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Bacteria as vitamin suppliers to their host: a gut microbiota perspective

Jean Guy LeBlanc[1], Christian Milani[2], Graciela Savoy de Giori[1,3], Fernando Sesma[1], Douwe van Sinderen[4] and Marco Ventura[2]

Food-related lactic acid bacteria (LAB) as well as human gut commensals such as bifidobacteria can *de novo* synthesize and supply vitamins. This is important since humans lack the biosynthetic capacity for most vitamins and these must thus be provided exogenously. Although vitamins are present in a variety of foods, deficiencies still occur, mainly due to malnutrition as a result of insufficient food intake and because of poor eating habits. Fermented milks with high levels of B-group vitamins (such as folate and riboflavin) can be produced by LAB-promoted and possibly bifidobacteria-promoted biosynthesis. Moreover, certain strains of LAB produce the complex vitamin cobalamin (or vitamin B_{12}). In this review, fermented foods with elevated levels of B-group vitamins produced by LAB used as starter cultures will be covered. In addition, genetic abilities for vitamin biosynthesis by selected human gut commensals will be discussed.

Addresses
[1] Centro de Referencia para Lactobacilos (CERELA-CONICET) Chacabuco 145, (T4000ILC) Tucumán, Argentina
[2] Laboratory of Probiogenomics, Department of Genetics, Biology of Microorganisms, Anthropology and Evolution, University of Parma, Parma, Italy
[3] Cátedra de Microbiología Superior, Universidad Nacional de Tucumán (UNT), (T4000INI) Tucumán, Argentina
[4] Department of Microbiology & Alimentary Pharmabiotic Centre, Bioscience Institute, National University of Ireland Cork, Western Road, Cork, Ireland

Corresponding author: van Sinderen, Douwe (d.vansinderen@ucc.ie)

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Introduction

Vitamins are essential micronutrients that are normally found as precursors of various enzymes that are necessary for vital biochemical reactions in all living cells. Humans are incapable of synthesizing most vitamins and they consequently have to be obtained exogenously. The use of vitamin-producing microorganisms may represent a more natural and consumer-friendly alternative to fortification using chemically synthesized pseudo-vitamins, and would allow the production of foods with elevated concentrations of vitamins that are less likely to cause undesirable side-effects. The biochemical pathways involved in B-vitamin biosynthesis by food microorganisms have previously been described in detail [1••].

The human gastrointestinal tract (GIT) is colonized by a vast array of microorganisms known as the gut microbiota, with up to 10^{11} bacteria per gram of intestinal content [2]. Apart from its impact on different human functions [2], the intestinal microbiota plays a pivotal role in food digestion and energy recovery, while it can also act as an important supplier of vitamins. In humans it has been shown that members of the gut microbiota are able to synthesize vitamin K as well as most of the water-soluble B vitamins, such as biotin, cobalamin, folates, nicotinic acid, panthotenic acid, pyridoxine, riboflavin and thiamine [3]. In contrast to dietary vitamins, which are adsorbed in the proximal tract of the small intestine, the predominant uptake of microbially produced vitamins occurs in the colon [4,5].

