MS. ROMINA LEVIT (Orcid ID : 0000-0002-6398-2510)
MR. JEAN GUY LEBLANC (Orcid ID : 0000-0002-4634-8630)

Article type   : Review Article

Recent update on lactic acid bacteria producing riboflavin and folates: Application for food fortification and treatment of intestinal inflammation

Uses of vitamin-producing LAB

Romina Levit [1], Graciela Savoy de Giori [1,2], Alejandra de Moreno de LeBlanc [1,*,†], Jean Guy LeBlanc [1,*,†]

[1] Centro de Referencia para Lactobacilos (CERELA-CONICET). Chacabuco 145, (T4000ILC) San Miguel de Tucumán, Tucumán, Argentina
[2] Cátedra de Microbiología Superior, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, San Miguel de Tucumán, Tucumán, Argentina
[†] Participated equally in this work

* Corresponding authors:
Dr. Jean Guy LeBlanc, E-mail: leblanc@cerela.org.ar, leblancjeanguy@gmail.com
Dr. Alejandra de Moreno de LeBlanc, E-mail: demoreno@cerela.org.ar
Tel/Fax: +54 (381) 4310465

Summary

Lactic acid bacteria (LAB), widely used as starter cultures for the fermentation of a large variety of food, can improve the safety, shelf life, nutritional value and overall quality of the fermented products. In this regard, the selection of strains delivering health-promoting compounds is now the main objective of many researchers. Although most LAB are auxotrophic for several vitamins, it is known that certain strains have the capability to synthesize B-group vitamins. This is an important property since humans cannot synthesize most vitamins, and these could be obtained by consuming LAB fermented foods. This review discusses the use of LAB as alternative to fortification by the chemical synthesis to increase riboflavin and folates concentrations in food. Moreover, it provides an overview of the recent applications of vitamin-producing LAB with anti-inflammatory/antioxidant activities against gastrointestinal tract inflammation. This review shows the potential uses of riboflavin and folates producing LAB for the biofortification of food, as therapeutics against intestinal pathologies and to complement anti-inflammatory/anti-neoplastic treatments.

Keywords: lactic acid bacteria, riboflavin, folates, intestinal inflammation

Introduction

Lactic acid bacteria (LAB) encompass a heterogeneous group of microorganisms having as a common metabolic property, the production of lactic acid as the majority end-product from the fermentation of carbohydrates (Hatti-Kaul *et al.* 2018; Takahashi *et al.* 2019). LAB are found in a large variety of nutrient-rich environments where carbohydrates and proteins are abundant (Vivek *et al.* 2019). LAB are Gram (+), non-sporulating, catalase negative, acid-tolerant, facultative anaerobic organisms. Except for a few species, LAB members are nonpathogenic organisms with a reputed Generally Recognized As Safe (GRAS) status. Many species are used for the manufacture and preservation of fermented feed and foods from raw agricultural materials in which they are either present as contaminants or deliberately added as starter cultures. Enzymatic activities of these microorganisms contribute to the final organoleptic, rheological and nutritional properties of fermented products (dos Santos Cruxen *et al.* 2019).

Some LAB strains have been shown to possess health-promoting functions such as immunomodulation, improvement of intestinal integrity, pathogens resistance, prevention of lactose intolerance, anticarcinogenic effects, reversal of depression and anxiety symptoms, anti-obesity and anti-diabetic activities and reduction of serum cholesterol levels (de Melo Pereira *et al.* 2018). Due to their safety and beneficial effects on health, in addition to their biotechnological potential (Capozzi *et al.* 2012), many strains of LAB can be considered probiotics which are defined as "live microorganisms that when administered in adequate amounts, confer a health benefit to the host" (WHO 2002).

In addition to their intrinsic properties, certain strains of LAB have the capability of producing /releasing and/or increasing specific beneficial compounds in foods. While LAB are usually auxotrophic for several vitamins, it has been reported that certain strains have the ability to synthesize B group vitamins (riboflavin, folates, thiamine and cobalamin) (LeBlanc *et al.* 2011).

Vitamins are organic compounds necessary for many physiological functions essential for life. Unlike other nutrients, vitamins neither fulfill structural functions nor are they an energy source; they act as enzymatic cofactors, participate in oxide-reduction metabolic reactions or act as hormones (Combs Jr, 2012). Certain vitamins, such as folates have even been considered functional food constituents due to their biological functions (Iyer andTomar 2009). Although vitamins are found in a wide variety of foods, unbalanced diets are an important cause of deficiencies, present in all parts of the world (LeBlanc *et al.* 2011). For this reason, vitamin-producing microorganisms could be used to produce foods with high concentrations of natural forms of vitamins and avoid undesirable side effects associated to chemical vitamins normally used for food fortification (FAO/WHO, 2005). In addition, bio-fortified foods represent a less expensive alternative than fortification and could help consumers meet their daily recommended intakes (LeBlanc *et al.* 2013). In addition, consumers currently tend to choose foods that not only provide nutrients but also have additional properties that benefit their health (Burgess *et al.* 2004). In this sense, LAB that produce vitamins have the advantage of increasing the nutritional value of a food (LeBlanc *et al.* 2013). The chemical synthesis of a vitamin, such as riboflavin, can be replaced by fermentation processes, showing economic and environmental advantages, in addition that microbial synthesis uses of renewable sources, is more ecological and achieve high quality end products (Thakur *et al.* 2016b, Zhu *et al.* 2020).

This review will discuss the use of LAB as a natural alternative to increase riboflavin and folates concentrations in foods and provide an overview of the recent applications of LAB as a source of vitamins with anti-inflammatory/antioxidant activities against gastrointestinal tract (GIT) inflammation.

Methods

The authors have searched relevant literature in the electronic databases of the U.S. National Library of Medicine (Pubmed) and Google Scholar, by the use of the following search terms: lactic acid bacteria, riboflavin, folates, vitamins, probiotic, intestinal inflammation, inflammatory bowel disease and intestinal mucositis. No double-reviewed studies, issued in a language other than English and published before 1990 were excluded. Works published between 1990 and 2020, and one official web-site were extracted as fulfilling the inclusion criteria.

Riboflavin-producing LAB

Riboflavin (vitamin B2) is the precursor of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), two coenzymes that play a central role in metabolism by acting as hydrogen carriers in biological redox reactions (Fraaije and Mattevi 2000).

Riboflavin is a key nutrient for all aerobic forms of life, essential for normal cellular functions and growth (Aili *et al.* 2013). Although riboflavin is found in a variety of foods, suboptimal intakes can lead to ariboflavinosis. Riboflavin deficiency is associated with eye-related problems, cardiac risk, preeclampsia, anemia, liver and skin damage and changes in cerebral glucose metabolism (Levit *et al.* 2018a).

In addition to the fortification of foods with chemically synthesized vitamin, the use of riboflavin-producing microorganisms, as was explained above, has gained importance in recent years as a biofortification strategy. This is due to its economic advantages compared to the chemical synthesis process added to the ”nature-friendly” characteristics of the fermentation process (Thakur *et al.* 2016b; Revuelta *et al.* 2017).

Riboflavin is synthesized in seven enzymatic stages from guanosine triphosphate (GTP) and d-ribulose-5-phosphate precursors (Bacher *et al.* 2000) (Figure 1), and the synthesis is strain dependent.

Some LAB strains are able to synthesize riboflavin and their use during fermentation improves the nutritional value of foods (Table 1). Furthermore, riboflavin concentrations can sometimes vary in certain products due to processing technologies and the action of the microorganisms utilized during food processing. In this regard, it was shown that the addition of supplements, such as dietary fibers from fruit sources can increase riboflavin production (Albuquerque *et al.* 2020).

Riboflavin-producing LAB have been isolated from different ecological niches and showed the ability to increase the concentrations of this vitamin in food matrices such as milk, soymilk, whey and pseudocereals (Ewe *et al.* 2010; Juarez del Valle *et al.* 2014; Thakur *et al.* 2016a, Rollán *et al.* 2019). Recently, it was reported the riboflavin fortification of different kefir-like cereal-based beverages using selected LAB strains (Yépez *et al.* 2019).

