggers cellular inflammation as well as oxidative stress due to the generation of reactive oxygen species. In turn, these cause endothelial damage and insulin resistance, which increases the risk for diseases such as the metabolic syndrome, atherosclerosis, type 2 diabetes (T2D), hypertension and stroke[58–60].

A recent placebo-controlled study on healthy overweight people by Professor Serafini's group demonstrated that a dietary intervention in the form of fruit juice prevented an increase in plasma levels of pro-inflammatory cytokines (TNF-α, IL-6 and IL-17) that occurred after eating a high-fat meal[61]. Further studies have shown that eating high-fat meals increases plasma levels of uric acid and thiols; this also can be prevented by simultaneous consumption of fruit juice[60,62]. These effects may be due to the presence of flavonoid in the fruit juice; these plant metabolites are found in many foods[63]. Flavonoid bioavailability *in vivo* (i.e. their antioxidant capability within the human body) can vary enormously in different types of food and can also differ significantly from antioxidant levels detected by *in vitro* testing of the food. Polyphenol flavonoids found in chocolate, for instance, have high antioxidant levels, but the interference of milk with their absorption *in vivo* explains why dark chocolate has a far greater antioxidant benefit *in vivo*[64]. After ingestion, as well as being digested by host enzymes, dietary polyphenols are metabolised by the gut microbiota to produce a range of bioactive derivatives that can be absorbed by the intestinal cells[65,66]. The profile of bacterial species present in any individual's gut microbiota determines what bioactive metabolites will be derived from dietary polyphenols.

There is potential for probiotics to enhance microbial production of bioactive flavonoid metabolites in the gut[67]. Several animal studies have also indicated that certain *Lactobacillus* and *Bifidobacterium* probiotic strains can reduce oxidative stress via mechanisms such as reducing hydrogen peroxide and hydroxyl radicals[68–72]. The one human study conducted to date showed a similar positive trend: consumption of a probiotic yogurt improved the antioxidant status of people with T2D[73].

Professor Nathalie Delzenne (Catholic University Louvain, Belgium) suggested the gut dysbiosis as a target for management of obesity and related disorders[74]. In obese and overweight people, a gut microbiota of low diversity has been linked to these people's propensity for metabolic disturbances and chronic low-grade inflammation[75]. Animal models show that the aberrant microbiota, often with lower levels of bifidobacteria and *Akkermansia muciniphila*, triggers disruption to the gut barrier, increasing permeability and promoting translocation of bacteria lipopolysaccharide (LPS) to cause endotoxaemia[76,77]. In diet-induced obese mice, live *A. muciniphila* administration decreased serum LPS, reduced adipose tissue inflammation and increased expression of the antimicrobial peptide RegIIIγ in the colon. This intervention also reverses the thinning of the mucus layer observed in obesity[77]. Obesity-related disease is also associated with an altered profile of microbial metabolites, such as lower levels of SCFA and bile salt hydrolases, and raised numbers of sulphide-reducing bacteria. Recent animal studies also underline the ability of the gut microbiota to metabolise dietary PUFA[78].

In diet-induced obese animals, probiotic administration results in loss of body weight and/or fat mass and may also improve blood lipid levels, glycaemia or other metabolic disorders[79–82]. In mice fed a high-fat diet, probiotics shifted the aberrant gut microbiota profile towards that of lean mice on a normal diet. Strains differentially attenuated the induced obesity, inflammation and the metabolic syndrome[83]. Human studies, however, have not been as convincing. Only a few have shown probiotic benefit, with only minor effects on the body weight and fat mass but better effects on blood lipids and metabolic response to diet[84–86]. A recent study with *Lactobacillus casei* Shirota has indicated the potential of certain probiotics in a preventative strategy for overfeeding-induced insulin resistance[87]; however, Professor Delzenne recommended development of novel commensal strains as well as butyrate-producing[88] and CLA-producing probiotics[89].