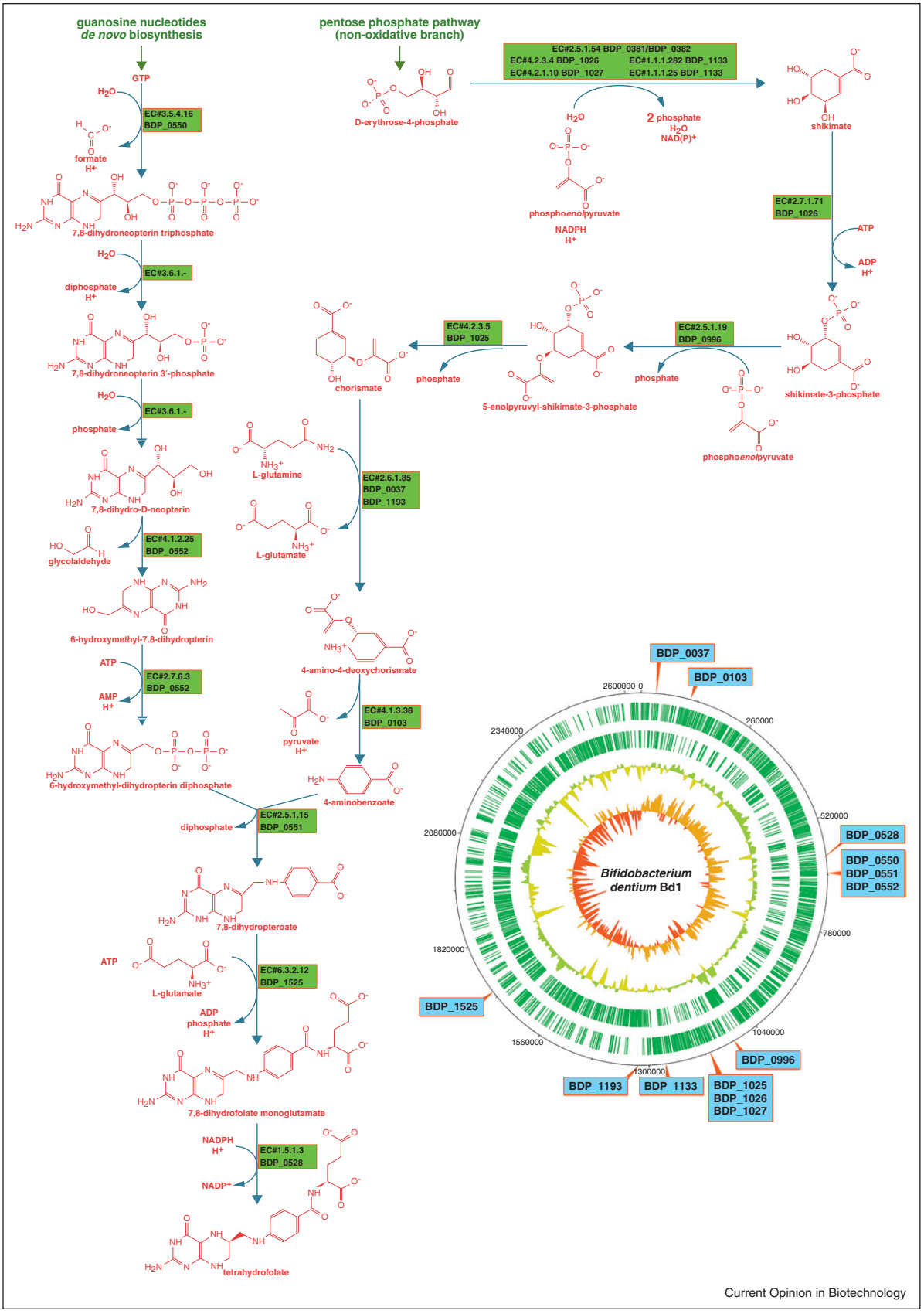
The genus *Bifidobacterium* currently encompasses 39 species (reviewed in [6]) and its members represent key components of the human gut microbiota [7,8,9•]. Several reports have highlighted the importance of bifidobacteria in regulating intestinal homeostasis, modulating local and systemic immune responses, and protecting against inflammatory diseases and infections [10,11]. In addition, some bifidobacterial species are claimed to convert a number of dietary compounds into health-promoting bioactive molecules, such as conjugated linoleic acid and certain vitamins [12,13]. Particular bifidobacterial strains have been shown to exhibit vitamin production [14–16], although their biosynthetic abilities have not been examined in detail and will be discussed here.

Biosynthesis of folate by human gut commensals

The B-group vitamin folate is involved in various essential metabolic functions such as DNA replication, repair and methylation, and synthesis of nucleotides, vitamins and certain amino acids. *De novo* synthesis of folate requires both 6-hydroxymethyl-7,8-dihydropterin pyrophosphate (DHPPP) and para-aminobenzoic acid (pABA).