Furthermore some bacteria are capable of riboflavin overproduction (Burgess *et al.* 2009). This trait can be achieved either by metabolic engineering (Jiménez *et al.* 2005) or by exposure to purine analogues and/or the toxic riboflavin analogue roseoflavin (Perkins *et al.* 1991). Particularly, in *Lactococcus* (*L.*) *lactis* both of these approaches have been used with success (Burgess *et al.* 2004). Recent studies have reported on the selection of riboflavin-overproducing strains for potential food applications, for example, the manufacture of vitamin B2-enriched dairy products (Burgess *et al.* 2006), which were found to improve the riboflavin status of deficient rats (LeBlanc *et al.* 2006). These strains are acceptable by the consumer because they are not considered genetically modified. Natural strains overproducing riboflavin obtained by exposure to roseoflavin, a riboflavin toxic analog, were used to obtain bioenriched bread, pasta and soymilk (Capozzi *et al.* 2011; Juarez del Valle *et al.* 2014; Russo *et al.* 2014). This strategy allows increasing the nutritional value of these foods, for example in the case of soymilk. This matrix is rich in proteins and contains high levels of unsaturated fatty acids; the fermentation with riboflavin-overproducing strains was used to obtain a bio-enriched food with improved nutritional, organoleptic and sensorial properties (Juarez del Valle *et al.* 2014). Furthermore the bioavailability of riboflavin produced by some LAB has been demonstrated in different animal models (LeBlanc *et al.* 2005; Juarez del Valle *et al.* 2016; Carrizo *et al.* 2020). On the other hand, metabolic engineering was used to construct recombinant strains and also to improve the riboflavin production (Koizumi *et al.* 2000; Burgess *et al.* 2004; Jayashree *et al.* 2011). Genetically modified *L. lactis* has been shown to be efficient in reverting riboflavin deficiencies in rodents (LeBlanc *et al.* 2005a; LeBlanc *et al.* 2005b). In these studies, it was shown that the administration of spontaneous and engineered *L. lactis* that overproduces riboflavin and a milk fermented by these strains was able to eliminate most physiological manifestations of ariboflavinosis, such as stunted growth, elevated erythrocyte glutathione reductase activation coefficient values and hepatomegaly in depleted rats. These findings were similar to those obtained with commercial riboflavin whereas a non riboflavin-producing strain did not show beneficial results (LeBlanc *et al.* 2005). Furthermore, the administration of soymilk bio-enriched with riboflavin produced by *Lactobacillus* (*Lact.) plantarum* CRL 2130 was able to revert riboflavin deficiency in depleted mice as evidenced by normal growth, riboflavin status and morphology of the small intestines. The same tendency it was observed in a prevention model where mice did not show signs of riboflavin deficiency (Juarez del Valle *et al.* 2016). Another study showed that the administration of pasta made with quinoa sourdough and fermented by *Lact. plantarum* strains that produce both vitamins B2 and B9 was able to increase the levels of these vitamins in mice blood compared to depleted animals, similar to the results obtained in animals that received pasta supplemented with commercial vitamins (Carrizo *et al.* 2020). These results showed that vitamins produced by LAB have a bioavailability similar to synthetic vitamins and it could be useful to prevent vitamins deficiency. In another study, as many as 60 lactobacilli were screened for the ability of riboflavin overproduction via screening of the genes responsible for riboflavin synthesis by a PCR-based method (Thakur *et al.* 2016c). Among the lactobacilli screened, the presence of genes responsible for riboflavin synthesis was strain-specific across different species. The isolates possessing incomplete *rib* structural genes could not survive in the riboflavin-deficient medium. On contrary, the isolates KTLF1 (*Lact. fermentum*) KTLP13 (*Lact. plantarum*) and KTLF3 (*Lact. fermentum*) were not only able to grow well on riboflavin-deficient medium agar but also supported the growth of the riboflavin auxotroph strain (Liu *et al.* 2020).

Folates-producing LAB

The generic term folates or vitamin B9/B11 is used to describe folic acid and related compounds that exhibit the same biological activity. This vitamin is involved in numerous vital biological reactions, including DNA synthesis and methylation, and participates in the synthesis of some amino acids, nucleotides and other vitamins (Nazki *et al.* 2014). Folates possess antioxidant properties that protect the genome by inhibiting free radical attack of DNA, in addition to their role in DNA repair and replication mechanisms (Duthie *et al.* 2002). Folates are key nutrients for human health and for ensuring normal development, growth, and the maintenance of optimal health (Bailey *et al.* 2015). Folates deficiency causes severe abnormalities in one-carbon metabolism, which is considered risk factor for some chronic diseases and developmental disorders, including autism (Lyall *et al.* 2014), Alzheimer's disease (Hinterberger and Fischer 2013), senile dementia (mental deterioration in old age) (Araújo *et al.* 2015), and neural tube defects (NTDs) (Copp *et al.* 2013).

Although folates are present in various foods, the intake of this vitamin through the diet may be insufficient to meet daily requirements. To address this problem, foods fortified by addition of folic acid have been developed. Unfortunately, food fortification with synthetic molecules may present some downsides. It has been shown that the absorption of high amounts of folic acid can mask the symptoms of vitamin B12 deficiency, which may result in the progression of neuropathy to an irreversible point (FAO/WHO, 2005). Furthermore, it was reported that synthetic folic acid can cause alterations of the dihydrofolate reductase enzymatic activity in the liver (Bailey and Ayling 2009). Therefore several studies seem to raise doubts about the safe use of the chemically synthetized folic acid in foods (Saubade *et al.* 2017), addressing the fortification through biological approaches (Rad *et al.* 2016). In this sense the use of natural folates could represent an alternative to synthetic folic acid to avoid the adverse effects associated with its consumption (Scaglione and Panzavolta 2014).To this regard, some species of LAB are able to accumulate folates (see biosynthetic pathway in Figure 2) in different substrate after a fermentation process (Table 2). However, the ability of microorganisms to produce folates is strain-specific and influenced by the growth conditions (Laiño *et al.* 2013; Kariluoto *et al.* 2014; Laiño *et al.* 2015; Saubade *et al.* 2017). In this sense, a preview review discussed that folates production can also be affected by symbiosis between starter cultures, the presence of prebiotics, the external pH of the growth media (food), incubation temperature and the presence of the chemical precursors guanosine triphosphate (GTP) and 4-aminobenzoate, a product of shikimate biosynthesis pathway (Savoy de Giori and LeBlanc, 2018).

It was previously believed that *Streptococcus* (*Strep.*) *thermophilus* are folates producers while *Lact. delbrueckii* subsp. *bulgaricus* utilize folates for their growth. However, Laiño *et al.* (2012) found that some strains of *Lact. delbrueckii* subsp. *bulgaricus*, isolated from artisanal Argentinean fermented dairy products, were able to grow in a folates-free culture medium and to produce high folates levels. It was demonstrated that *Lact. bulgaricus* CRL 871 was able to synthesize both intra-and extra-cellular folates. This strain inoculated with *Strep. thermophilus* (CRL 803 and CRL 415) produced a yogurt naturally bio-enriched in folates with a four-fold increase in vitamin content compared to unfermented milk and a two-fold increase compared to conventional yogurts (Laiño *et al.* 2013). It has been also demonstrated that the addition of *Lact. amylovorus* CRL 887 to this yogurt starter culture was efficient in producing a yogurt with even higher folates concentrations (six-fold increase, 260 μg l^{-1}) making this new bio-enriched product a very interesting alternative to fortification with chemical folic acid (Laiño *et al.* 2014). A recent study combined five *Strep. thermophilus* strains with *Lact. plantarum* 16cv, and the combination that produced the highest levels of folates was used for preparation of bioenriched fermented milk under controlled conditions (pH 6.0, 42 °C, 70 rpm, 24 h). The bioavailability and intestinal benefits of this milk fermented by folates-producing LAB were demonstrated in a depletion/repletion mice model (Cucick *et al.* 2020). Some folates-producing strains were also able to increase the concentration of this vitamin in goat's milk (Da Silva *et al.* 2016). Several studies showed that LAB strains, isolated from cereals and seeds, were also able to overproduce folates leading to a bioenriched end product (Salvucci *et al.* 2016; Carrizo *et al.* 2016). Furthermore, *Lact. plantarum* CRL 2106 and CRL 2107 synthesized the highest concentration of folates during amaranth sourdough demonstrating the potential of LAB to improve the nutritional and functional values of pseudocereal-derived foods (Carrizo *et al.* 2017). Fermentation process of oats and soybean with LAB to improve folates content was also successful (Kariluoto *et al.* 2014; Carrizo *et al.* 2020). *Lact. rhamnosus* LGG and *Strep. thermophilus* TH-4, used as culture starter for fermented soymilk production, were able to produce folates. Also, the addition of passion fruit by-product and fructooligosaccharides stimulated the folates production by these strains during fermentation process (Albuquerque *et al.* 2017). Moreover, LAB strains isolated from a traditional Andean fermented potato product, tocosh, were selected to ferment tubers-oca, papalisa and potato supplemented with amaranth and chia flour and a product with high folates content and nutritional value was obtained (Mosso *et al.* 2018).

