Fermentation of food components by microbes occurs both during certain food production processes and in the gastro-intestinal tract. In these processes specific compounds are produced that originate from either biotransformation reactions or biosynthesis, and that can affect the health of the consumer. In this review, we summarize recent advances highlighting the potential to improve the nutritional status of a fermented food by rational choice of food-fermenting microbes. The vast numbers of microbes residing in the human gut, the gut microbiota, also give rise to a broad array of health-active molecules. Diet and functional foods are important modulators of the gut microbiota activity that can be applied to improve host health. A truly multidisciplinary approach is required to increase our understanding of the molecular mechanisms underlying health beneficial effects that arise from the interaction of diet, microbes and the human body.

Introduction

Microbes play an important role in human food production. Since ancient times, fermentation has been used to improve both preservation and organoleptic properties. The importance of food in health was already acknowledged more than 2500 years ago when Hippocrates espoused the ‘food as medicine’ philosophy. In many traditional cultures, fermentation has been long associated with general health benefits but it has recently been employed to introduce specific health attributes, for instance through the enrichment with specific vitamins or the introduction of probiotics, ‘live microorganisms which when administered in adequate amounts confer a health benefit on the host’ (http://www.who.int).

The impact of microbes on our ability to harvest energy and nutrients from food is extended to the gastro-intestinal (GI) tract. Microbial degradation of complex carbohydrates and proteins is key for food digestion but we have only recently begun to appreciate the full extent of the role of the GI microbiota in human health and disease. This is largely driven by recent technological advances in sequencing and other –omics technologies that provide tools to study the complex GI microbial communities as well as host responses related to dietary interventions and gut microbiota perturbations.

In this paper, we will review selected advances in the field of microbial fermentation in food production and the human GI tract with a focus on microbial metabolites that affect host health (Figure 1).

Fermentation in food processing

Fermented foods — dietary relevance and health beneficial attributes

Fermentation is widely applied to a broad range of food substrates and today there is an array of food products produced artisanally or at industrial scale (for recent reviews see [1,2]). At the heart of most fermented foods are ancient processes that date back to the introduction of agriculture and animal husbandry approximately 10,000 years ago [3]. Today, fermentation technology has moved from artisanal practices and empirical science to industrialised and life-science driven technology. Fermented foods and beverages are estimated to make up approximately 1/3 of the human diet [4]. The microbial activity impacts on the composition and nutritional status which can be valorised by the introduction of specific health-beneficial attributes.

Primary carbon metabolism in fermenting microbes is typically designed for transforming sugars into simple acids, alcohols and carbon dioxide as major end products. In addition a whole range of secondary metabolites, including vitamins, polyols, or antioxidants, can be produced which may bring specific health benefits. Targeting, amongst others, lactic acid bacteria for dairy foods, a number of groups have been very active in studying the underlying pathways and improving productivity by metabolic engineering or by selecting spontaneous or induced mutants. This work has been reviewed extensively
elsewhere covering the main principles in the field [5–7]. Building on these approaches multi-vitamin producing strains have been reported that are able to produce different ratios of folate and cobalamin depending on the food substrate used [8,9].

Functional attributes can also be introduced or altered through biotransformation reactions. Many substrates of vegetal origin contain polyphenols or flavonoids to which health-beneficial activities are attributed. In plants these are often glycosylated which can decrease their bioavailability. Recent papers have demonstrated that α-rhamnosidase [10,11] or β-galactosidase [12] activity produced by food-fermenting LAB can effectively remove glycoside-residues.

Fermentation can also be applied as a means to remove unwanted compounds from food substrates. Cassava forms a major dietary component for a large part of the world population and contains highly toxic cyanogens. Levels can be decreased with fermentation processes probably through the involvement of linamarase activity [13]. Other examples come from α-galactosides such as raffinose and stachyose that are flatulogenic and frequently found in plant-derived substrates. The levels of these sugars in soy milk have been successfully reduced using either wild-type, recombinant or transconjugant LAB expressing α-galactosidase [14,15].

Finally, fermented foods are an important vehicle for the delivery of probiotics. A rapidly growing number of well conducted, prospective, randomized, controlled, clinical trials (RCTs) are published to substantiate health benefits ([16] and references therein). Mechanistic studies initially focused on the direct cross-talk of
probiotic strains and host, in particular the immune system. Recent analyses on the gut microbiota alterations in RCTs provide evidence that probiotic intervention may induce subtle changes in the GI microbiota community structure that may play a role in the observed effect [17*].