Folate biosynthetic properties of bifidobacteria have been verified, though folate biosynthesis appears to be

Figure 1


guanosine nucleotides
de novo biosynthesis
GTP
EC#3.5.4.16
BDP_0550
formate
7,8-dihydroneopterin triphosphate
EC#3.6.1.-
diphosphate
7,8-dihydroneopterin 3′-phosphate
EC#3.6.1.-
phosphate
7,8-dihydro-D-neopterin
EC#4.1.2.25
BDP_0552
glycolaldehyde
6-hydroxymethyl-7,8-dihydropterin
ATP
EC#2.7.6.3
BDP_0552
AMP
6-hydroxymethyl-dihydropterin diphosphate
pentose phosphate pathway
(non-oxidative branch)
D-erythrose-4-phosphate
EC#2.5.1.54 BDP_0381/BDP_0382
EC#4.2.3.4 BDP_1026
EC#1.1.1.282 BDP_1133
EC#4.2.1.10 BDP_1027
EC#1.1.1.25 BDP_1133
2 phosphate
shikimate
EC#2.7.1.71
BDP_1026
ATP
ADP
shikimate-3-phosphate
EC#2.5.1.19
BDP_0996
phosphate
phosphoenolpyruvate
5-enolpyruvyl-shikimate-3-phosphate
EC#4.2.3.5
BDP_1025
phosphate
chorismate
L-glutamine
L-glutamate
EC#2.6.1.85
BDP_0037
BDP_1193
4-amino-4-deoxychorismate
pyruvate
EC#4.1.3.38
BDP_0103
4-aminobenzoate
diphosphate
EC#2.5.1.15
BDP_0551
7,8-dihydropteroate
ATP
L-glutamate
EC#6.3.2.12
BDP_1525
ADP
phosphate
7,8-dihydrofolate monoglutamate
NADPH
EC#1.5.1.3
BDP_0528
NADP+
tetrahydrofolate
Bifidobacterium
dentium Bd1
BDP_0037
BDP_0103
BDP_0528
BDP_0550
BDP_0551
BDP_0552
BDP_1525
BDP_0996
BDP_1193
BDP_1133
BDP_1025
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BDP_1027
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restricted to certain species/strains, leading to the identification of high level (e.g. *Bifidobacterium bifidum* and *Bifidobacterium longum* subsp. *infantis*) and low level folate-producing species (e.g. *Bifidobacterium breve*, *Bifidobacterium longum* subsp. *longum* and *Bifidobacterium adolescentis*) [16]. Such findings have been confirmed by *in vivo* studies: administration of high-producing folate strains was shown to cause an increased faecal level of folate in both rats and humans [17,18].

With the advent of microbial genomics it is now possible to interrogate the genetic make-up of microorganisms for specific features (reviewed in [19]). The first decade of molecular exploration of gut commensals, in particular bifidobacteria and lactobacilli, has afforded unprecedented insights into the genetic adaptation of these bacteria to the human gut through the decoding of their genome sequences (probiogenomics) [20].

Genomic adaptation is obvious in many bifidobacterial genomes where over 9% of annotated genes encode enzymes involved in carbohydrate metabolism [21,22]. However, the dissection of bifidobacterial genomes has also revealed interesting features with respect to vitamin biosynthetic capabilities (Figure 1). No complete pathways for the biosynthesis of biotin, panthothenate, pyridoxine, cobalamin and menaquinone are present in any of the so far sequenced bifidobacterial genomes, yet they are predicted to encode complete pathways for the synthesis of shikimate and thus are expected to produce chorismate [23–27,28•,29,30], a precursor for folate biosynthesis. However, although all available complete bifidobacterial genomes are expected to specify aminodeoxychorismate synthase (EC 2.6.1.85), a gene specifying a putative 4-amino-4-deoxychorismate lyase (EC 4.1.3.38) can only be found on the genome of *B. adolescentis* ATCC15703 and *B. dentium* Bd1 [27], which are thus expected to accomplish *de novo* biosynthesis of pABA (Figure 2). By contrast, *B. animalis* subsp. *lactis* does not appear to possess the entire pathway for DHPPP biosynthesis or the gene encoding dihydropteroate synthase (EC 2.5.1.15) (Figure 2). Thus, *B. animalis* subsp. *lactis* is expected to be auxotrophic for folates or DHP, and would therefore be incapable of folate biosynthesis, even in the presence of pABA (Figure 2).