It was also demonstrated that folates production may be influenced by nutritional and environmental conditions. In this regard, different parameters such as the fermentation period, temperature, concentration of precursors such as para-aminobenzoic acid (*p*ABA) and glutamate, prebiotics and reducing agents influenced the production and bioavailability of this vitamin in skim milk and fruit juices (Gangadharan and Nampoothiri 2011). It was also demonstrated that passion fruit by-products and fructooligosaccharides increased the folates production in soymilk by selected bacterial strains (Albuquerque *et al.* 2017). Recently, an increase in folates content has been observed by Thompson *et al.* (2020) in a cauliflower-white beans mixture after fermentation with *Lact. plantarum* strains. The genes for folates biosynthesis have been identified in this species (Kleerebezem *et al.* 2003). In the last year, it was reported that all of the genes encoding enzymes involved in the folates biosynthesis pathway in *Lact. plantarum* strain 4_3 genome sequences were expressed and detected in transcriptomic data throughout the culture period in folic acid casei medium (FACM) and fermented soybean (Liu *et al.* 2019). All of the genes involved in the four *de novo* biosynthesis pathways (DHPPP, chorismate, *p*ABA, and THF–polyglutamate biosynthesis) were expressed at different levels in both media. This strain grew slower in fermented soybean than in FACM, which was confirmed by the final pH values of the media and the transcriptomic patterns. However, the high folates production in fermented soybean could be explained by the high expression of the *de novo* biosynthesis genes in the *para*-aminobenzoate pathway. Moreover, it is known that hyperhomocysteinemia is associated to folates deficiency. Recently a study performed in hyperhomocysteinemic mice, showed that supplementation with folates-enriched fermented milk (folates-producing strains: *Strep. thermophilus* 563 and *Lact. delbrueckii* subsp. *lactis* 1021 used as starter) restored homocysteine and S-adenosyl-methionine levels in folates deficient mice (Zinno *et al.* 2020).

Vitamin production by LAB to counteract intestinal inflammation

This review summarizes the studies concerning the effect of vitamin-producing LAB against intestinal inflammation in pathologies such as inflammatory bowel diseases and intestinal mucositis.

Inflammatory bowel diseases (IBD) are a group of chronic disorders characterized by inflammation of the GIT. Crohn's disease (CD) and ulcerative colitis (UC), the two main manifestations of IBD, share common characteristics but exhibit physiopathological differences (Yangyang and Rodriguez 2017). Successful management of IBD includes pharmacological, surgical and nutritional therapies. Some studies have shown that a large percentage of patients with IBD have nutritional deficiencies, mainly of vitamins and minerals (iron, vitamin B12, vitamin D, vitamin K, folates, selenium, zinc, vitamin B6 and vitamin B1) (Montgomery *et al.* 2015; Weisshof and Chermesh 2015). For this reason, patients with IBD under treatment frequently receive nutritional supplementation to correct deficiencies of macro and micronutrients and receive extra calories and proteins to maintain a positive nitrogen balance and thus promote mucosal healing (Martínez Gómez *et al.* 2016).

Another intestinal inflammatory condition is mucositis, a severe inflammation of the gastrointestinal mucosa that occurs, among other causes, as a result of cancer treatments. In general terms, antineoplastic therapies damage the mucous membranes leading to structural and functional changes compromising the absorption of fluids and nutrients and the intestinal barrier. This can cause malnutrition and bacterial translocation contributing to systemic inflammation that is associated with the morbidity and/or mortality of cancer patients (Vanhoecke *et al.* 2015). Because of malnutrition, many patients undergoing chemotherapy treatment often take nutritional supplements and receive mega vitamin therapy (Branda *et al.* 2004).

LAB have shown that can counteract inflammatory processes in the GIT through different mechanisms, this was demonstrated using animals models of IBD and intestinal mucositis (IM) and in clinical trials, being the intestinal microbiota and host's immune response modulation the most evaluated (dos Santos *et al.* 2016; Ferreira dos Santos *et al.* 2016; Tang *et al.* 2017; Yokota *et al.* 2018; Choi *et al.* 2019).

LAB can also produce different vitamins, and some vitamins' supplementation have proven to be effective against intestinal inflammation; so vitamin production has also been associated to the anti-inflammatory properties of certain LAB, in addition to the nutrition value of these strains, against intestinal pathologies (Table 3).

Recent studies about the effect of vitamin-producing LAB on intestinal inflammation are summarized below. A riboflavin-overproducing strain, *Lact. plantarum* CRL 2130, attenuated the chemical induced intestinal inflammation in mice, in a similar way as the commercial riboflavin, which was used as a control. These benefits were obtained when the LAB was administered in a fermented aqueous soy extract or as a bacterial suspension. The administration of the unfermented aqueous soy extract or fermented with a non riboflavin-producing strain did not show the anti-inflammatory effect, demonstrating that the benefit of LAB against colitis was due to the riboflavin production (Levit *et al.* 2017a; Levit *et al.* 2017b). It should be noted that the non-riboflavin-producing strain used in this study is not an isogenic strain of *Lact. plantarum* CRL 2130, so it is possible that both are genetically and phenotypically different; and these differences could affect the response in the host, regardless of riboflavin production. A strain of *Lact. plantarum* CRL 2130 in which riboflavin biosynthesis genes are non-functional should be used in future studies to avoid the possible effect of the genetic background.

In addition, *in vitro* assays demonstrated that this strain exerted a protective mechanism against oxidative stress (Levit *et al.* 2018b). The anti-oxidant/anti-inflammatory effects of *Lact. plantarum* CRL 2130 were also evaluated in a mouse model of intestinal mucositis. Oral administration of this riboflavin-producing strain was effective in attenuating damages associated with 5-fluorouracil (5-FU) induced IM (Levit *et al.* 2018b). In this study, authors used *Lact. plantarum* CRL 725 as control. This is the bacterial strain from which *Lact. plantarum* CRL 2130 was derived, after exposing it to roseoflavin, so the only difference between both strains is the amount of riboflavin produced. The lack of effect reported from mice administered *Lact. plantarum* CRL 725 showed that riboflavin was associated to the anti-inflammatory effect attributed to *Lact. plantarum* CRL 2130.

Regarding folates, a folates-producing strain, *Strep. thermophilus* CRL 808, was also effective in preventing IM induced by 5-FU in mice. Administration of this folates-producing strain showed an anti-inflammatory effect similar to commercial folic acid that was used as a control. (Levit *et al.* 2018c).

In order to improve the individual properties of these two vitamin-producing strains, they were mixed with *Strep. thermophilus* CRL 807, a LAB with immunomodulatory properties (del Carmen *et al.* 2014). This bacterial blend was studied as a complement to the conventional treatment against chronic inflammation. The bacterial mixture was administered to mice with chronic colitis during the remission period together or not with the anti-inflammatory drug mesalazine. The anti-inflammatory effect of the bacterial blend was by attenuating the symptoms after inducing the recurrence of inflammation. It is important to highlight that the selected LAB mixture did not affect the primary treatment in mice that received the anti-inflammatory drug; however, prevented undesirable side effects induced by the chronic treatment with mesalazine (Levit *et al.* 2019).

All these reports demonstrate that the use of vitamin-producing LAB could represent a potential tool to reduce inflammation in patients with intestinal pathologies and to provide patients with essential nutrients that are normally deficient in these diseases; however, future studies are need in order to elucidate the exact mechanism by which these vitamin-producing strains exert their benefits.

Conclusions

This review summarized the use of LAB that produce riboflavin or folates for food biofortification as a natural and more economical alternative than fortification by chemical synthesis. This would enable the food industry to produce foods with high concentrations of these bioactive compounds by selecting appropriate microorganisms and culture conditions.

Taking into account that consumers demand foods that in addition to providing nutrients have some health benefits, this review provided an overview of recent studies where vitamin-producing LAB were shown to counteract inflammatory processes in the GIT and to complement anti-inflammatory/anti-neoplastic treatments (Figure 3). From these studies, vitamin-producing LAB have the potential to improve the effects and/or to reduce the unwanted side effects associated with conventional treatments as well as represent a source of vitamins for these patients that usually have nutritional deficiencies. In this sense, vitamin-producing LAB could be used as adjunct treatments and eliminate the need of using synthetic forms of vitamins (such as folic acid) that have been shown to cause unwanted side effects.

Acknowledgments: This work was supported by ANPCyT (Agencia Nacional de Promoción Científica y Tecnológica, Project 0301), CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas, PIP 0687), and CYTED (Programa Iberoamericano de Ciencia y Tecnología para el Desarrollo, Project 917PTE0537).

Conflict of interest: The authors have no conflict of interest to declare.