Dairy fermentation — paradigm for food microbiology
Food fermentations do not only deserve attention due to their relevance for application. They also provide highly attractive model systems for microbial ecologists. Food fermentations are typically carried with microbial consortia that can exist of two species, each represented by one strain, as for instance in the case of yoghurt production, or undefined microbial consortia made up of multiple eukaryotic and prokaryotic species with cheese and sourdough fermentation as the best studied examples. As in other areas in microbiology the emergence of genomics and related technologies has deepened our understanding of how the fermenting microbes interact with the substrate and other microbes.

Yoghurt is typically produced with bi-cultures of strains of Lactobacillus bulgaricus subsp. delbrueckii (L. bulgaricus) and Streptococcus thermophilus that mutually stimulate growth in a process also referred to as proto-cooperation. Some of these interactions have been known for decades and include the action of cell wall-bound proteases, produced by L. bulgaricus which in turn benefits from formate and carbon dioxide supplied by S. thermophilus [18]. Harvesting on the availability of multiple genome sequences, Liu et al. [19] demonstrated that during the long history of co-cultivation in milk multiple events of horizontal gene transfer have occurred, as for instance the transfer of a gene cluster for the production of sulfur-containing amino acids from L. bulgaricus to S. thermophilus that probably optimize their combined growth [20]. In a study by Hervé-Jiminez et al. [21*], transcriptomic and proteomic analysis revealed regulatory responses in purine biosynthesis related to co-cultivation. In addition the production of H2O2 by L. bulgaricus modifies iron metabolism in S. thermophilus likely protecting it from the generation of reactive oxygen species. These post-genomic approaches confirm findings by others that the physiological state and activity of a given micro-organism are largely determined by environmental stimuli imposed by fermented dairy substrates and co-cultivated strains [22–24]. For instance, striking differences were observed in the modulation of NF-κB-dependent immune signalling pathways in the human duodenum after consumption of living L. plantarum WCFS1 bacteria harvested at different growth phases [25]. This is of particular importance for the design of fermented foods containing probiotic strains as both their ability to survive gastric stress and the production of probiotic effector molecules may be highly dependent on growth conditions.

Fermentation in gastro-intestinal tract
The gut — a continuous fermentor
The human gut is densely populated with a vastly diverse microbial community [26,27,28***]. The gut microbiota closely interacts with the host through a broad array of molecular interactions affecting nutrition, immunity and metabolism throughout the lifespan [29]. With approximately 1.5 kg of bacteria in the colon [30] and a density of 10^{12} cells per gram of intestinal content [31], the gut microbiota has been recognized as an important metabolic organ comparable to the liver [32]. It can be regarded as a continuous cultivation system, where undigested parts of the diet work as a feeding medium to maintain the high density and diversity of the gut microbiota. A major difference with a conventional chemostatic bioreactor is that the reactor itself (the human body) extracts, and competes for, nutrients with the gut microbiota [33] while concomitantly supplying it with mucus as an additional feedstock [34]. The composition and activity of the gut microbiota is controlled both by diet as well as through modulation by the host mainly via the mucosal immune system. Disturbance of this ecosystem by environmental factors for example diet, pathogens or antibiotic treatment coupled with genetic pre-depositions in the host, may lead to ‘dysbiosis’ and impaired activity that can have a negative impact on health [35].

From all dietary components that are ingested especially those which are non-digestible by host enzymes, such as dietary fibers, or those escaping from upper digestion and absorption, such as overeaten proteins, can be metabolized and transformed by the gut microbiota. Recently, it has been shown that diet plays a dominating role in shaping gut microbiota, overriding host genetics [36]. It has been estimated that approximately 60% of total gut microbiota variations may be explained by change of diet while only about 10% can be attributed to a host genetic variation. Major changes in diet directly impact on the microbiota composition and specific phylogenetic groups may bloom within a few days in response to a change in carbohydrate and protein intake [37]. Metagenomic sequencing of the gut microbiome (collective genome of gut microbiota) has revealed a huge genetic repertoire with vast arrays of genes for breakdown and transformation of diverse dietary components [27,28***,38]. Dietary carbohydrates are major sources of energy for both the microbiota and human. Hence it is no surprise that the gut microbiome is in particular rich in genes involved in carbohydrate catabolism and uptake. Especially, complex carbohydrates such as plant cell wall polysaccharides (including cellulose, pectin, and hemicellulose) and resistant starch can escape host digestion and reach the colon to serve as a substrate for fermentation by bacteria residing in the gut.

Metabolic products of the gut fermentation process
The most important end products of fermentation of undigestible hydrocarbons reaching the colon are short
chain fatty acids (SCFAs), principally acetate, propionate and butyrate and gases such as carbon dioxide, hydrogen and methane [39,40*]. As a result of the intense metabolic activity of the gut microbiota on average 13 l of hydrogen is daily produced during colonic fermentation [41]. The majority is recycled through bacterial cross-feeding reactions, amongst others leading to the production of methane and hydrogen sulfide. A fraction of the gases is trapped in the intestine and needs to be excreted. Abnormalities in the physiological processes leading to the handling of these fermentative gases could lead to the generation of abdominal distension and bloating [42]. Interestingly, a fermented milk containing *Bifidobacterium animalis* subsp. *lactis* DN-173 010 was shown to improve abdominal distension in constipated women with irritable bowel syndrome though a direct effect on gas metabolism in these subjects remains to be established [43].