Lactobacilli are another common group of human gut commensals and have recently been investigated as possible folate producers [31]. The genus *Lactobacillus* contains more than 100 recognized species displaying a remarkable phylogenetic, phenotypic and ecological diversity [32,33]. The genetic characterization of lactobacilli is better developed than that of bifidobacteria, but the molecular mechanisms driving their interaction with the human gut still remain largely unknown (reviewed in [20]). Owing to their commercial potential, the ability to produce folate has been investigated in several lactobacilli from a variety of ecological origins. In this context, lactobacilli from various fermented foods have been investigated as starter cultures for the manufacture of folate-fortified dairy products, while lactobacilli isolated from the human gut have been explored as folate-producing probiotics [34–38,39•]. The availability of genome sequences of various lactobacilli provided an important contribution to the genetics underlying folate biosynthesis in this group of microorganisms [40]. For example, lactobacilli do not appear to harbour the genetic determinants for *de novo* pABA synthesis, with the exception of *Lactobacillus plantarum* WCFS1 [41], suggesting that the vast majority of lactobacilli are unable to synthesize folate in the absence of pABA.

Biosynthesis of riboflavin

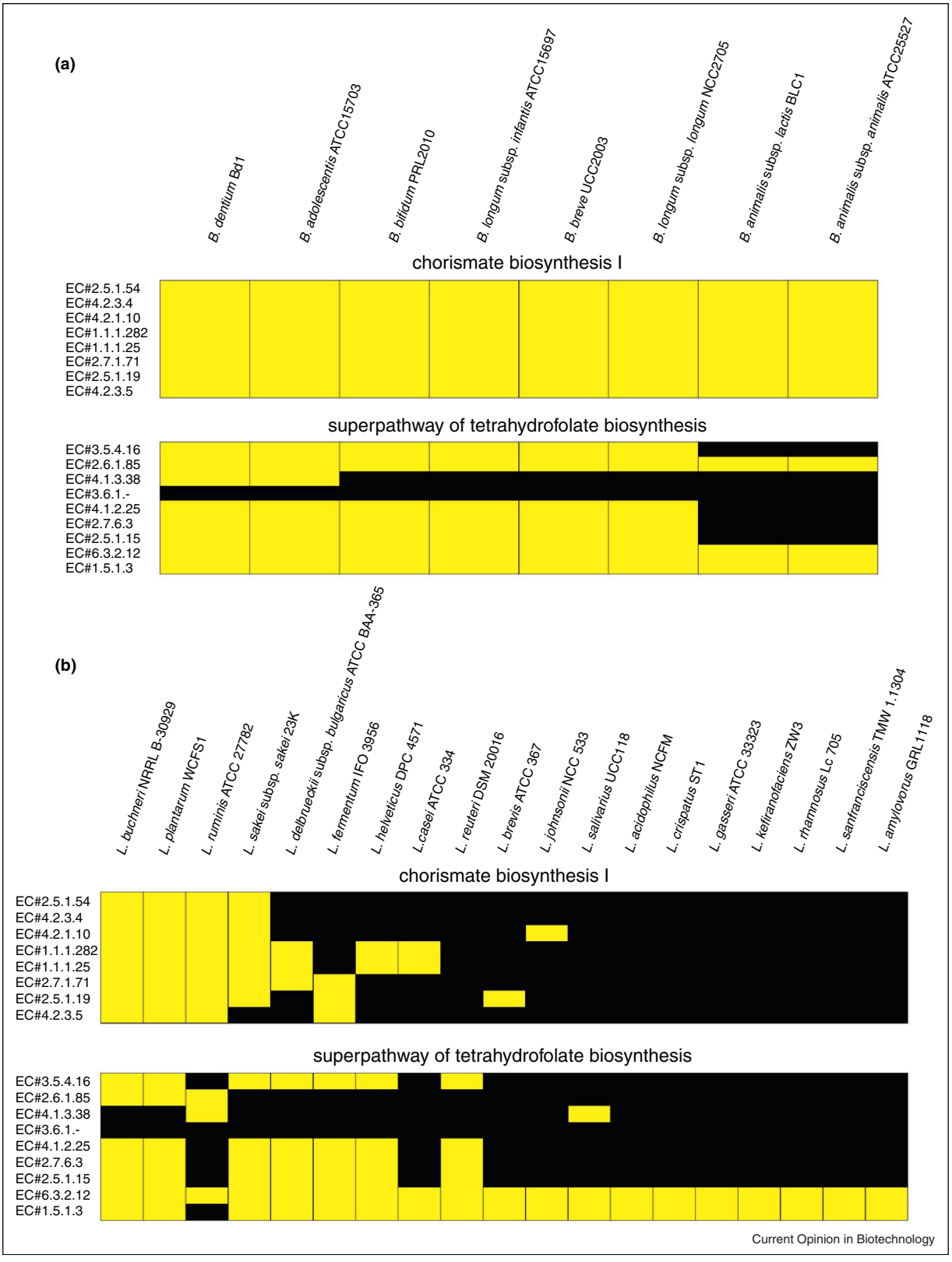
Riboflavin (vitamin B_2) plays an essential role in cellular metabolism, being the precursor of the coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which both act as hydrogen carriers in many biological redox reactions. Microbial riboflavin biosynthesis from the precursors guanosine triphosphate (GTP) and D-ribulose 5-phosphate occurs through seven enzymatic steps, with detailed studies performed for *Bacillus subtilis* [42] and *Escherichia coli* [43], and reviewed recently [44].