References

Aili, A., Hasim, A., Kelimu, A., Guo, X., Mamtimin, B., Abudula, A. and Upur, H. (2013) Association of the plasma and tissue riboflavin levels with C20orf54 expression in cervical lesions and its relationship to HPV16 infection. *PloS One* **8**(11), e79937.

Albuquerque, M. A. C., Bedani, R., LeBlanc, J. G. and Saad, S. M. I. (2017) Passion fruit by-product and fructooligosaccharides stimulate the growth and folate production by starter and probiotic cultures in fermented soymilk. *Int J Food Microbiol* **261**, 35-41.

Albuquerque, M., Candelaria, A.C., Garutti, L., Teran, M.M., LeBlanc, J.G., Franco, B.G.M., Saad, S. (2020) B-group vitamin producing lactic acid bacteria as a tool to bio-enrich foods and improve human vitamin content. In: Lactic Acid Bacteria: A functional approach. Eds. Albuquerque, M.A.C., de Moreno de LeBlanc, A., LeBlanc, J.G., Bedani,

R. Chapter 7, pp. 106-123. CRC Press, LLC, Boca Raton, Fl, USA ISBN 978-113-839-163-5.

Araújo, J. R., Martel, F., Borges, N., Araújo, J. M., and Keating, E. (2015) Folates and aging: Role in mild cognitive impairment, dementia and depression. *Ageing Res Rev* **22**, 9-19.

Bacher, A., Eberhardt, S., Fischer, M., Kis, K. and Richter, G. (2000) Biosynthesis of vitamin B2 (riboflavin). *Annu Rev Nutr* **20**, 153-167.

Bailey, S. W. and Ayling, J. E. (2009) The extremely slow and variable activity of dihydrofolate reductase in human liver and its implications for high folic acid intake. *Proc Natl Acad Sci* **106**(36), 15424-15429.

Bailey, L. B., Stover, P. J., McNulty, H., Fenech, M. F., Gregory Iii, J. F., Mills, J. L., Pfeiffer, C. M., Fazili, Z., Zhang, M. and Ueland, P. M. (2015) Biomarkers of nutrition for development-folate review. *J Nutr* **145**(7), 1636S-1680S.

Branda, R. F., Naud, S. J., Brooks, E. M., Chen, Z. and Muss, H. (2004) Effect of vitamin B12, folate, and dietary supplements on breast carcinoma chemotherapy-induced mucositis and neutropenia. *Cancer* **101**, 1058-1064.

Burgess, C., O'Connell-Motherway, M., Sybesma, W., Hugenholtz, J. and Van Sinderen, D. (2004) Riboflavin production in *Lactococcus lactis*: potential for in situ production of vitamin-enriched foods. *Appl Environ Microbiol* **70**, 5769-5777.

Burgess, C., Eddy, M., Smid, M. J., Rutten, G., and Van Sinderen, D. (2006) A general method for selection of riboflavin-overproducing food grade micro-organisms. *Microb Cell Fact* **5**, 24.

Burgess, C. M., Smid, E. J., and Van Sinderen, D. (2009) Bacterial vitamin B2, B11 and B12 overproduction: an overview. *Int J Food Microbiol* **133**, 1-7.

Capozzi, V., Menga, V., Digesu, A. M., De Vita, P., Van Sinderen, D., Cattivelli, L., Fares, C. and Spano, G. (2011) Biotechnological production of vitamin B2-enriched bread and pasta. *J Agric Food Chem* **59**, 8013-8020.

Capozzi, V., Russo, P., Dueñas, M. T., López, P. and Spano, G. (2012) Lactic acid bacteria producing B-group vitamins: a great potential for functional cereals products. *Appl Microbiol Biotechnol* **96**, 1383-1394.

Carrizo, S. L., de Moreno de LeBlanc, A., LeBlanc, J. G. and Rollan, G. C. (2020) Quinoa pasta fermented with lactic acid bacteria prevents nutritional deficiencies in mice. *Food Res Int* **127**, 108735.

Carrizo, S. L., Montes de Oca, C. E., Hebert, M. E., Saavedra, L., Vignolo, G., LeBlanc, J. G. and Rollan, G. C. (2017) Lactic acid bacteria from andean grain amaranth: a source of vitamins and functional value enzymes. *J Mol Microbiol Biotechnol* **27**, 289-298.

Carrizo, S. L., Montes de Oca, C. E., Laiño, J. E., Suarez, N. E., Vignolo, G., LeBlanc, J. G. and Rollan, G. (2016) Ancestral andean grain quinoa as source of lactic acid bacteria capable to degrade phytate and produce B-group vitamins. *Food Res Int* **89**(Pt 1), 488-494.

Combs Jr, G. F. (2012) *The vitamins,* Academic Press.

Choi, E.-J., Lee, H. J., Kim, W.-J., Han, K.-I., Iwasa, M., Kobayashi, K., Debnath, T., Tang, Y., Kwak, Y.-S. and Yoon, J.-H. (2019) *Enterococcus faecalis* EF-2001 protects DNBS-induced inflammatory bowel disease in mice model. *PloS One* **14**, e0210854.

Copp, A. J., Stanier, P., Greene, N. D. (2013) Neural tube defects: Recent advances, unsolved questions, and controversies. *Lancet Neurol* **12**, 799–810.

Cucick, A. C., Gianni, K., Todorov, S. D., de Moreno de LeBlanc, A., LeBlanc, J. G. Franco, B. D.G.M. (2020) Evaluation of the bioavailability and intestinal effects of milk fermented by folate producing lactic acid bacteria in a depletion/repletion mice model. *J Funct Foods* **66**, 103785.

Da Silva, F. F. P., Biscola, V., LeBlanc, J. G. and de Melo Franco, B. D. G. (2016) Effect of indigenous lactic acid bacteria isolated from goat milk and cheeses on folate and riboflavin content of fermented goat milk. *LWT-Food Sci Technol* **71**, 155-161.

de Melo Pereira, G. V., de Oliveira Coelho, B., Júnior, A. I. M., Thomaz-Soccol, V. and Soccol, C. R. (2018) How to select a probiotic? A review and update of methods and criteria. *Biotechnol Adv* **36**, 2060-2076.

del Carmen, S., de Moreno de LeBlanc, A., Martin, R., Chain, F., Langella, P., Bermúdez-Humarán, L. G. and LeBlanc, J. G. (2014) Genetically engineered immunomodulatory *Streptococcus thermophilus* strains producing antioxidant enzymes exhibit enhanced anti-inflammatory activities. *Appl Environ Microbiol* **80**, 869-877.

dos Santos Cruxen, C. E., Funck, G. D., Haubert, L., da Silva Dannenberg, G., de Lima Marques, J., Chaves, F. C., da Silva, W. P. and Fiorentini, Ã. M. M. (2019) Selection of native bacterial starter culture in the production of fermented meat sausages: Application potential, safety aspects, and emerging technologies. *Food Res Int* **122**, 371-382.

dos Santos, T. F., Melo, T. A., Santos, D. S., Rezende, R. P., Dias, J. C. T. and Romano, C. C. (2016) Efficacy of oral administration of lactic acid bacteria isolated from cocoa in a fermented milk preparation: reduction of colitis in an experimental rat model. *Genet Mol Res* **15**(3).

Duthie, S. J., Narayanan, S., Brand, G. M., Pirie, L. and Grant, G. (2002) Impact of folate deficiency on DNA stability. *J Nutr* **132**(8), 2444S-2449S.

Ewe, J.-A., Wan-Abdullah, W.-N. and Liong, M.-T. (2010) Viability and growth characteristics of *Lactobacillus* in soymilk supplemented with B-vitamins. *Int J Food Sci Nutr* **61**, 87-107.

FAO/WHO (2005) Vitamin and mineral requirements in human nutrition, Second edition. Available from http://www.who.int/nutrition/publications/micronutrients/9241546123/en/.

Ferreira dos Santos, T., Alves Melo, T., Almeida, M. E., Passos Rezende, R. and Romano, C. C. (2016) Immunomodulatory effects of *Lactobacillus plantarum* Lp62 on intestinal epithelial and mononuclear cells. *BioMed Res Int*, 2016

Fraaije, M. W. and Mattevi, A. (2000) Flavoenzymes: diverse catalysts with recurrent features. *Trends Biochem Sci* **25**, 126-132.

Gangadharan, D. and Nampoothiri, K. M. (2011) Folate production using *Lactococcus lactis* ssp *cremoris* with implications for fortification of skim milk and fruit juices. *LWT-Food Sci Technol* **44**, 1859-1864.

Hatti-Kaul, R., Chen, L., Dishisha, T. and Enshasy, H. E. (2018) Lactic acid bacteria: From starter cultures to producers of chemicals. *FEMS Microbiol Lett* **365**(20), fny213.