Short-chain fatty acids (SCFAs) are an important class of metabolites. While acetate and propionate are reabsorbed by the portal vein and metabolized by the liver as an energy source, butyrate is rapidly absorbed by the host as the major energy source for colonocytes [44]. It is estimated that SCFAs produced by the gut microbiota may constitute ten percent of the energy extracted from food in human. In fact, the ability of the gut microbiota to transform otherwise undigestible food components into molecules that serve as an energy source for the host was proposed to be involved in obesity pathogenesis due to an excess energy intake [45*,46].

Besides their role in energy metabolism, other effects have been assigned to SCFAs [47]. This is particularly true for butyrate that plays a role in maintaining the integrity of gut mucosa. Butyrate may reduce risk for colon cancer development by reducing cell proliferation and stimulating cell differentiation and it is also associated with a maturation of enteric neurons [48,49] (Table 1). Moreover, SCFAs are important modulators of the immune system [50], for example they may help resolve chronic inflammation associated with IBD by the production of compounds that can be disadvantageous and even toxic to the host, including ammonia, amines, phenols, thiols, indoles, N-nitroso-compounds, branched chain fatty acids and sulfide [65,66] which has been associated with colon cancer [67] and inflammatory bowel diseases [68].

**Consequences of the Western diet**

The diet in early human civilization was rich in complex carbohydrates but nowadays in industrialized societies it has typically shifted to the so-called ‘Western diet’ which is characterized by high fat, high proteins and low fibre intake [3]. A recent study showed that an African rural diet was associated with profound differences in gut microbiota including an overrepresentation of plant polysaccharides-degrading genera such as *Prevotella* and *Xylanibacter* and this was correlated to higher faecal SCFA concentrations compared to European Children consuming a Western diet [64*]. When proteins and peptides reach the colon they can serve as substrates for fermentation by gut microbiota. Dissimilatory metabolism of proteins in the gut can lead to the production of compounds that can be disadvantageous and even toxic to the host, include ammonia, amines, phenols, thiols, indoles, N-nitroso-compounds, branched chain fatty acids and sulfide [65,66] that has been associated with colon cancer [67] and inflammatory bowel diseases [68].

Owing to the steady increase of obesity and related metabolic disorders in western societies several groups have studied the impact of a high fat diet on the gut microbiota over the past years. Analysis of the gut microbiota from human and mice showed different proportional representation of major bacterial phyla correlated to the obese and metabolic syndrome phenotype. Amongst other things it suggests that intestinal bacteria from obese
subjects extract energy more efficiently from the diet [69]. A unique class of products promoted by high-fat, low fibre diet is free antigens released by increased population levels of opportunistic pathogens in the gut. Also the amount of lipopolysaccharides (LPS) from gram-negative bacteria that reaches bloodstream via an impaired gut barrier seems to be promoted on a high fat diet. This may provoke inflammation that through multiple pro-

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<td>Short chain fatty acids (SCFAs) from carbohydrates (plant cell wall polysaccharides, resistant starch, polysaccharides, oligosaccharides)</td>
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| **II Metabolic products from gut microbiota biosynthesis** |
| Unknown fermentation product | Faecalibacterium prausnitzii | Anti-inflammatory through blocking NF-κB activation | [53*] |
| Unknown fermentation product | Lactobacillus acidophilus NCFM | Modulator intestinal visceral pain Induction of opioid and cannabinoid receptors | [54] |
| Vitamin K₂ (menaquinone) | Bacteroides fragilis, Escherichia coli, Propionibacterium spp., Eubacterium spp., Veillonella parvula, Lactococcus lactis, Leuconostoc lactis | Modulation of bone mineralisation and blood coagulation | [55,56] |
| Vitamine B₉ (folic acid) | Bifidobacterium spp. | Modulation of cellular cycle, cell proliferation, | [85] |
| Vitamin B₁₂ (Cobalamin) | Lactobacillus reuteri | Stimulation of nervous system development | [8,57] |
| Conjugated linoleic acid (CLA) | Bifidobacterium breve Bifidobacterium longum | Modulation of the immune system Decrease of the risk of development symptoms related to metabolic syndrome | [61,62] |
| γ-Aminobutyric acid (GABA) | Lactobacillus brevis Lactobacillus paracasei | Central nervous system inhibition Relief of hypotension and diuresis | [58] |

| II Cellular components |
| Polysaccharide A (PSA) | Bacteroides fragilis | Decrease of pro-inflammatory molecules such as TNF-α, IL-1β levels, increase of anti-inflammatory IL-10 level, decrease neutrophil infiltration, decrease of epithelial cell hyperplasia | [59,60] |
| Lipopolysaccharides (LPS) | Gram-negative bacteria | Pro-inflammatory activity through NF-κB activation Maturation of dendritic cells | [86] |
| Teichoic acids | Gram-positive bacteria | Modulation of proinflammatory or antiinflammatory immune responses | [87] |

