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MiniReview

Oxalobacter formigenes and its role in oxalate metabolism in the human gut

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Abstract

Oxalate is ingested in a wide range of animal feeds and human foods and beverages and is formed endogenously as a waste product of metabolism. Bacterial, rather than host, enzymes are required for the intestinal degradation of oxalate in man and mammals. The bacterium primarily responsible is the strict anaerobe *Oxalobacter formigenes*. In humans, this organism is found in the colon. *O. formigenes* has an obligate requirement for oxalate as a source of energy and cell carbon. In *O. formigenes*, the proton motive force for energy conservation is generated by the electrogenic antiport of oxalate^{2-} and formate^{1-} by the oxalate–formate exchanger, OxlT. The coupling of oxalate–formate exchange to the reductive decarboxylation of oxalyl CoA forms an 'indirect' proton pump. Oxalate is voided in the urine and the loss of *O. formigenes* may be accompanied by elevated concentrations of urinary oxalate, increasing the risk of recurrent calcium oxalate kidney stone formation. Links between the occurrence of nephrolithiasis and the presence of *Oxalobacter* have led to the suggestion that antibiotic therapy may contribute to the loss of this organism from the colonic microbiota. Studies in animals and human volunteers have indicated that, when administered therapeutically, *O. formigenes* can establish in the gut and reduce the urinary oxalate concentration following an oxalate load, hence reducing the likely incidence of calcium oxalate kidney stone formation. The findings to date suggest that anaerobic, colonic bacteria such as *O. formigenes*, that are able to degrade toxic compounds in the gut, may, in future, find application for therapeutic use, with substantial benefit for human health and well-being.
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Keywords: Oxalate; Kidney stone; Anaerobic bacterium; Colon; Probiotic; *Oxalobacter formigenes*; Antibiotic

1. Introduction

Oxalic acid (ethanedioic acid) is a highly oxidised and corrosive compound with strong chelating activity that is synthesised by a wide range of plants, animals and microorganisms. The ingestion of gram quantities of oxalate can result in paralysis of the nervous system, corrosion of the skin and precipitation of blood calcium [1]. Oxalate is excreted in urine, and the most widespread adverse consequence of elevated urinary oxalate concentrations (hyperoxaluria) is the development of calcium oxalate kidney stone disease (reviewed in [2,3]). This painful and debilitating disease affects around 10% of the people of North America, Europe and other regions of the world at some time in their lives. In a small minority of cases, kidney stone disease results from genetic abnormalities such as the lack of key enzymes [4], or from the ingestion of toxic compounds. However, most cases of calcium oxalate kidney stone disease do not have an obvious clinical cause, and this review concerns this idiopathic condition.

In humans, oxalate is ingested with many common foods and beverages including coffee, chocolate, rhubarb, spinach, nuts and other fruits and vegetables [5–7]. Some typical values are cited in Table 1. In addition, it is formed endogenously as a waste product of metabolism [4]. Oxalate homeostasis is maintained by oxalate degradation by the action of bacterial, rather than host, enzymes. The responsible bacteria reside in the gastro-intestinal tract and their degradative activity can be shown to respond to the amount of oxalate present. Allison and Cook [8] first showed that increasing the amount of oxalate in the diet increases the rate of microbial oxalate degradation in gut contents from herbivores (Fig. 1) and similar effects

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Table 1
Oxalate content of some foods

Food	Mean oxalate (mg 100 g^{-1})	Range (mg 100 g^{-1})
Wheat bran	130	84–193
Wholewheat bread	27	25–29
French fries (oven-bake, frozen)	22	20–23
Tomatoes	7	2–13
Chocolate (Hershey)	58	50–67
Chocolate (cream, Swiss)	34	21–43
Potatoes	18	6–29
Sweet potatoes (yams)	29	0.2–87
Rhubarb	450	379–511
Peanuts (raw)	72	44–105
Almonds (raw)	192	160–238
Tea (2 g in 100 ml)	7	Not recorded
Spinach	794	537–987

have also been recorded in humans [9–11]. The bacterium believed to be primarily responsible for oxalate breakdown in these animals and humans is the anaerobe *Oxalobacter formigenes* [12]. It now seems that *O. formigenes* contributes to oxalic acid homeostasis and that its absence may predispose individuals to idiopathic calcium oxalate kidney stone disease (reviewed in [13]). This leads to the intriguing possibility that re-colonising these deficient individuals with *O. formigenes* might provide protection against recurrent kidney stone disease.