Riboflavin concentrations can vary in certain dairy products due to processing technologies and to the action of microorganisms during food processing [1••]. It has been shown that most yoghurt starter cultures decrease riboflavin concentrations whereas others can increase levels of this essential vitamin up to 160% of the initial concentration present in unfermented milk [45]. Selection of spontaneous roseoflavin-resistant mutants was found to be a reliable method to obtain natural riboflavin-overproducing strains of various species commonly used in the food industry [46].

So far fragmentary information is available on the *de novo* synthesis of riboflavin by enteric bacteria, in the case of bifidobacteria the enzymes needed for the biosynthesis of this vitamin seem to be partially or completely absent from the majority of currently available bifidobacterial genomes [19]. However, one cannot exclude

(Figure 1 Legend Continued) Folate biosynthesis pathway of *Bifidobacterium dentium* Bd1 as obtained from the Metabolic Pathway reconstruction software. In the inset on the right genes involved in folate biosynthesis are mapped on the *B. dentium* Bd1 genomes. The metabolic pathway describes how pABA is synthesized from chorismate as a branchpoint of the aromatic amino acid biosynthesis pathway. Folate synthesis continues through a condensation reaction between DHPPP and pABA. This complex pathway is catalysed by various enzymes such as dihydropteroate synthase (EC 2.5.1.15), dihydrofolate synthase (EC 6.3.2.12) and dihydrofolate reductase (EC 1.5.1.3).

Figure 2



Heat map representing the distribution of genes involved in the biosynthesis of folate in bifidobacterial genomes (panel **a**) and lactobacilli (panel **b**). Yellow indicates presence and black represents absence.

the possibility that multiple, co-existing microbial species are capable of *de novo* synthesis.

Biosynthesis of vitamin B_{12}

The term vitamin B_{12} is generally used to describe a type of cobalt corrinoid, particularly of the cobalamin (cbl) group. Animals, plants and fungi are incapable of cobalamin production and it is the only vitamin that is exclusively produced by microorganisms, particularly by anaerobes [47–49]. One of the first model organisms used for the study of biosynthesis was *P. freudenreichii* that is used in the commercial production of vitamin B_{12}.

Lactobacillus reuteri CRL1098 was shown to be the first LAB strain able to produce a cobalamin-like compound with an absorption spectrum closely resembling that of standard cobalamin but with a different elution time, while cobalamin production was confirmed using different bioassays [50]. However, the biological activity of this pseudovitamin B12 is still not clear.

Genetic evidence of cobalamin biosynthesis by *L. reuteri* CRL 1098 was then obtained and it was shown that at least 30 genes are involved in the *de novo* synthesis of the vitamin (Figure 3). The genetic organization (*cob* and *cbi* genes) are very similar to those of *Salmonella enterica* and *Listeria innocua* [51]. Recently, the genetic pathway responsible of the *de novo* synthesis of vitamin B12 by *L. reuteri* was described for two *L. reuteri* strains [52].

One distinctive characteristic of the *cob* cluster of *L. reuteri* is the presence of *hem* genes in the middle of the cluster. In the respiratory organisms *Listeria* and *Salmonella* with similar *cob* clusters, the *hem* genes are located at a different position on their genome. The presence of the *hem* genes in the *cob* cluster is a characteristic that has only been observed in certain genomes of *Clostridium* [53]. Recently, the transcription of a vast set of genes involved in cobalamin synthesis in sourdough prepared with strain *L. reuteri* ATCC 55730 was described [54].