Food biotechnology

Processes contribute to obesity and insulin resistance [70,71,72,73]. There is evidence that the inclusion of fibers or prebiotics in a high fat diet can help to maintain gut barrier integrity so that the antigen load crossing the gut barrier entering is restricted [74,75] preventing mice from getting obese and insulin-resistant even though they were fed a high fat diet.

Concluding remarks and future prospects
There is a rapid increase in the understanding of how microbes can be employed to deliver health benefits by means of food intake. Much of this is fueled by emerging evidence on the close interrelation of host physiology and GI microbiota community structure and metabolism. Current efforts in this regard have largely been limited to correlating phylogenetic community composition to dietary habits or diseased phenotypes. This has revealed important correlations but typically has failed to establish cause–effect relationships. At best it may allow the formulation of a hypothesis that can explain a causative role of the gut microbiota. The availability of high quality metagenomic gene catalogue will aid the functional interpretation of microbial genes and human phenotypes [28]. As functional meta–omics technologies are rapidly maturing, true systems biology approaches are within reach. Co-variation analysis of microbial community structure and their metabolic products upon dietary modulation can help to establish specific associations between a microbial population in the gut and its metabolic products [76]. Concomitant holistic interrogation of the host response, for instance through metabonomics of diverse biofluids such as urine, blood or even tissues, should facilitate the elucidation of molecular interactions between diet, microbes and host. This emphasizes the importance of a balanced and systems concept for managing diet so that metabolic products and population composition of gut microbiota would favour high level of beneficial compounds and bacteria and suppress population levels of pathogens and toxin producers. A stable gut microbiota with significantly reduced inflammation-provoking and toxin-producing activities should become the target for dietary manipulation and functional foods to enable consumers to live a long and healthy life.

A deeper understanding on how food and food microbes interact with the GI microbiota and host metabolism opens new avenues both to demonstrate efficacy and to innovate in functional foods. In this respect fermented foods can be regarded as an ‘upstream’ extension of the food digestion and fermentation process that can be highly steered and controlled to introduce health beneficial attributes. A truly integral approach combining nutrition science, microbiology and food technology will be required to identify how functional foods can compensate dysbiosis in the highly complex and ‘open’ GI fermentor.

References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

17. Salonen A, Palva A, de Vos W: Microbial functionality in the human intestinal tract. Front Biosci 2009, 14:.
18. Sieweverts S, de Bok FAM, Hugenholtz J, van Hylckama Vlieg JET: Unraveling microbial interactions in food fermentations: from
Impact of microbial transformation of food on health van Hylckama Vlieg et al.


Post-genomics study employing transcriptomics and proteomics to provide novel insights on the interactions between yoghurt bacteria.


Meta genomic sequencing using next generation sequencing technology to generate a reference data set comprising the gene complement in of the gut microbiota, host genetics and diet relevant to development of metabolic syndromes in mice. ISME J 2010, 4:232-241.


Demonstration in mice that fermentation activity of the gut microbiota modulates the adiposity via the SCFA binding GPR41 receptor.


Pioneering study that showed that gut microbiota increases fat storage in conventional germ-free mice, by suppressing the fasting-induced adipocyte factor (Fiaf).


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Impact of microbial transformation of food on health van Hylckama Vlieg et al.

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Demonstration of efficacy of a fermented milk containing a probiotic in ameliorating spontaneous colitis resembling ulcerative colitis in T-bet−/− and Rag2−/− mice. The intervention results in increased butyrate production and creates a non-permissive environment for colitogenic Enterobacteriaceae.


Elegant study demonstrating the capacity of a resident gut bacterium, Faecalibacterium prausnitzii, to exhibit antiinflammatory properties in cellular and mice colitis model, via metabolite production.


A study comparing human intestinal microbiota from children exposed to a modern western diet and a rural diet high in complex polysaccharides. The marked enrichment in micro-organisms capable of utilizing plant-derived polysaccharides for the rural diet which was correlated with a significantly increase in levels of short-chain fatty acids in feces.


Evidences that high-fat diet increases bacterial lipopolysaccharide endotoxin, that in turn triggers low grade inflammation, body weight and insulin resistance.