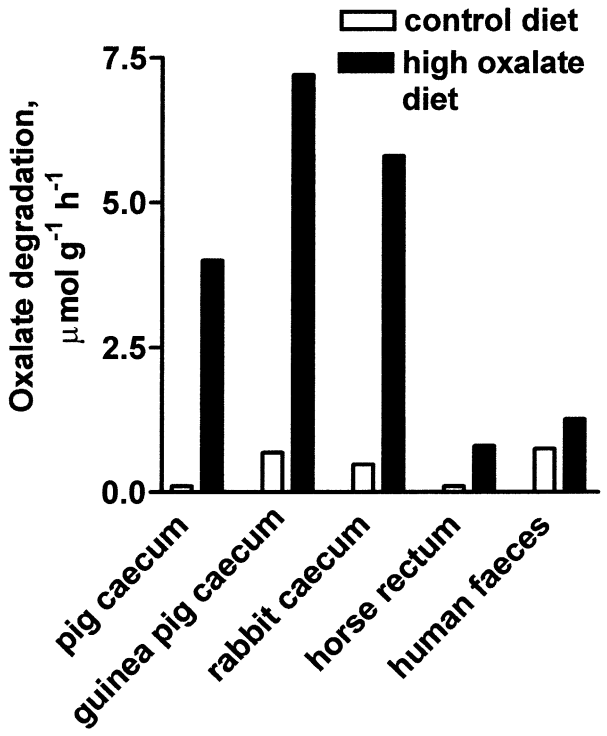

Fig. 1. Effect of high and low oxalate dietary intakes on the rate of oxalate degradation in samples from the gut of animals and human faeces. Data: animals [8], humans [9].

2. *O. formigenes*, phylogeny, physiology and metabolism

O. formigenes is a Gram-negative obligately anaerobic member of the β-Proteobacteria. The current understanding of the phylogenetic relatedness of *O. formigenes* is summarised in Fig. 2 [14]. A related species, *O. vibrioformis*, has been isolated from freshwater sediments, a habitat also colonised by *O. formigenes* [2]. 16S rDNA sequencing, lipid analysis and the use of oligonucleotide probes and primers complementary to sequences in key enzymes have revealed that a range of strains of *O. formigenes* could be allocated to two groups. No obvious relationship was found between this grouping and the host source [3]. *O. formigenes* relies on oxalate as a source of carbon and energy. It requires a small amount of acetate in the growth medium as additional carbon source. There is no growth on sugars, and glycolytic pathways are absent [12].

2.1. Oxalate transport, activation and decarboxylation

The absolute reliance of *O. formigenes* on the supply of oxalate has been confirmed in detailed studies of its energy metabolism and biosynthetic pathways (summarised in Fig. 3). In *O. formigenes*, oxalate is activated and converted to formate and CO_2 by the combined action of two cytosolic enzymes. First, oxalyl CoA is formed by the transfer of CoA from formyl CoA to oxalate. This reaction is catalysed by formyl-CoA:oxalate CoA transferase (frc), a 45-kDa monomer and founder member of a group (family III) of CoA transferases which operate by the formation of a ternary complex between the two substrates and the enzyme [15]. The active native form of the enzyme is a modified protein that, as a result of transformation and removal of the usual terminal methionine residue, has a threonine residue in position 1 of the N-terminus [16]. Oxalyl CoA decarboxylase (oxc) catalyses the formation of formyl CoA and CO_2 from oxalyl CoA in a reductive reaction with close to 100% stoichiometry