Prebiotics affect not just bifidobacteria numbers but also the overall richness and diversity of microbial functions and species in the gut. Obesity studies in mice have shown that prebiotics changed the ratio of Firmicutes:Bacteroidetes and modified over 100 microbial taxa[90]. High-fat diet and prebiotic intervention also influence host antimicrobial peptides[91] and gut endocrine function[92]. A 2013 review, which identified six prebiotic trials in overweight or obese people and three in non-alcoholic steatohepatitis or T2D, concluded that prebiotics increased feelings of satiety and reduced postprandial plasma glucose and insulin concentrations[93]. Effects of prebiotics on endotoxaemia, fat mass, gut hormones, cardiovascular and hepatic health were also reported, which may be linked to microbial changes. A study on obese women found that an inulin-type fructans changed the gut microbiota by increasing levels of *Bifidobacterium* and *F. prausnitzii* (which correlated with reduced serum LPS) and reducing *Bacteroides intestinalis*, *Bacteroides vulgatus* and *Propionibacterium* (which correlated with slight decreases in fat mass, and plasma lactate and phosphatidylcholine levels)[94]. Another study on obese women showed levels of *B. longum* and *A. muciniphila* correlated with reductions in plasma LPS, and *Bifidobacterium adolescentis* with decreases in fat mass. Perhaps surprisingly, the prebiotic also decreased SCFA levels (raised SCFA could be a metabolic risk factor for obese women)[95]. The observed link between some PUFA-derived bacterial metabolites (e.g. CLA), certain gut species and host metabolism is not yet fully understood[96].

Dr Carl Hulston (Loughborough University, UK) continued the theme of the gut microbiota's influence on obesity and T2D[97,98] in a short talk on his recent study into the effects of a probiotic on health risks associated with poor diet[87]. In an RCT, a test group drank an *L. casei* Shirota fermented milk twice daily for 4 weeks, whereas a control group received no supplementation. All ate their normal diet for 3 weeks and then consumed a high-fat high-energy diet for 1 week. Compared with baseline, the week of overfeeding resulted in an increase of glucose AUC in an oral glucose tolerance test and elevated

fasting plasma glucose, as well as a decrease in insulin sensitivity for the control group, whereas all these parameters were maintained in the probiotic group. Fasting serum insulin did not change in either group. These results suggest that probiotics may be useful in preventing diet-induced metabolic diseases but need to be confirmed in a larger study; the mechanism of activity also needs to be investigated.

Manipulation of the gut–brain axis: the emergence of psychobiotic therapy

Professor John Cryan (University College Cork, Ireland) reviewed research into the microbiota-gut–brain axis and explained that the gut microbiota is considered 'the conductor of the orchestra of immune-neuroendocrine communication', essential for normal stress, antidepressant and anxiety responses[99,100]. Unfortunately, the majority of evidence in this field is still from animal studies: either germ-free, subjected to early-life modulation of the gut microbiota, or exposed to specific pathogens, probiotics or antibiotics[101]. These studies have shown that stress in early life not only alters behaviour but also causes key changes in the immune system and the gut microbiota, which are themselves linked to changes in colonic transit time and morphology, intestinal permeability and mucosal inflammation[102,103]. Perturbation of the postnatal microbial colonisation process affects neurodevelopment, with possible consequences for later mental health[104].

The gut microbiota influences the brain and nervous system via several routes, including the vagus nerve, the spinal cord, and the immune and neuroendocrine systems. Gut bacteria produce several neuroactive molecules – for example, γ-aminobutyric acid, serotonin, catecholamines and acetylcholine[105] – and may also affect the early development of the hypothalamic–pituitary–adrenal reaction to stress[106]. The importance of the gut microbiota for brain development and behaviour is clearly shown by germ-free animals, which display less anxiety and neurochemical differences[107,108]. Male germ-free animals show changes in the hippocampal serotonergic system, not reversible with restoration of the gut microbiota[109]. Animal models have also shown that the gut microbiota influences regulation of neurogenesis in the adult hippocampus[110] and blood–brain barrier permeability (reversible with the introduction of butyrate-producing bacteria)[111]. It may also act as an epigenetic regulator of brain function, which may have a bearing on neurodevelopmental disorders such as autism. SCFA such as butyrate can alter the function of enzymes that modify histone proteins, thus changing gene expression[112].

Development of probiotics with psychotrophic effects would be facilitated by charting developmental events in the brain through life and relating this to parallel changes in the gut microbiota and its metabolites[104]. Animal studies indicate the benefit of lactobacilli and bifidobacteria probiotics, with the vagus nerve as the key route for effects[113,114].