Hinterberger, M. and Fischer, P. (2013) Folate and Alzheimer: When time matters. *J Neural Transm* (Vienna) **120**, 211-224.

Iyer, R. and Tomar, S.K. (2009) Folate: a functional food constituent. *J Food Sci* **74**, :R114-22.

Jayashree, S., Rajendhran, J., Jayaraman, K., Kalaichelvan, G. and Gunasekaran, P. (2011) Improvement of riboflavin production by *Lactobacillus fermentum* isolated from yogurt. *Food Biotechnol* **25**, 240-251.

Jimenez, A., Santos, M. A., Pompejus, M., and Revuelta, J. L. (2005) Metabolic engineering of the purine pathway for riboflavin production in *Ashbya gossypii. Appl Environ Microbiol* **71**, 5743-5751.

Juarez del Valle, M., Laiño, J. E., de Moreno de LeBlanc, A.,Savoy de Giori, G. and LeBlanc, J. G. (2016) Soyamilk fermented with riboflavin-producing *Lactobacillus plantarum* CRL 2130 reverts and prevents ariboflavinosis in murine models. *Brit J Nutr* **116,** 1229-1235.

Juarez del Valle, M., Laiño, J. E., Savoy de Giori, G. and LeBlanc, J. G. (2014) Riboflavin producing lactic acid bacteria as a biotechnological strategy to obtain bio-enriched soymilk. *Food Res Int* **62**, 1015-1019.

Kariluoto, S., Edelmann, M., Nystrom, L., Sontag-Strohm, T., Salovaara, H., Kivela, R., Herranen, M., Korhola, M. and Piironen, V. (2014) In situ enrichment of folate by microorganisms in beta-glucan rich oat and barley matrices. *Int J Food Microbiol* **176**, 38-48.

Kleerebezem, M., Boekhorst, J., van Kranenburg, R., Molenaar, D., Kuipers, O. P., Leer, R., Tarchini, R., Peters, S. A., Sandbrink, H. M., Fiers, M. W., Stiekema, W., Lankhorst, R. M., Bron, P. A., Hoffer, S. M. ,Groot, M. N., Kerkhoven, R., de Vries, M., Ursing, B., de Vos, W. M., Siezen, R. J. (2003) Complete genome sequence of *Lactobacillus plantarum* WCFS1. *Proc Natl Acad Sci USA* **100**, 1990-1995.

Koizumi, S., Yonetani, Y., Maruyama, A. and Teshiba, S. (2000) Production of riboflavin by metabolically engineered *Corynebacterium ammoniagenes*. *Appl Microbiol Biotechnol* **53**, 674-679.

Laiño, J. E., Juarez del Valle, M., Savoy de Giori, G. and LeBlanc, J. G. (2013) Development of a high folate concentration yogurt naturally bio-enriched using selected lactic acid bacteria. *LWT-Food Sci Technol* **54**, 1-5.

Laiño, J. E., Zelaya, H., Juarez del Valle, M., Savoy de Giori, G., LeBlanc, J. G. (2015) Milk fermented with selected strains of lactic acid bacteria is able to improve folate status of deficient rodents and also prevent folate deficiency. *J Funct Foods* **17**, 22-32.

Laiño, J. E., LeBlanc, J. G. and Savoy de Giori, G. (2012) Production of natural folates by lactic acid bacteria starter cultures isolated from artisanal Argentinean yogurts. *Can J Microbiol* **58**, 581-588.

Laiño, J.E., Juarez del Valle, M., Savoy de Giori, G., LeBlanc, J.G. (2014) Applicability of a *Lactobacillus amylovorus* strain as co-culture for natural folate bio-enrichment of fermented milk. *Int J Food Microbiol* **191**,10-16.

LeBlanc, J.G., Burgess, C., Sesma, F., Savoy de Giori, G., and Van Sinderen, D. (2005a) Ingestion of milk fermented by genetically modified *Lactococcus lactis* improves the riboflavin status of deficient rats. *J Dairy Sci* **88**, 3435-3442.

LeBlanc, J. G., Burgess, C., Sesma, F., Savoy de Giori, G., and Van Sinderen, D. (2005b) *Lactococcus lactis* is capable of improving the riboflavin status in deficient rats. *Br J Nutr* **94**, 262-267.

LeBlanc, J. G., Laiño, J. E., Juarez del Valle, M., Vannini, V., van Sinderen, D., Taranto, M. P., Font de Valdez, G., Savoy de Giori, G., and Sesma, F. (2011) B-group vitamin production by lactic acid bacteria-current knowledge and potential applications. *J Appl Microbiol* **111**, 1297-309.

LeBlanc, J. G., Milani, C., Savoy de Giori, G., Sesma, F., van Sinderen, D. and Ventura, M. (2013) Bacteria as vitamin suppliers to their host: a gut microbiota perspective. *Curr Opin Biotechnol* **24**, 160-168.

LeBlanc, J. G., Rutten, G., Bruinenberg, P., Sesma, F., Savoy de Giori, G., and Smid, E. J. (2006) A novel dairy product fermented with *Propionibacterium freudenreichii* improves the riboflavin status of deficient rats. *Nutrition* **22**, 645-651.

Levit, R., Savoy de Giori, G., de Moreno de LeBlanc, A. and LeBlanc, J. G. (2017a) Evaluation of the effect of soymilk fermented by a riboflavin-producing *Lactobacillus plantarum* strain in a murine model of colitis. *Benef Microbes* **8**, 65-72.

Levit, R., Savoy de Giori, G., de Moreno de LeBlanc, A. and LeBlanc, J. G. (2017b) Effect of riboflavin-producing bacteria against chemically induced colitis in mice. *J Appl Microbiol* **124**, 232-240.

Levit, R., Savoy de Giori, G., de Moreno de LeBlanc, A. and LeBlanc, J. G. (2018a) Increasing B vitamins in foods to prevent intestinal inflammation and cancer. In: Nutrients in dairy and their implications on health and disease. Eds. Watson, R. R., Collier, R. J., and Preedy, V. R. pp. 193-204, Elsevier.

Levit, R., Savoy de Giori, G., de Moreno de LeBlanc, A. and LeBlanc, J. G. (2018b) Protective effect of the riboflavin-overproducing strain *Lactobacillus plantarum* CRL2130 on intestinal mucositis in mice. *Nutrition* **54**, 165-172.

Levit, R., Savoy de Giori, G., de Moreno de LeBlanc, A. and LeBlanc, J. G. (2018c) Folate-producing lactic acid bacteria reduce inflammation in mice with induced intestinal mucositis. *J Appl Microbiol* **125**, 1494-1501.

Levit, R., Savoy de Giori, G., de Moreno de LeBlanc, A. and LeBlanc, J. G. (2019) Beneficial effect of a mixture of vitamin-producing and immune-modulating lactic acid bacteria as adjuvant for therapy in a recurrent mouse colitis model. *Appl Microbiol Biotechnol* **103**, 8937-8945.

Liu, S., Hu, W., Wang, Z., and Chen, T. (2020) Production of ribofavin and related cofactors by biotechnological processes. *Microb Cell Fact* **19**, 1-16.

Liu, C-J., Wu, B., Zhang, S-Y., Li, Q-K., Zeng, X-Q., Yang, E., Luo, Y-Y. and Li, X-R. (2019) Transcriptomic analysis of de novo folate biosynthetic genes in *Lactobacillus plantarum* strain 4_3 in fermented soybean. *Food Funct* **10**(5), 2426-2438.

Lyall, K., Schmidt, R. J., and Hertz-Picciotto, I. (2014) Maternal lifestyle and environmental risk factors for autism spectrum disorders. *Int J Epidemiol* **43**, 443-464.

Martínez Gómez, M. J., Melián Fernández, C. and Romeo Donlo, M. (2016) Nutrición en enfermedad inflamatoria intestinal. *Nutr Hosp* **33***,* 59-62.

Montgomery, S. C., Williams, C. M. and Maxwell, P. J. (2015) Nutritional support of patient with inflammatory bowel disease. *Surg Clin* **95**, 1271-1279.

Mosso, A. L., Jimenez, M. E., Vignolo, G., LeBlanc, J. G. and Samman, N. C. (2018) Increasing the folate content of tuber-based foods using potentially probiotic lactic acid bacteria. *Food Res Int* **109**, 168-174.

Nazki, F. H., Sameer, A. S. and Ganaie, B. A. (2014) Folate: metabolism, genes, polymorphisms and the associated diseases. *Gene* **533**, 11-20.

Perkins, J. B., Pero, J., and Sloma, A. (1991) Riboflavin overproducing strains of bacteria. *European Patent Aplication* 405370, 1991-2001.