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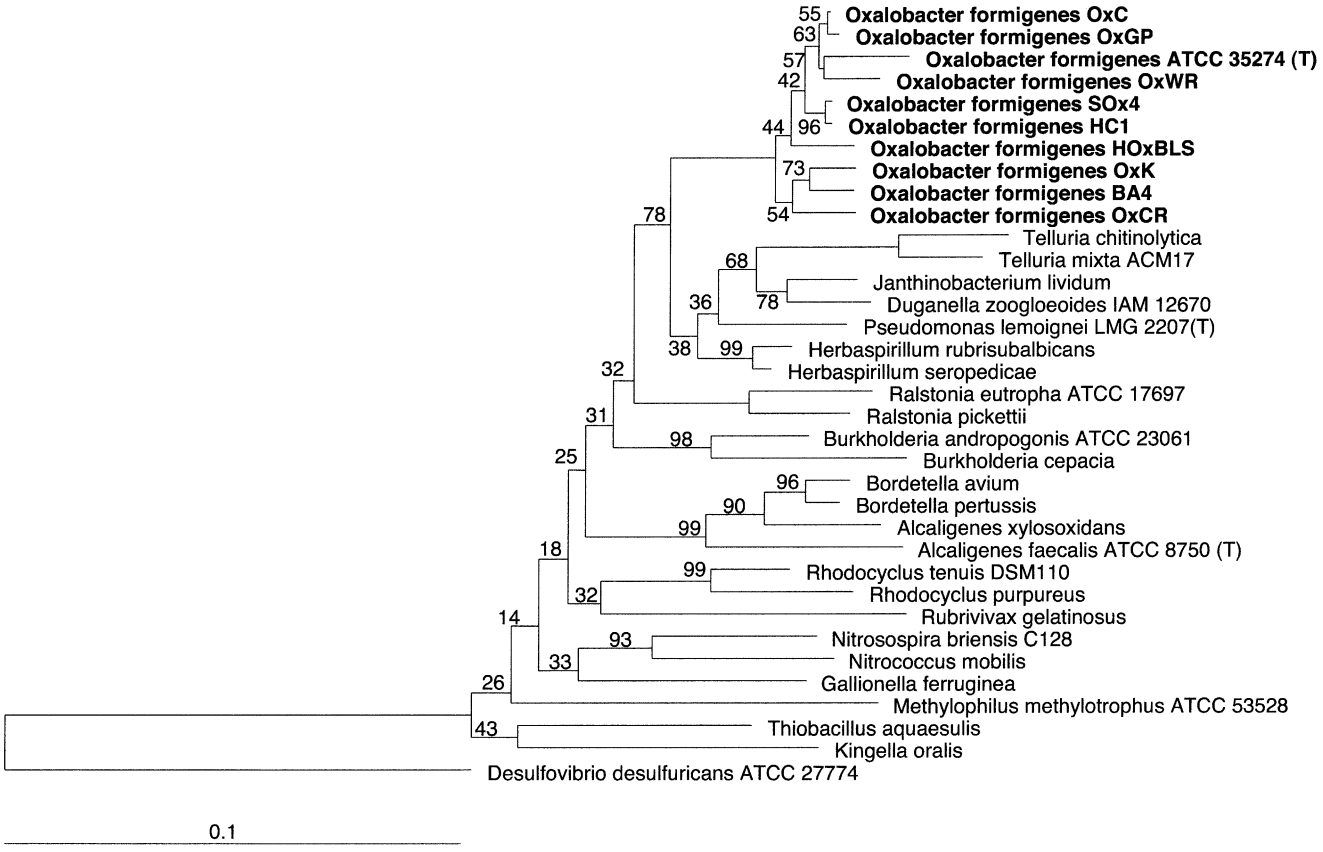

Fig. 2. Phylogenetic tree based on analysis of 16S rDNA showing *O. formigenes*, selected members of the β-Proteobacteria and *Desulfovibrio desulfuricans* of the δ-Proteobacteria. Bootstrap values (expressed as percentages of 1000 replications) are shown at branch points. The scale bar represents genetic distance (10 substitutions per 100 nucleotides). Reproduced from [14] with the permission of Bergey's Manual Trust.

(Fig. 3). This enzyme accounts for around 10% of the soluble protein of the cell and in cultures of *O. formigenes* it exists as a four-subunit tetramer of molecular mass around 265 kDa. Thiamine pyrophosphate (TPP) is required, and Mg^{2+} is stimulatory [17]. When oxc is overexpressed in *Escherichia coli*, the enzyme forms homodimers spontaneously. A putative TPP binding motif common to TPP binding enzymes had been identified around 100 amino acids upstream of the C-terminus [18]. Although the actions of frc and oxc are linked and are co-ordinated by the cell, the genes *frc* and *oxc* are not part of a polycistronic operon. They have independent promoter regions and termination sequences and are located several kilobases apart on the chromosome [16].