Dysbiosis may be an underlying factor in the gut problems frequently experienced by people with autism spectrum disorder (ASD). The gut microbiota is essential for normal social development in mice[115], and alterations in the gut microbiota and function were associated with autism-like behaviour in a murine ASD model, particularly in males[116]. This has promoted interest in probiotics for ameliorating both gut and behavioural symptoms[117,118]. FMT has also been shown to change brain chemistry and behaviour in germ-free recipient mice[119]. One outcome from transplanting from mice on a high-fat diet to those on normal feed was a negative change in neurocognitive behaviour[120].

IBS is considered a disorder of the gut–brain axis, and microbiome research confirms this. Transient dysbiosis early in life affects visceral sensitivity, increasing the risk for later developing IBS[121]. The gut microbiota profile also correlates with the clinical phenotype of patients[122]. Probiotic benefit has been demonstrated in several animal studies[123], and human study evidence is accumulating, particularly with lactic acid bacteria[124]. IBS patients also appear to have a subtle deficit in cognitive behaviour linked to changes in cortisol levels; perhaps this is another target for probiotics[125].

Finally, Professor Cryan introduced the term 'psychobiotics' for probiotics aimed at psychiatric illness[126]. Since a first report in 1910[127], data are now accumulating on improvements in mental health parameters associated with lactobacilli and bifidobacteria[128–131].

Prostate cancer

Professor Hideyuki Akaza (The University of Tokyo, Japan) began by outlining the geographical differences in prostate cancer (PC) incidence. Although PC is much lower in Asian countries compared with Western countries[132], Japanese migrants in Hawaii have an increased PC incidence compared with Japanese people still living in Japan. This observation suggested that lifestyle factors, and not just diet, may be involved in determining disease risk[133]. As this cancer progresses very slowly (up to 27 years), preventive interventions are worth pursuing[134]. It may not be cost-effective or even safe for drugs such as 5α-reductase inhibitors[135] to be taken over long periods, making dietary interventions a better option.

In 1998, a detailed examination of UN data for fifty-nine countries on PC-related mortality, dietary intake and lifestyle factors highlighted the particular benefit of soya consumption in protecting against the disease[136], a finding confirmed by later meta-analysis[137]. The active ingredients in soya are isoflavones, such as genistein, daidzein and equol[138]. The anti-androgen activity of equol is explained by its ability to bind to oestrogen receptor β and the sex hormone-binding globulin (SHBG), thus inhibiting the growth of sex hormone-dependent tumours such as PC[139].

Certain bacterial species in the colon are able to metabolise daidzein to produce equol, but these species are only carried by about 30 % of people consuming a Western diet, which is low in soya[140]. Professor Akaza's group conducted a study on healthy Japanese people (both equol producers and non-producers), given 60 mg soya isoflavones daily for 3 months, which caused an increase in serum levels of equol and SHBG and a decrease in free testosterone and dihydrotestosterone[141]. The results also indicated that prolonged consumption of soya isoflavone might convert non-equol producers to equol producers; this may be an important finding as the lack of ability to metabolise daidzein

to equol in the colon appears to be a PC risk factor[142]. Numbers of equol producers are low in Western countries, where PC risk is higher compared with countries such as Japan and Korea. Worryingly, the ability to produce equol appears to be dropping in the Japanese population, which could be linked to a parallel rise in PC incidence[143]. A phase II placebo-controlled pilot trial in men with rising prostate-specific antigen showed that dietary supplementation of isoflavone for 12 months significantly increased serum levels of equol in subjects known to produce this metabolite. In subjects aged 65 years or older, the incidence of PC was also lower in the test group compared with the placebo group[144].

A novel *Slackia* spp. strain NATTS – a gram-positive bacterium belonging to the phylum Actinobacteria and isolated from Japanese adults – can produce high levels of equol[145]. Research is now focusing on understanding the global distribution of equol-producing strains and comparing this with PC risk. Large-scale intervention studies are also planned, which will investigate men at high risk of PC and the potential benefit of intervention with soya foods, equol supplements and/or foods containing the *Slackia* strain or its daidzein-metabolising enzymes. The realisation of a link between components of the intestinal microbiota and cancer risk reduction will support the development of a new research field[146].