In addition to strain CRL1098, other *L. reuteri* strains were shown to be capable of producing some corrinoids such as *L. reuteri* DCM 20016 [55], JCM1112 [36] and CRL 1324 and 1327, strains isolated from human vagina [56]. Recently, a reuterin-producing strain of *L. coryniformis* was shown to produce a cobalamin-type compound [57]. Notably, propionibacteria and *L. reuteri* normally occur in the human intestine and may thus (partially) fulfil the vitamin B12 requirement of the host.

Biosynthesis of other B-group vitamins

Besides riboflavin, folate and vitamin B_{12}, increased levels of other B-group vitamins, for example, niacin and pyridoxine, have been reported for certain LAB used in yoghurt, cheese, and fermentations [58,59]. For example, increases in thiamine and pyridoxine concentration were demonstrated as a result of soy fermentation with *Streptococcus thermophilus* ST5 and *Lactobacillus helveticus* R0052, or *B. longum* R0175 [60].

De novo synthesis of vitamin K by gut bacteria

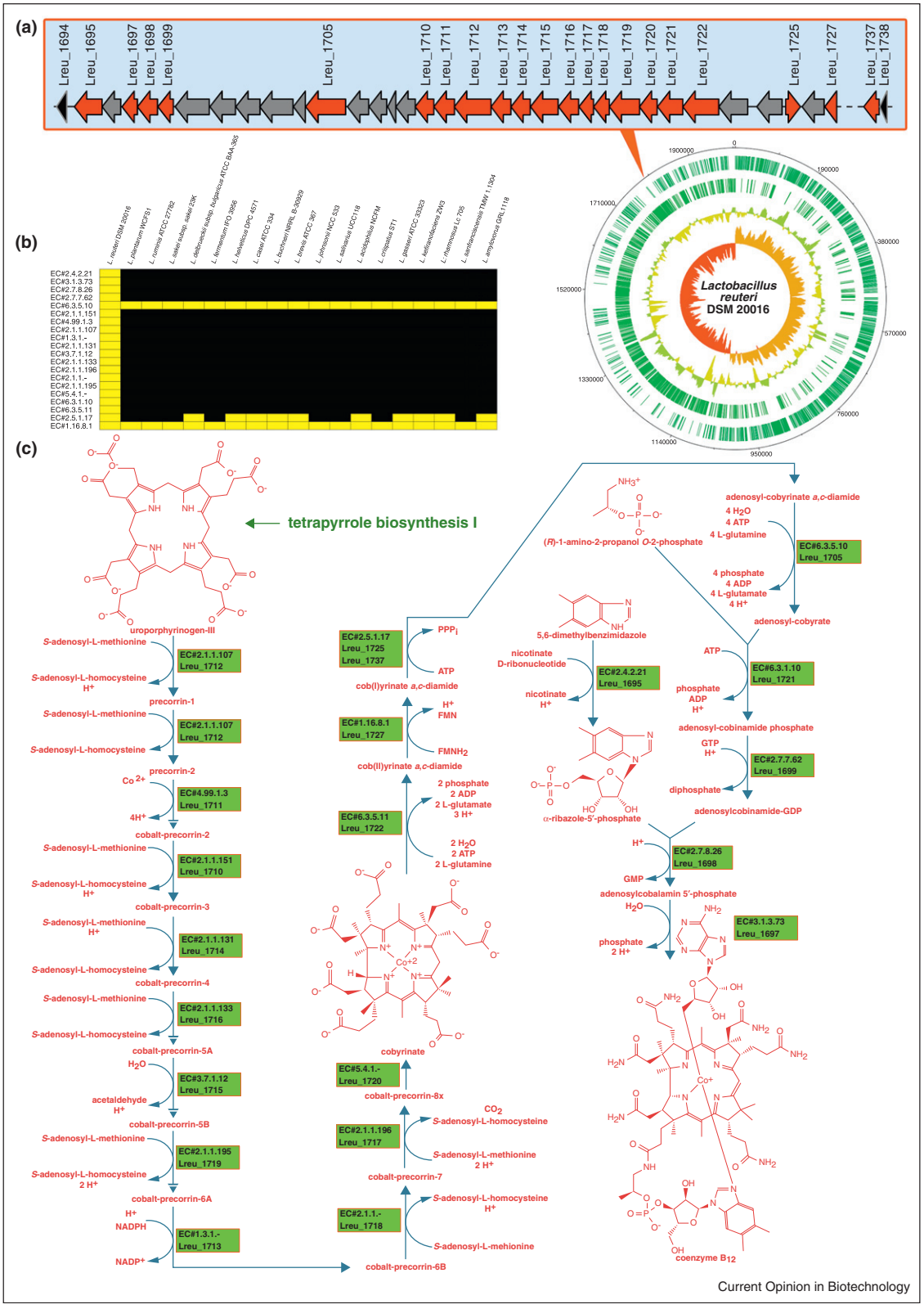
Vitamin K acts as a co-factor for the enzyme that converts specific glutamyl residues in a limited number of proteins to γ-carboxyglutamyl (Gla) residues. The daily requirement for vitamin K is fulfilled by dietary phylloquinone that is present in plants, and, to an undetermined extent, by bacterially produced polyisoprenyl-containing compounds known as menaquinones synthesized in the human gut [61]. However, menaquinone synthesis may not be fully dependent on the gut microbiota as animals lacking a gut microbiota can still produce menaquinone [62].