The decarboxylation of oxalyl CoA consumes a proton. *O. formigenes* operates an 'indirect' proton pump by linking this reaction to the electrogenic exchange of $oxalate^{2-}$ and $formate^{1-}$. The $oxalate^{2-}$:$formate^{1-}$ antiport carrier of *O. formigenes* is the integral membrane protein OxlT [19]. Purification and reconstitution of OxlT in proteoliposomes has revealed that it is a secondary carrier that mediates oxalate:formate exchange without a requirement for energy, and that it catalyses a high velocity of transport [20]. Allowing for inactivation during purification and the difficulty of sustaining initial rate conditions, Ruan et al. [20] calculated that their measured turnover number of around 420 s^{-1} probably corresponds to a value of at least 1000 s^{-1} for the native protein. The binding of oxalate to OxlT provides energy that is considered to stabilise the substrate:transporter complex [21]. OxlT comprises as much as 10% of the membrane protein of *O. formigenes*, potentially providing hundreds of thousands of copies per cell. This, combined with the high turnover number, renders *O. formigenes* essentially 'transparent' to oxalate [20]. When overexpressed in *E. coli* the protein contains 418 amino acids with a predicted molecular mass of 44.1 kDa [22].

Studies of the structure and molecular architecture of OxlT have revealed the presence of 12 transmembrane α-helical segments with the C- and N-terminal regions located on the cytoplasmic side. Electron crystallographic analysis suggests the presence, in each molecule, of a central cavity presumed to be part of the oxalate transport pathway [23]. Hirai et al. [24] suggested that the symmetrical conformation of OxlT which was consistent with their analysis might be an intermediate between asymmet-

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ric conformations which would allow preferential accessibility of the transporter from one side of the membrane or the other.

2.2. Measurement of proton motive force

Rapid oxalate^{2-}:formate^{1-} exchange, via OxlT, generates the proton motive force (Δp) for energy conservation [20,25]. The transmembrane electrical potential ($\Delta\psi$) was found to be quantitatively the main component of the proton motive force measured in non-growing cells of *O. formigenes* energised by the presence of 20 mM oxalate [25]. At external pH 7.0 for example, the $\Delta\psi$ contributed around −80 mV of the approx. −100 mV Δp; the ΔpH was 0.35 (internal alkaline). During changes in external pH between pH 5.0 and 8.0, the internal pH of energised, non-growing cells of *O. formigenes* appeared to be more regulated than that of acid-tolerant anaerobes such as lactobacilli, but less regulated than in *E. coli*. The generation of $\Delta\psi$ by *O. formigenes* was inhibited by the protonophore CCCD but not by the Na^+ ionophore monensin, supporting the view that in this bacterium energy is conserved in a proton, rather than a sodium ion, gradient. The optimum pH for oxalate degradation (measured by proton consumption) by cell-free extracts was 6.4 [25].

The Δp is assumed to support ATP synthesis via the action of a putative F_0F_1 ATPase. This enzyme would have ATP synthesis as its main function, rather than the extrusion of protons at the expense of ATP, a common reaction in many anaerobes. In this respect, Ruan et al. [20] drew a functional comparison with the ATPases of some methanol-utilising methanogens, which also serve to generate ATP.

2.3. Biosynthetic pathways

Oxalate carbon enters common bacterial biosynthetic pathways following reduction to 3-phosphoglycerate by the glycerate pathway [26] (Fig. 3). The origin of the reducing equivalents needed for the reductive reactions remains unclear. Although cells extracts of *O. formigenes* reduced viologen dye in the presence (but not the absence) of formate, NAD(P)-linked formate dehydrogenase activity was not detected.

When cells were grown to early exponential phase in non-labelled medium, then washed and grown for 0.5 generations in medium containing labelled substrates, around 55% of the cell carbon of *O. formigenes* was found to be derived from isotopically labelled oxalate. This probably underestimates the incorporation of oxalate into biosynthetic pathways, due to the relatively large amount of cell carbon present before exposure to labelled substrates. In the same experiments, acetate provided between 6% and 10% of cell carbon and carbonate was also used. Enzymes involved in α-ketoglutarate production were detected [27]. The labelling patterns of cell amino acids determined by