Rad, A. H., Khosroushahi, A. Y., Khalili, M. and Jafarzadeh, S. (2016) Folate bio-fortification of yoghurt and fermented milk: A review. *Dairy Sci Technol* **96**(4), 427-441.

Revuelta, J. L., Ledesma-Amaro, R., Lozano-Martínez, P., Díaz-Fernández, D., Buey, R. M. and Jiménez, A. (2017) Bioproduction of Riboflavin: A bright yellow history**.** *J Ind Microbiol Biotechnol* **44**(4-5), 659-665.

Rollan, G. C., Gerez, C. L. and LeBlanc, J. G. (2019) Lactic fermentation as a strategy to improve the nutritional and functional values of pseudocereals. *Front Nutr* **6**, 98.

Russo, P., Capozzi, V., Arena, M. P., Spadaccino, G., Dueñas, M. T., López, P., Fiocco, D. and Spano, G. (2014) Riboflavin-overproducing strains of *Lactobacillus fermentum* for riboflavin-enriched bread. *Appl Microbiol Biotechnol* **98**, 3691-3700.

Salvucci, E., LeBlanc, J. G. and Pérez, G. (2016) Technological properties of lactic acid bacteria isolated from raw cereal material. *LWT-Food Sci Technol* **70**, 185-191.

Saubade, F., Hemery, Y. M., Guyot, J.-P. and Humblot, C. (2017) Lactic acid fermentation as a tool for increasing the folate content of foods. *Crit Rev Food Sci Nutr* **57**, 3894-3910.

Savoy de Giori, G. and LeBlanc, J.G. (2018) Folate production by lactic acid bacteria. In: Polyphenols: Prevention and treatment of human diseases. Eds. Watson, R.R., Preedy, V.R. and Zibad, S. Chapter 22, pp.15-29.
Oxford, UK: Academic Press (Elsevier) Oxford, UK, ISBN: 978-012-813-008-7.

Scaglione, F. and Panzavolta G. (2014) Folate, folic acid and 5-methyltetrahydrofolate are not the same thing. Xenobiotica **44**(5), 480-488.

Takahashi, K., Orito, N., Tokunoh, N. and Inoue, N. (2019) Current issues regarding the application of recombinant lactic acid bacteria to mucosal vaccine carriers. *Appl Microbiol Biotechnol* **103**, 5947-5955.

Tang, Y., Wu, Y., Huang, Z., Dong, W., Deng, Y., Wang, F., Li, M. and Yuan, J. (2017) Administration of probiotic mixture DM# 1 ameliorated 5-fluorouracil- induced intestinal mucositis and dysbiosis in rats. *Nutrition* **33**, 96-104.

Thakur, K., Lule, V. K., Rajni, C. S., Kumar, N., Mandal, S., Anand, S., Kumari, V. and Tomar, S. K. (2016a) Riboflavin producing probiotic Lactobacilli as a biotechnological strategy to obtain riboflavin-enriched fermented foods. *J Pure Appl* **10**, 161-166.

Thakur, K., Tomar, S. K. and De, S. (2016b) Lactic acid bacteria as a cell factory for riboflavin production. *Microb Biotechnol* **9**, 441-451.

Thakur, K., Tomar, S. K., Brahma, B. and De, S. (2016c) Screening of ribofavin-producing lactobacilli by a polymerase-chain-reaction-based approach and microbiological assay. *J Agric Food Chem* **64**, 1950-1956.

Thompson, H. O., Önning, G., Holmgren, K., Strandler, H. S., and Hultberg, M. (2020) Fermentation of cauliflower and white beans with *Lactobacillus plantarum*–impact on levels of riboflavin, folate, vitamin B12, and amino acid composition. *Plant Foods Hum Nutr* 1-7.

Vanhoecke, B., Bateman, E., Mayo, B., Vanlancker, E., Stringer, A., Thorpe, D. and Keefe, D. (2015) Dark Agouti rat model of chemotherapy-induced mucositis: establishment and current state of the art. *Exp Biol Med* **240**, 725-741.

Vivek, N., Hazeena, S. H., Rajesh, R. O., Godan, T. K., Anjali, K. B., Nair, L. M., Mohan, B., Nair, S. C., Sindhu, R. and Pandey, A. (2019) Genomics of lactic acid bacteria for glycerol dissimilation. *Mol Biotechnol* 1-17.

Weisshof, R. and Chermesh, I. (2015) Micronutrient deficiencies in inflammatory bowel disease. *Curr Opin Clin Nutr Metab Care* **18**, 576-581.

WHO/FAO. (2002) Probiotics in food: health and nutritional properties and guidelines for evaluation. *Food and Agriculture Organization of the United Nations and World Health Organization Expert Consultation Report*. Available from: http://www.fao.org/3/a-a0512e.pdf

Yangyang, R. Y. and Rodriguez, J. R. (2017) Clinical presentation of Crohn's, ulcerative colitis, and indeterminate colitis: symptoms, extraintestinal manifestations, and disease phenotypes. *Semin Pediatr Surg* **26**, 349-355.

Yépez, A., Russo, P., Spano, G., Khomenko, I., Biasioli, F., Capozzi, V. and Aznar, R. (2019) In situ riboflavin fortification of different kefir-like cereal-based beverges using selected andean LAB strains. *Food Microbiol* **77**, 61-68.

Yokota, Y., Shikano, A., Kuda, T., Takei, M., Takahashi, H. and Kimura, B. (2018) *Lactobacillus plantarum* AN1 cells increase caecal *L. reuteri* in an ICR mouse model of dextran sodium sulphate-induced inflammatory bowel disease. *Int Immunopharmacol* **56**, 119-127.

Zhu, Y.Y., Thakur, K., Feng, J.Y., Cai, J.S., Zhang, J.G., Hu, F., Russo, P., Spano, G. and Wei, Z.J. (2020) Riboflavin-overproducing lactobacilli for the enrichment of fermented soymilk: insights into improved nutritional and functional attributes. *Appl Microbiol Biotechnol* **104**, 5759-5772.

Zinno, P., Motta, V., Guantario, B., Natella, F., Roselli, M., Bello, C., Comitato, R., Carminati, D., Tidona, F., Meucci, A., Aiello, P., Perozzi, G., Virgili, F., Trevisi, P., Canali, R. and Devirgiliis, C. (2020) Supplementation with dairy matrices impacts on homocysteine levels and gut microbiota composition of hyperhomocysteinemic mice. *Eur J Nut* **59**, 345-358.

Figure legends

Figure 1: Riboflavin biosynthesis pathway in lactic acid bacteria

Figure 2: Folates biosynthesis pathway in lactic acid bacteria

Figure 3: Vitamin-producing lactic acid bacteria can be used in the industry for food biofortification (riboflavin and folates) and as probiotics to counteract inflammatory processes in the gastrointestinal tract (inflammatory bowel diseases and intestinal mucositis)

Table 1 Examples of LAB strains with ability of increasing the riboflavin concentration in different food matrices

Strains	Matrices	Vitamin concentration	References
Lact. acidophilus ATCC 314	soymilk	6.57±0.36 mg l^{-1}	Ewe et al. 2010
Lact. acidophilus FTDC 8833	soymilk	2.43±0.05 mg l^{-1}	Ewe et al. 2010
Lact. acidophilus FTDC 8633	soymilk	1.13±0.02 mg l^{-1}	Ewe et al. 2010
Lact. plantarum CRL 725	soymilk	700.00±20.00 ng ml^{-1}	Juarez del Valle et al. 2014
Lact. platarum CRL 725 (G)	soymilk	1860.00±20.00 ng ml^{-1}	Juarez del Valle et al. 2014
Lact. fermentum KTLF1	milk	1.50 mg l^{-1}	Thakur et al. 2016a
Lact. muocase KTLF5	whey	0.83 mg l^{-1}	Thakur et al. 2016a
Lact. plantarum M5MA1-B2	maize kefir-like	0.50 mg l^{-1}	Yepéz et al. 2019
Lact. plantarum M5MA1-B2	oat kefir-like	1.50 mg l^{-1}	Yepéz et al.2019
Lact. plantarum UNIFGPL104 and UNIFGPL209	bread	6.81 µg g^{-1}	Capozzi et al. 2011
Lact. plantarum UNIFGPL104 and UNIFGPL209	pasta	4.01 µg g^{-1}	Capozzi et al. 2011