Antibiotic-associated diarrhoea in spinal cord injury patients

People with spinal cord injury (SCI) face a range of health problems, explained Dr Samford Wong (Stoke Mandeville Hospital, UK), which is why quality of life is a key research focus. Patients are encouraged to participate in sport and supported into re-employment but complications, such as malnutrition, affect clinical outcome[147]. Use of catheters increases risk of urinary tract infection as well as diarrhoea resulting from antibiotic treatment delays rehabilitation, which affects the patient and increases their health-care costs. This is why cost-effective, reliable and simple measures are needed. Probiotics have potential for preventing AAD[148] and in fact are already being used in some SCI centres. However, medical staff are not always aware that efficacy can be strain-, dose- and disease-specific.

Recently an RCT in SCI patients conducted by Dr Wong demonstrated that a fermented milk drink containing *L. casei* Shirota significantly prevented AAD, which developed in 54·9 % of patients in the control group compared with 17·1 % given the probiotic[149]. To fully evaluate probiotic potential for SCI patients, Dr Wong proposed a three-step approach: (i) to evaluate current AAD and *C. difficile*-AD (CDAD) practices in UK and European SCI centres; (ii) to conduct a systematic review and meta-analysis on their effectiveness in preventing AAD/CDAD in SCI patients; and (iii) to conduct a multicentre, double-blind, placebo-controlled trial to confirm the benefit of *L. casei* Shirota.

HIV

HIV/AIDS is characterised by a progressive depletion of CD4+ T-cells and a severe impairment of the immune system. This is often accompanied by an alteration of the gut mucosal barrier, which allows translocation of microbes and their products, and provokes a chronic state of inflammation[150]. In view of the immunomodulatory effects reported for probiotics in non-immunocompetent and HIV+ subjects[151,152], Dr D'Angelo's ('G. d'Annunzio' University of Chieti-Pescara, Italy) group conducted a pilot study on the effects of *L. casei* Shirota (at $1{\cdot}3 \times 10^{10}$ CFU/d for 4 weeks) on clinically stable HIV+ patients on antiretroviral therapy[153]. Blood samples were taken before and after the intervention. Haematological (lymphocyte subsets), immunological (circulating cytokine levels and mRNA expression in peripheral blood mononuclear cells) and other parameters (LPS and cystatin C) were measured. The probiotic was associated with slightly reduced plasma LPS, significantly increased CD56+ and significantly reduced mRNA levels of IL-1β, IL-10, IL-12 and transforming growth factor-β in peripheral blood mononuclear cells, while plasma IL-23 increased. An observed decrease of cystatin C also indicated that cardiovascular risk might be reduced. Dr D'Angelo concluded that this preliminary evidence of an improvement in systemic inflammatory cytokines suggests that this probiotic could improve the management of antiretroviral therapy-treated HIV+ patients and warrants further investigation.

Probiotic mechanisms: immune modulation and effects of metabolites

Faecalibacterium prausnitzii

This bacterium, the subject of a talk by Professor Philippe Langella (INRA, France), is an ubiquitous commensal species comprising >3·5 % of the gut microbiota, which is extremely oxygen sensitive and a butyrate producer. It belongs to the gram-positive Firmicutes phylum and *Clostridium leptum* group cluster IV[154–156]. Reduced abundance and diversity of Firmicutes occur in CD patients. A landmark study by Sokol *et al.*[157] in 2008 also demonstrated an association between reduced *F. prausnitzii* numbers and increased risk for postoperative recurrence of ileal CD. The species was also shown to have anti-inflammatory effects in cellular and animal models of gut inflammation[157]. A different strategy to find new probiotic strains was proposed: identify candidate species whose presence/absence is linked to health in diseases linked to microbial dysbiosis. One such species is *F. prausnitizii*. Having been linked to dysbiosis in CD, UC, colorectal cancer and IBS, it could almost be an indicator of intestinal health[158–160]. A novel, chronic dinitrobenzene sulphonic acid (DNBS)-induced acute and chronic colitis model in mice developed by Professor Langella's group showed that *F. prausnitzii* and its supernatant protected the gut epithelium during episodes of chronic colitis and its reactivation[161].

The same DNBS-induced mouse model was adapted to better mimic IBS, with cycles of low-grade, sub-clinical inflammation and then a period of recovery followed by reactivation of inflammation. The IBS mice had raised levels of serotonin and inflammatory cytokines in the colon (IL-6, interferon-γ (IFN-γ), IL-4 and IL-22), as well as impaired gut permeability, but these effects were reversed following treatment with *F. prausnitzii* or its supernatant[162]. The species was also tested in a neonatal

maternal separation model that induces colonic hypersensitivity and increases gut permeability. In this pain model, *F. prausnitzii* strain A2–165 (now patented) demonstrated antinociceptive effects and restored gut permeability.