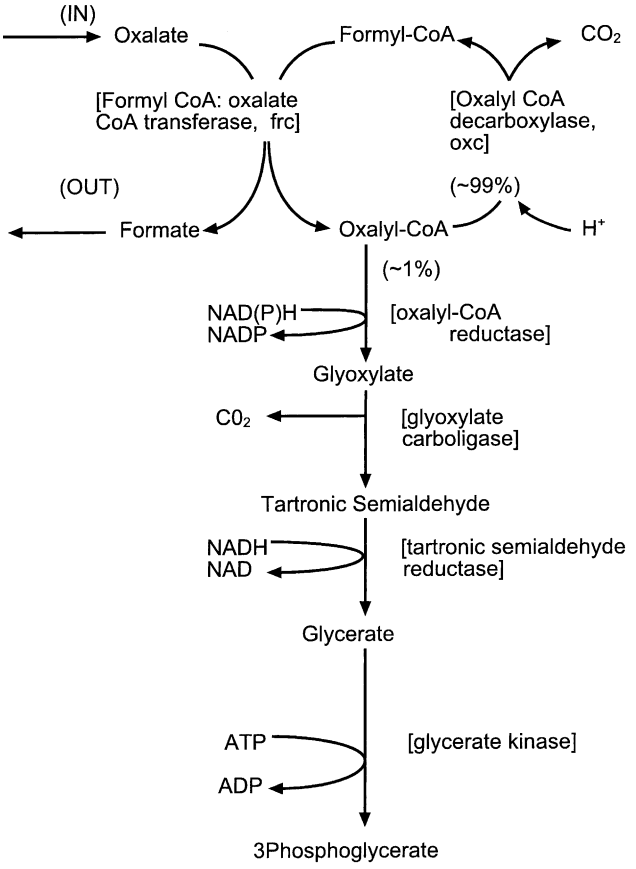

Fig. 3. Principal steps in the pathways of oxalate metabolism to formate and 3-phosphoglycerate in *O. formigenes*. Around 99% of C from oxalate is converted to formate and CO_2. Energy is conserved from the electrogenic exchange, by the carrier OxlT, of oxalate^{2-} (IN) and formate^{1-} (OUT). Oxalate C enters biosynthetic pathways via 3-phosphoglycerate. Each mole of tartronic semialdehyde is formed by the condensation of 2 moles of glyoxylate.

nuclear magnetic resonance were consistent with their derivation from central precursor metabolites, including pyruvate, phosphoenolpyruvate, oxaloacetate, α-ketoglutarate and 3-phosphoglycerate. Thus, for example, the labelling of glutamate family amino acids was consistent with glutamate production from oxaloacetate plus acetate via α-ketoglutarate. Glutamate labelling was consistent with the presence of a citrate synthase sharing the stereospecificity of the enzyme present in yeast, rather than that found in *Clostridium kluyveri* [28]. C1 of isoleucine was labelled by both oxalate and carbonate, suggesting either the presence of an additional pathway or the occurrence of exchange reactions. Carbon from both acetate and oxalate appears in leucine, supporting the operation of the isopropylmalate pathway. About 60% of acetate carbon was recovered as C2 units in proline, arginine, glutamate and leucine, and single methyl or carboxy carbons were found in other amino acids. In the latter cases, acetate C was always accompanied by oxalate C, suggesting some rearrangement ('scrambling') of label between intermediates common to oxalate and acetate [27].

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3. Factors influencing carriage of *O. formigenes*

Sidhu et al. [29] used genus- and group-specific oligonucleotide sequences corresponding to homologous regions in the *oxc* gene to study the natural colonisation of the human gut by *O. formigenes*. Children in the Ukraine were selected because of their limited prior exposure to therapeutic antibiotics and other drugs. It was revealed that faeces from the newborn and from infants less than 9 months old lacked *O. formigenes*. The bacterium began to be detected in the faeces when the children started to crawl. Almost all children between 6 and 8 years old were colonised, but the rate of colonisation declined in older children, to around that found in the adult population (around 70%). Other reports confirm this adult prevalence in other parts of the world [30]. Why some of the older children in the Ukraine, and a substantial minority of the adult population worldwide, lack *O. formigenes* is not clear. However, it has been shown that health status can affect colonisation markedly. Faeces from individuals with inflammatory bowel disease, Crohn's disease, and from patients who have undergone jejunoileal bypass surgery, often show little or no oxalate-degrading activity. These individuals carry an increased risk of hyperoxaluria [2]. Cystic fibrosis (CF) patients have low rates of colonisation with *O. formigenes* [31] and many of these patients are hyperoxaluric. The incidence of kidney stone formation in CF patients is around 20-fold higher than that in the general population. The causative links between hyperoxaluria and specific diseases are not fully understood. In some cases, accompanying diarrhoea might prevent the maintenance of a fully functioning colonic microbiota; increased concentrations of bile salts may also have a role [11]. The most credible hypothesis is that the loss of *Oxalobacter* is the consequence of a common treatment, the therapeutic use of antibiotics [31]. If this is true, then in the future we might expect to reveal new links between specific diseases and hyperoxaluria, resulting from the use of particular antibiotic therapies. For example, clarithromycin, commonly used in the treatment of *Helicobacter pylori*, is among the antibiotics to which some human strains of *O. formigenes* are sensitive [32]. If it can be demonstrated that antibiotic therapy contributes to the loss of such an important symbiotic bacterium, then we should reconsider the freedom with which these compounds are used [33], or consider the use of replacement therapy.