Human gut microbiome and vitamin biosynthesis

Although whole genome sequencing and assembly have historically been used for the study of single organisms, recent reports have shown the validity of this approach to investigate mixed microbial communities [2,63,64]. In this context, sampling genetic information of the human gut microbiota, also known as human gut microbiome, allowed us to obtain insights into the genetic features of enteric bacteria [64]. In order to delineate if and to what extent the enteric microbiome provides physiological features that were not evolved by its human host, the metabolic potential of microbial sequences was analysed through the classification of all identified microbial genes based on the Kyoto Encyclopedia of Genes and Genomes as well as the Clustered Orthologous Groups (COG). These analyses showed that the distal gut microbiome of two subjects is enriched for a variety of COGs involved in synthesis of essential amino acids and vitamins, such as those required for the synthesis of deoxyxylulose 5-phosphate (DXP), a precursor of the vitamins thiamine and pyrodoxal [64]. Recently, the combination of 22 newly sequenced faecal metagenomes of individuals from four countries allowed the identification of three robust clusters, named enterotypes, which are not nation or continent-specific [65••]. Notably, vitamin metabolism pathways were shown to be highly represented in all enterotypes, while two enterotypes were particularly enriched in genes that specify the biosynthetic enzymes for biotin, riboflavin, pantothenate, ascorbate, thiamine and folate production. These phylogenetic and functional differences among enterotypes thus reflect different combinations of microbial trophic chains with a probable impact on synergistic interrelations with the human host [65••].

Recently, transcriptomic studies directed to explore upregulated genes of bifidobacteria residing in faecal samples of adult subjects identified the presence of bifidobacterial genes predicted to be involved in the biosynthesis of several B-vitamins and folate that are highly expressed

Figure 3



Cobalamin biosynthesis pathway identified in certain lactobacilli. Panel **a** represents the relative position of genes involved in the cobalamin biosynthesis as identified on the genome of *Lactobacillus reuteri* DSM20016. Panel **b** represents the distribution of genes involved in *de novo* cobalamin synthesis among various lactobacilli. Panel **c** depicts the presumed metabolic pathway followed for the cobalamin biosynthesis in certain lactobacilli.

when bifidobacteria are in their natural ecological niche [66–68]. Since it is nearly impossible to quantify or demonstrate vitamin production by individual organisms of the human microbiome using traditional methods (e.g. HPLC, microbiological assays), these and other 'omics' approaches can provide evidence for such *in situ* vitamin production, while also allowing the development of methodologies to increase their production.

Conclusions

The increase of B-group vitamin concentrations in fermented/functional foods is possible through judicious selection of microbial species and cultivation conditions. It is expected that the food industry will exploit novel and efficient vitamin-producing strains to produce fermented products. Such products are expected to provide economic benefits to food manufacturers since increased 'natural' vitamin concentrations would be an important value-added trait without increasing production costs.

With the expanding availability of genome sequences it is not only possible to identify potential vitamin-producing strains, but also to understand the intertwined mechanisms for their biosynthesis, all of which will be exploited to increase the vitamin producing capacities in the GIT of humans.

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- • of special interest
- •• of outstanding interest

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