A further *F. prausnitzii* gnotobiotic model has been developed, where dual colonisation with *E. coli* achieves more effective and stable colonisation by *F. prausnitzii*. When colitis was induced in this model, disease activity and other parameters were improved by the presence of *F. prausnitzii*. The species was also shown to be very metabolically active, producing several compounds with beneficial effects, such as shikimic and salicylic acids. Both have anti-pain activity; the latter is also a precursor of 5-aminosalicylic acid, the anti-inflammatory drug used to treat IBD[163].

The next stage is to develop *F. prausnitzii* as a human probiotic, but first a few obstacles have to be overcome. The species is considered to be a novel food; hence, full toxicology assays and characterisation of the strain are needed for regulatory approval. The freeze-drying process for commercial preparation of the strain also needs to be optimised. Work is continuing to fully understand the species, its mode of action and its bioactive metabolites.

Immunomodulatory mechanisms of probiotics

Dr Liam O'Mahony (Swiss Institute of Allergy and Asthma Research, Switzerland) explained that immune tolerance in the gut evolved in order to minimise the impact on the host of dealing with an invader and to allow the commensal microbiota to colonise the gut, where it provides nutrients to and protection for the host[164,165]. In conditions of gut dysbiosis, however, even commensal bacteria can trigger an inappropriate inflammatory response[166]. For example, recognition of segmented filamentous bacteria by the mucosal immune system can, in the wrong circumstances, lead to T-helper 17 cell-mediated inflammation[167]. Gut microbiota colonisation in early-life influences the risk for immune disorders; allergy development in germ-free mice, for instance, depends on what age the mice are colonised with bacteria[168]. Mice studies show that food allergy is associated with a particular profile of gut microbiota; this triggered allergy and anaphylaxis when transplanted to germ-free mice[169].

Dr O'Mahony's group is elucidating the cellular and molecular effects of an immunoregulatory probiotic, *B. longum* subsp. *infantis* 35624[170]. In mice, its consumption resulted in induction of T-regulatory cells and attenuation of NF-κB activation, preventing excessive inflammation induced by *Salmonella* infection[171]. Induction of T-regulatory cells by the strain has also been shown in humans, as well as reduction of systemic pro-inflammatory biomarkers in patients with psoriasis, IBS, chronic fatigue syndrome or UC[172]. In the peripheral blood, the strain stimulates dendritic cell-induced T-regulatory cells to produce IL-10 and also enhances *Foxp3* expression. It also reduces production of IL-12 and TNF-α, effects which all appear to be strain-specific[173,174].

Bacterial metabolites can also exert immunological effects. The biogenic amine histamine, which is found at particularly high levels in the gut mucosa of IBS and IBD patients, can promote either pro- or anti-inflammatory effects depending on which of its four receptors are activated[175,176]. Not all commensal bacteria, however, express histidine decarboxylase (the enzyme needed to convert histidine to histamine). The isolate *Lactobacillus saerimneri* strain 30a produces high levels of biologically active histamine[177]. Feeding this strain to wild-type mice and mice lacking histamine receptor 2, for example, resulted in a deterioration in health. Current research is investigating the ability of the gut microbiota to produce histamine, as this may have health implications.

SCFA are other types of bacterial metabolites with immunoregulatory effects. Dr O'Mahony's group has used an ovalbumin-challenge respiratory allergy model to show that butyrate reduces pro-inflammatory cytokines such as IL-17, which can be raised with asthma. Dr O'Mahony stressed that the timing of any intervention relating to immune benefit may be critical; the greatest effects are likely to be early in life[178].