4. Therapeutic potential of *O. formigenes*

The hypothesised role of *O. formigenes* in oxalate homeostasis in humans suggests that this bacterium may have application as a probiotic. In humans, its ecological niche is in the colon; thus it is unlikely to have any impact on soluble oxalate that may be readily absorbed by the small intestine. However, its contribution to the degradation of colonic oxalate may be vitally important. Support for this concept comes from the observation that an intact colon is required for enteric hyperoxaluria to develop. Removal of the entire colon eliminates the condition. The nearly complete absence of *O. formigenes* in inflammatory bowel disease patients, the classic condition that predisposes to enteric hyperoxaluria, further supports this hypothesis [34]. Secondly, it has been demonstrated in rats that there is bi-directional flux of oxalate across the colonic epithelium [35]. If confirmed in man, this could provide an opportunity for reducing hyperoxalaemia and hence hyperoxaluria.

The potential for such manipulation has been investigated in animal and fermentor models and in an experiment with human volunteers. *O. formigenes* is found in the large bowel (but not the small intestine) of wild rats but many strains of laboratory rats lack this bacterium; these animals thus provide a convenient means of investigating the effect of colonisation on oxalate metabolism [36]. Strains of *O. formigenes* from pigs, guinea pigs, wild rats and humans were able to colonise the caecum of laboratory rats, but a rumen strain failed to do so [37]. The administration of *O. formigenes* reversed hyperoxaluria which had been induced in laboratory rats by feeding diets rich in oxalate. The establishment of *O. formigenes* in the treated rats was, however, transient. Five to 10 days after the oxalate supplement was withdrawn, the faecal population of *O. formigenes* had declined to a level which was undetectable by polymerase chain reaction [38].

Inoculation with *O. formigenes* (of either rumen or human origin) also established oxalate-degrading activity in continuous-flow incubations of mixed human faecal bacteria. The oral ingestion of biomass of *O. formigenes* restored faecal oxalate-degrading activity to two adult human volunteers who previously lacked this activity [32]. Co-administration of *O. formigenes* and an oxalate supplement was shown to decrease oxalate excretion. Most encouragingly, the *O. formigenes* biomass used to colonise these individuals was 1 g (wet weight) of cells which had been stored at −20°C before use and which was administered in a sandwich meal. The sensitivity to oxygen of the strain [32] did not prevent its use as a probiotic. A clinical trial designed to evaluate whether the ingestion of *O. formigenes* can reduce urinary oxalate in a larger number of individuals is in progress.

5. Conclusions

The concept of probiosis remains controversial. Berg [39], whilst supporting the probiotic concept, argued for rigorous investigation of the ecological principles operating in the digestive tract to validate the ecological basis of probiosis. At present, probiotics for human use are mostly incorporated into fermented foods such as yoghurt or

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health drinks and are consumed by many individuals who do not have any specific disease or malfunction. It is becoming clear that colonic anaerobes make a vital contribution to post-natal development and function in many different ways (reviewed in [40]). In future, the knowledge of which functions are performed by the different species of colonic bacteria should help us to target individuals lacking key bacteria and to provide therapy which could be administered under medically supervised conditions.

The degradation of toxic compounds has been shown to provide ecological niches which support specialised populations of anaerobic bacteria in the gut. Examples other than *O. formigenes* include the rumen mimosine degrader *Synergistes jonesii* and nitropropanol-degrading bacteria (reviewed in [41]). Oxalate degradation seems to offer an ecological niche in the human colonic ecosystem as well as in the gut of animals. Such a niche should offer an excellent opportunity for the use of probiotic preparations of *Oxalobacter* for replacement therapy [42]. Further investigation of the role of *Oxalobacter* is needed, particularly in relation to incidence of other diseases and the suggested link between the use of antibiotics and loss of this bacterium. Quantitative molecular methods [43] will aid these investigations. It also remains unclear whether or not other bacterial species also contribute to oxalate degradation in the gut [44,45]. The target of alleviating suffering from recurrent renal colic, a very painful and economically significant condition, renders continued detailed scrutiny of microbial intestinal oxalate degradation a worthy endeavour.

Acknowledgements

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