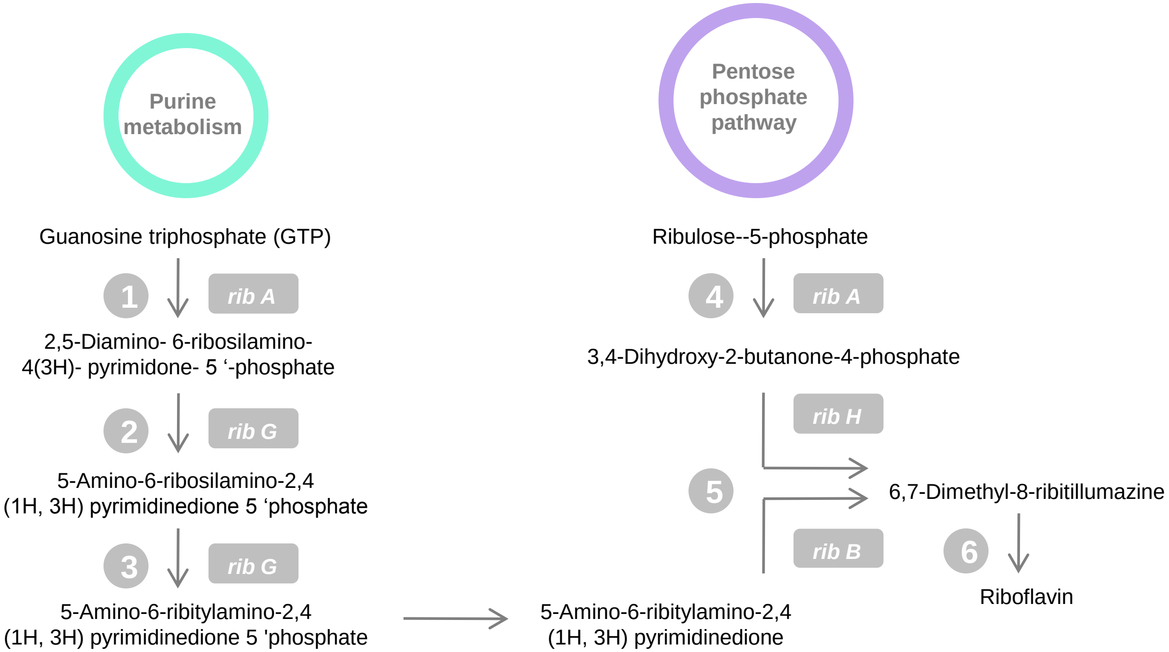
Table 2 Examples of LAB strains with ability of increasing the folates concentration in different food matrices

Strains	**Matrices**	**Vitamin concentration**	**References**
Strep. thermophilus CSCC2000	skim milk	40.00-50.00 ng g^{-1}	Crittenden et al. 2003
Strep. thermophilus CRL 803	nonfat milk	60.00-80.00 µg l^{-1}	Laiño et al. 2012
Strep. thermophilus 908	nonfat milk	76.61±3.29 ng ml^{-1}	Meucci et al. 2018
Strep. thermophilus ABM5097	oat flour	20.00-29.00 ng g^{-1}	Kariluoto et al. 2014
Strep. gallolyticus subsp. *macedonicus* CRL 415	nonfat milk	60.00-80.00 µg l^{-1}	Laiño et al. 2012
Lact. delbrueckii subsp. *bulgaricus* CRL 863	nonfat milk	60.00-80.00 µg l^{-1}	Laiño et al. 2012
Strep. thermophilus CRL 803, *Lact. delbrueckii* subsp. *bulgaricus* CRL 871, and *Strep. gallolyticus* subsp. *macedonicus* CRL 415	yogurt	180.00±10.00 µg l^{-1}	Laiño et al. 2013
Strep. thermophilus CRL 803, *Lact. delbrueckii* subsp. *bulgaricus* CRL 871, *Lact. amylovorus* CRL 887, and *Strep. gallolyticus* subsp. *macedonicus* CRL 415	yogurt	263.00±2.40 µg l^{-1}	Laiño et al. 2014
Strep. thermophilus TH-4 and *Lact. rhamnosus* LGG	soy milk with passion fruit and fructooligosaccharides	1927.00±49.00 ng ml^{-1}	Albuquerque et al. 2017
Lact. plantarum P2R3FA	wheat-based fermented bread	43.10±3.20 µg l^{-1}	Tamene et al. 2019
Lact. plantarum CRL2106 and *Lact. plantarum* CRL 2107	amaranth sourdough	138.00±7.50 ng ml^{-1}	Carrizo et al. 2017
Lact. plantarum CRL 2107 and *Lact. plantarum* CRL 1964	quinoa sourdough	1.60±0.20 µg g^{-1}	Carrizo et al. 2020
Lact. sakei CRL 2210	tuber puree with amaranth and chia flour	1.90 µg g^{-1}	Mosso et al. 2018
Lact. sakei CRL 2209 and *Lact. sakei* CRL 2210	andean tuber purees	730.00-1484.00 ng g^{-1}	Mosso et al. 2018
L. lactis subsp. *lactis* FP368	goat milk	313.00±81.00 µg l^{-1}	Da Silva et al. 2016
L. lactis subsp. *cremoris*	cucumber juice	60.00±1.90 ng ml^{-1}	Gangadharan et al. 2011
L. lactis subsp. *cremoris*	watermelon juice	26.00±1.60 ng ml^{-1}	Gangadharan et al. 2011

Table 3 Examples of beneficial effects of folates and riboflavin-producing LAB strains

Strains / product	**Vitamin produced**	**Beneficial effect**	**Host /model**	**References**
Lact. plantarum CRL 2130	riboflavin	Normalization of the intestinal morphology (villus size)	Riboflavin-depleted mice	Juarez del Valle et al. 2016
Lact. plantarum CRL 2130 / fermented soy milk	riboflavin	Reduction of IBD features (weight loss, intestinal inflammation, microbial translocation to liver and cytokines in intestinal fluids)	2,4,6-trinitrobenzene sulphonic acid (TNBS)-induced mice	Levit et al. 2017b
Lact. plantarum CRL 2130*, Lact. paracasei* CRL 76*, Lact. bulgaricus* CRL 871 *or Strep. thermophilus* CRL 803	riboflavin	Reduction of IBD features (intestinal inflammation, microbial translocation to liver, inducible nitric oxide synthase (iNOSs) enzyme producing cells, pro-inflammatory cytokines in intestinal fluids)	TNBS- induced mice	Levit et al. 2017a
Lact. plantarum CRL 2130	riboflavin	Reduction of intestinal mucositis (IM) features (diarrhea, alterations in the architecture of the small intestine, pro-inflammatory cytokines in serum and in intestinal fluids)	5-Fluorouracil (5-FU)-induced mice	Levit et al. 2018b
Lact. reuteri ATCC PTA 6475	folates	Reduction of IBD features (macroscopic intestinal inflammation, weight loss, serum amyloid protein A)	TNBS- induced mice	Thomas et al. 2016
Strep. thermophilus CRL 808	folates	Reduction of IM features (diarrhea, alterations in the architecture of the small intestine, pro- inflammatory cytokines in serum)	5-FU- induced mice	Levit et al. 2018a

Strep. thermophilus 34v and Lact. plantarum16cv / fermented milk	folates	Improve of hematological parameters and villi height/crypt depth ratio in the small intestine	Folates-depleted mice	Cucick et al. 2020
Lact. plantarum CRL 2130, *Strep. thermophilus* CRL 808 and *Strep. thermophilus* CRL 807	riboflavin-folates	Reduction of chronic IBD features (intestinal inflammation, and pro- inflammatory cytokines in intestinal fluids) and prevention of side effects of chronic anti- inflammatory therapy	TNBS- induced mice	Levit et al. 2019
Lact. plantarum CRL 2107 and *Lact. plantarum* CRL 1964 / fermented pasta	riboflavin-folates	Normalization of the intestinal morphology (number and length of villi)	Folates and riboflavin-depleted mice	Carrizo et al. 2020



Purine metabolism

Guanosine triphosphate (GTP)