Campylobacter *infection*

Although *Campylobacter* is the most common bacterial cause of enteritis in industrialised countries, Professor Stefan Bereswill (Charité – University Medicine Berlin, Germany) explained that its pathogenicity is still not fully understood. Although *Campylobacter jejuni* can asymptomatically colonise animals such as chickens and cows, it causes inflammation and acute diarrhoea in humans, and rare but serious post-infectious immune-related disorders such as Guillain–Barré syndrome[179]. Mice with a normal gut microbiota display a strong colonisation resistance to *C. jejuni*; hence, finding a good animal model for this infection has proved a challenge for researchers. Wild-type and GM mice models have now been developed, where the commensal microbiota has been eradicated or modified (e.g. to one resembling a human gut microbiota)[180]. Infant mice harbouring a conventional gut microbiota have much higher counts of commensal *E. coli* compared with adult animals, and overgrowth of the murine gut microbiota with *E. coli* abrogates colonisation resistance against *C. jejuni*[181,182]. The pathogen readily colonises antibiotic-treated gnotobiotic mice with a knockout for IL-10 expression (i.e. IL-10$^{-/-}$), causing enterocolitis and the development of LPS-mediated inflammation and specific T-cell responses that are characteristic of campylobacteriosis in humans[180,183]. Studies on these mice have shown that *C. jejuni* is a potent activator of the innate immune response via toll-like receptor (TLR) 4 and, to a lesser extent, TLR2 (these receptors detect LPS and lipoprotein molecules on the bacterial surface). TLR4 and TLR2 also appear to be involved in the ability of the pathogen to induce inflammation and apoptosis of enterocytes[184]. The addition of a sialic acid derivative to the surface LPS of *C. jejuni* also aggravates its interaction with TLR, increasing the invasion, translocation and inflammatory potential of the pathogen and thereby raising the risk of post-infectious complications[185]. Several cytokines have been identified as important in protection against *Campylobacter*. Investigations using human biopsy material, for example, have shown that the innate and adaptive T-cell immune responses to *C. jejuni* are associated with production of IFN-γ, IL-22 and IL-17A[186]. The Gastrointestinal Microbiology Research Group at Charité (headed by Markus Heimesaat and

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Stefan Bereswill) has shown that IL-22 and IL-23 are essential for maintaining the composition of the intestinal microbiota; this then helps maintain colonisation resistance. Current work is aimed at analysing the pro- and anti-inflammatory T-cell subsets in murine infections and in IL-22$^{-/-}$ and IL-23$^{-/-}$ mice. *C. jejuni* infection in 3-week-old mice results in a self-limiting illness, but neutrophil infiltration can be observed in the colon, liver, lungs and kidneys, and this can lead to a state of chronic inflammation in asymptomatic carriers. The similarity of this pathology to the human disease with its serious postoperative complications means that these novel animal models will be extremely useful tools for *C. jejuni* research and perhaps development of probiotics to help prevent this infection.

Butyric acid, the oral microbiota and ageing

A complex association exists between periodontal disease and certain systemic disorders such as arteriosclerosis, T2D, pneumonia, heart disease and premature childbirth[187–189]. Professor Kuniyasu Ochiai's (Nihon University School of Dentistry, Japan) group are researching into this, focusing on the role of butyric acid (BA), which is produced at high levels by periodontopathic plaque species such as *Porphyromonas gingivalis*, *Fusobacterium nucleatum* and *Eubacterium*[190,191]. BA accumulates in gingival crevices at higher levels in periodontitis cases compared with the healthy[190] and could serve as a promoter of periodontitis[192,193]. At low concentrations, BA stimulates cell growth, but at higher concentrations it induces apoptosis in neutrophils, T-cells and macrophages[191,194,195]. T-cells survive by adhering to fibroblasts[196] as a result of increased expression of adhesion molecules plus increased production of pro-inflammatory cytokines IL-6 and IL-11 by the gingival fibroblasts. Although fibroblasts are usually resistant to BA-induced apoptosis, cells from periodontitis patients are highly sensitive[197].

The complex signalling network associated with oral BA shows how it may elicit systemic effects, which could influence ageing and latent infections. Animal studies have shown that BA-induced primary signalling affects total haem, NADP and hydrogen peroxide[198]. These compounds are linked to the free radical theory of ageing[199], reactivation of latent infections such as HIV[200], periodontal disease[201] and promotion of *P. gingivalis*[202]. Secondary signalling affects cytosolic and serum levels of Ca (factors associated with osteoporosis and Alzheimer's disease)[203,204] and caspases (proteases important for apoptosis and inflammation)[205]. The late network caused by BA affects the oxidised form of NAD, sirtuin1 and the mammalian target of rapamycin, and is linked to the ageing processes[198,199].

In HIV-infected patients, degree of periodontitis correlates to HIV-RNA load in gingival crevicular fluid[206]. Professor Ochiai's group found that the BA produced by *P. gingivalis* acts as an epigenetic regulator, inhibiting the histone deacetylases that maintain HIV latency[207]. Severe periodontitis could trigger a synergy between the local effects of BA in gingival tissue and systemic effects of induced TNF-α. An epidemiological study has started in Japan to confirm whether periodontal disease is a risk factor for HIV reactivation. Epstein–Barr virus (EBV), another latent infection, has been isolated from patients with periodontitis[208,209], and BA in *P. gingivalis* culture supernatant can also reactivate EBV *in vitro* by a mechanism involving histone modification and chromatin remodelling[210].

These findings show BA in a new light. Although it is considered beneficial in the gut, Professor Ochiai debated whether we need to understand the effects of high levels of SCFA on gut tissues. His final message was very clear: brush your teeth!

A re-examination of the probiotic definition and category

'Probiotic' means different things to different people depending on their particular interest, explained Professor Colin Hill (University College Cork, Ireland). Some health conditions (e.g. AAD) respond to several different probiotics[50], yet some probiotics are associated with several health benefits[211], and a further complication is that some effects are very strain-specific[212]. In response to concerns about regulatory developments in Europe as well as misuse of the term 'probiotic' and confusion of general public about this category, the International Scientific Association for Probiotics and Prebiotics organised a meeting in 2013 to re-examine the probiotic concept. The panel comprised clinical and scientific experts, including members of an original FAO/WHO expert panel, who in 2001 had agreed what has been a widely accepted definition for probiotics[213]. Professor Hill gave an insight into these discussions and the resulting consensus statement, published in 2014[214].

The panel endorsed the 2001 probiotic definition, with just a small change to improve its grammar to: 'Live micro-organisms that, when administered in adequate amounts, confer a health benefit on the host'. They recommended that 'probiotic' should only apply to products containing a suitable number of live cells of well-defined and safe microbial strains and with a reasonable level of scientific evidence of health benefit from a body of research that included well-conducted human studies. The panel agreed that the process of assessment of scientific evidence for food probiotics should differ from the assessment for pharmaceutical drugs, and the process should be consistent with other foods that have approved health claims (e.g. vitamins). The panel agreed that changes are needed by regulators in jurisdictions such as the USA and Europe[215].

The panel identified three categories of probiotics:

- Microbial species, used in a food or food supplement, without a specific health claim
  - with reasonable evidence of a general benefit for humans associated with the species itself and/or its function, structure, activity or end product;
  - claim: 'contains probiotics'.
- Microbial strains, used in/as a food or food supplement, with a specific health claim
  - based on convincing evidence specific to the constituent strain(s) to satisfy the appropriate regulatory authority.
- Microbial strains, used as probiotic drugs
  - sufficient trial evidence to meet appropriate regulatory standards for drugs.

A very broad range of bacterial species have been associated with a robust gut microbiota; therefore, it is very likely that the

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probiotic framework could be extended to include new well-defined beneficial microbes, such as *A. muciniphila* and *F. prausnitzii*, but this could only happen when/if there is sufficient evidence of the benefit and understanding of mechanism of activity.

Dead microbes or microbial products are not probiotic. Live microbial cultures with no evidence of benefit that are traditionally associated with fermented foods also fall outside the probiotic framework and should be labelled as 'containing live and active cultures'. FMT cannot be considered probiotic, because this involves an undefined mixture of micro-organisms. FMT preparations containing defined mixtures of microbes, such as RePOOPulate, however, do meet the probiotic criteria[216].

The panel supported the concept that certain well-studied species could impart general benefits, particularly for gut health but not for immune health. This has been concluded in meta-analyses[217] and acknowledged in Canada where non-strain-specific claims are allowed for various species of *Lactobacillus* and *Bifidobacterium*[218]. There were more tentative conclusions regarding mechanisms of activity: some (such as colonisation resistance and SCFA production) were considered to be widespread among studied probiotics; others were considered to occur frequently within the same species (such as vitamin synthesis and gut barrier reinforcement); and others were considered to be rare and strain-specific (such as neurological and immunological effects).

The consensus statement is valuable in providing clear guidelines for all stakeholders in the probiotics sector, be they researchers, regulators, health-care professionals, manufacturers or consumers.

Acknowledgements

The 8th International Yakult Symposium was financially supported by Yakult Europe B.V., Almere, The Netherlands. The present report was commissioned by Yakult Europe B.V.

L. V. T., K. S. and J. Z. contributed to the writing of the report.

None of the authors has any conflicts of interest to declare.

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