1 *rib A*

2,5-Diamino- 6-ribosilamino-
4(3H)- pyrimidone- 5 ‘-phosphate

2 *rib G*

5-Amino-6-ribosilamino-2,4
(1H, 3H) pyrimidinedione 5 ‘phosphate

3 *rib G*

5-Amino-6-ribitylamino-2,4
(1H, 3H) pyrimidinedione 5 'phosphate

5-Amino-6-ribitylamino-2,4
(1H, 3H) pyrimidinedione

Pentose phosphate pathway

Ribulose--5-phosphate

4 *rib A*

3,4-Dihydroxy-2-butanone-4-phosphate

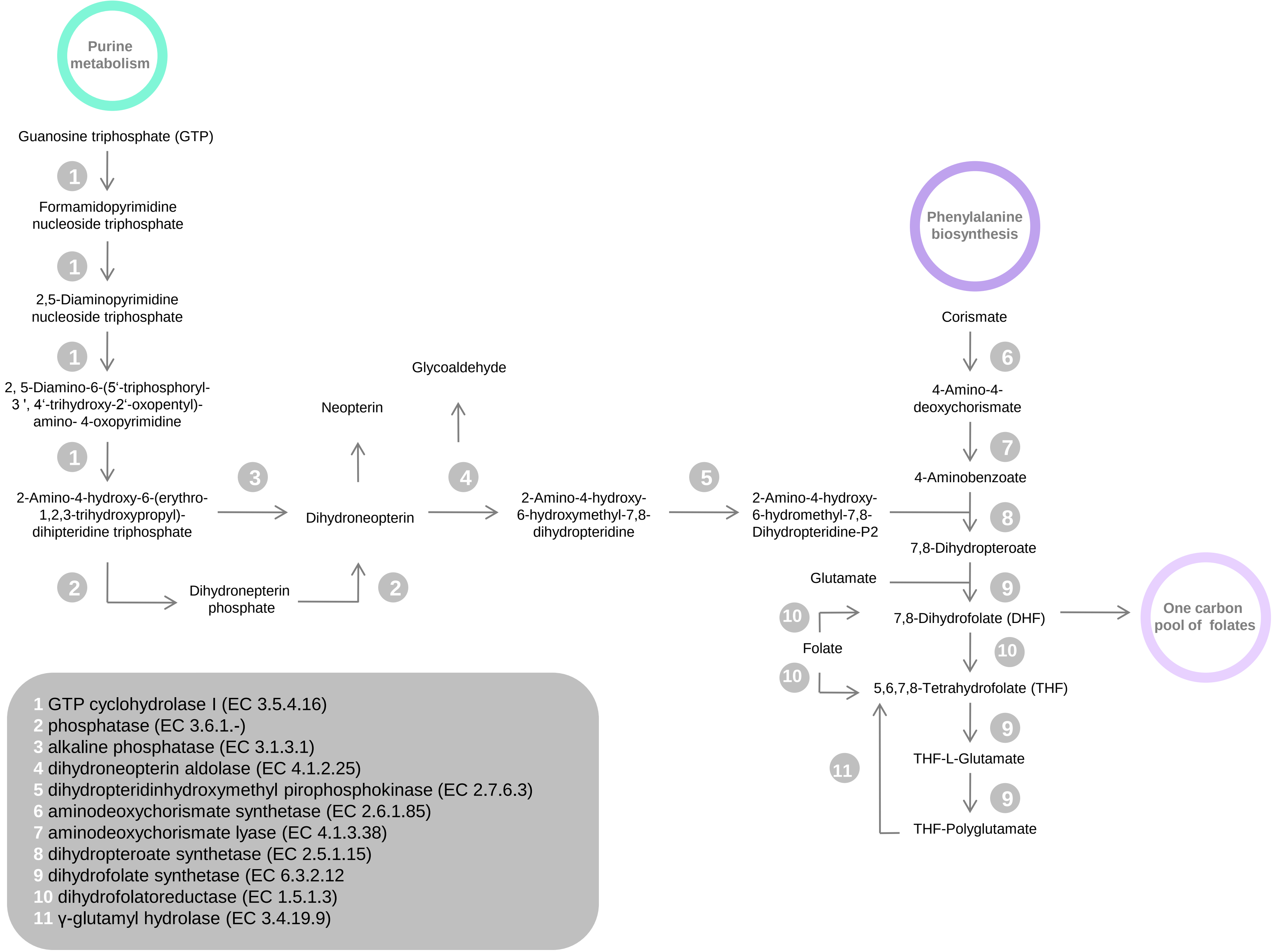
5 *rib H* *rib B*

6,7-Dimethyl-8-ribitillumazine

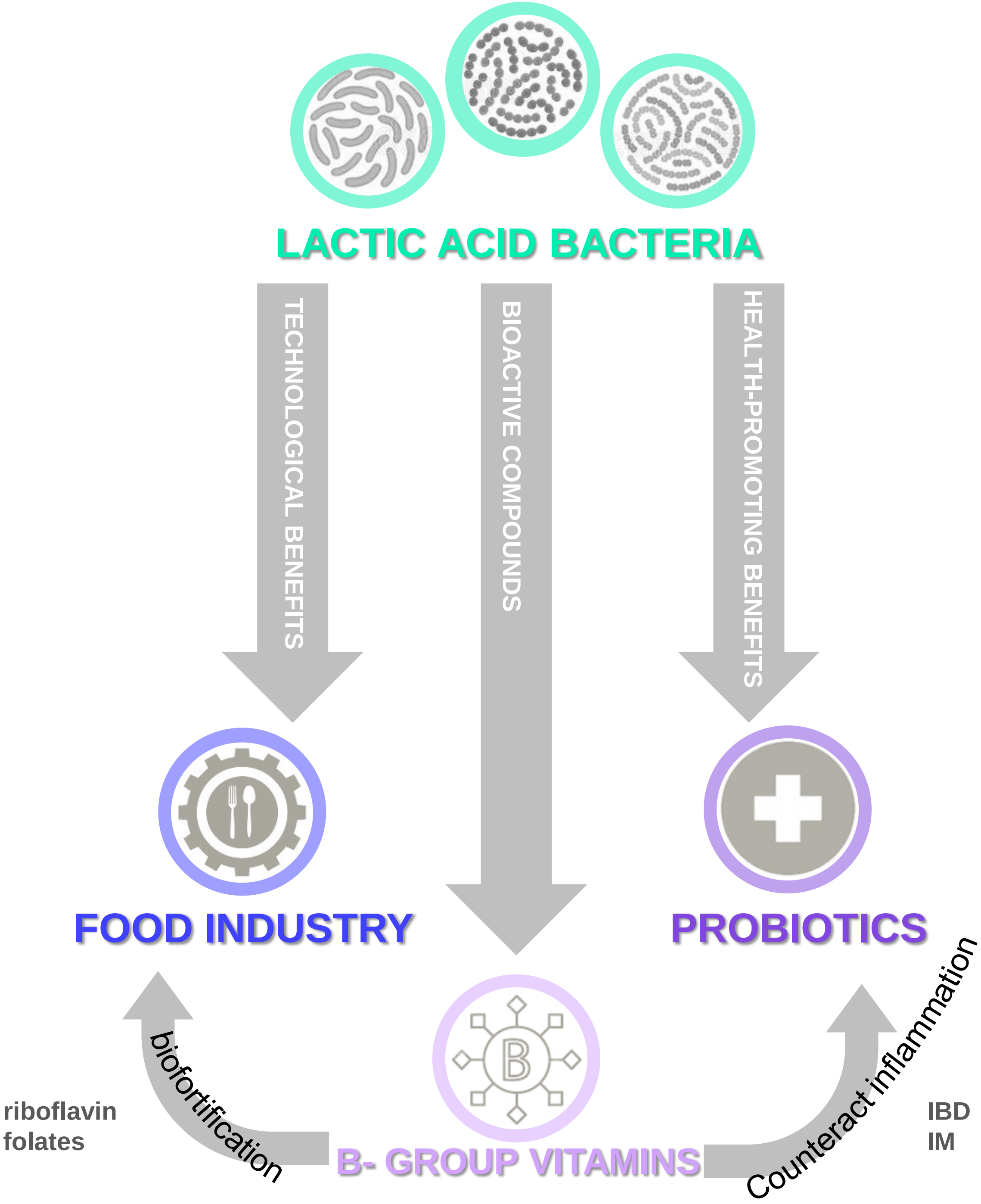
6

Riboflavin

1 cyclohydrolase II
2 desaminase
3 reductase
4 3,4-dihydroxy-2-butanone-4-phosphate synthase
5 lumazine synthase
6 riboflavin synthase



1 GTP cyclohydrolase I (EC 3.5.4.16)
2 phosphatase (EC 3.6.1.-)
3 alkaline phosphatase (EC 3.1.3.1)
4 dihydroneopterin aldolase (EC 4.1.2.25)
5 dihydropteridinhydroxymethyl pirophosphokinase (EC 2.7.6.3)
6 aminodeoxychorismate synthetase (EC 2.6.1.85)
7 aminodeoxychorismate lyase (EC 4.1.3.38)
8 dihydropteroate synthetase (EC 2.5.1.15)
9 dihydrofolate synthetase (EC 6.3.2.12
10 dihydrofolatoreductase (EC 1.5.1.3)
11 γ-glutamyl hydrolase (EC 3.4.19.9)


LACTIC ACID BACTERIA
TECHNOLOGICAL BENEFITS
BIOACTIVE COMPOUNDS
HEALTH-PROMOTING BENEFITS
FOOD INDUSTRY
PROBIOTICS
B
biofortification
riboflavin
folates
B- GROUP VITAMINS
Counteract inflammation
IBD
IM