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Current Perspectives on Antihypertensive Probiotics

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Abstract Hypertension is a major risk factor for cardiovascular diseases. Optimizing blood pressure results in an overall health outcome. Over the years, the gut microbiota has been found to play a significant role in host metabolic processes, immunity, and physiology. Dietary strategies have therefore become a target for restoring disturbed gut microbiota to treat metabolic diseases. Probiotics and their fermented products have been shown in many studies to lower blood pressure by suppressing nitrogen oxide production in microphages, reducing reactive oxygen species, and enhancing dietary calcium absorption. Other studies have shown that hypertension could be caused by many factors including hypercholesterolemia, chronic inflammation, and inconsistent modulation of the renin-angiotensin system. This review discusses the antihypertensive roles of probiotics and their fermented products via the reduction of serum cholesterol levels, anti-inflammation, and inhibition of angiotensin-converting enzyme. The ability of recombinant probiotics to reduce high blood pressure has also been discussed.

Keywords Hypertension · Microbiota · Renin-angiotensin system · Inflammation · Cholesterol

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Introduction

Hypertension (high blood pressure) is an important disease characterized by a sustained systolic blood pressure (BP) value of ≥140 mmHg and a diastolic pressure of ≥90 mmHg (140/90) in young persons. Meanwhile, BP increases with age and hence only elderly people ≥60 years with BPs above 150/90 mmHg may require treatment [1]. Many obese persons have high BP. In obesity, the increased visceral adiposity may physically compress the kidneys leading to impaired renal-pressure natriuresis and high BP [2]. Therefore, control of BP in obesity requires that the body mass index is first reduced [3, 4]. If left untreated, hypertension can lead to insufficient blood supply to vital organs, which can cause myocardial infarction, stroke, and eventually death. Common treatment regimens are aimed at reducing BP which eventually reduces the associated risks [5]. Current guidelines for managing arterial BP involve proper life style measures such as exercise and diet (reduced salt intake and low fat diets rich in vegetables) [6, 7]. Hypertension may be primary or secondary. The causes of primary hypertension, which accounts for about 95% of all hypertensive cases, remain elusive [8]. However, secondary hypertension may be as a result of pregnancy, diseases such as Cushing's syndrome, kidney malfunction as well as a side effect of various drugs. Several risk factors that increase the risks of primary hypertension include hypercholesterolemia, inflammation, sleep apnea, and obesity [9]. A number of pathways such as the fluid and electrolyte balance pathway, the renin-angiotensin system (RAS), the kinin-kallikrein system, the neutral endopeptidase system, and the endothelin-converting enzyme system are known to control human BP [10]. Of the physiological mechanisms of hypertension, the renin-angiotensin system has attracted much scientific attention. The RAS is maintained by two proteases, renin, and angiotensin-converting enzyme (ACE). Renin (EC

3.4.23.15) hydrolyzes the Leu10-Val11 peptide bond of angiotensinogen (a 55-kDa protein produced in the liver) to produce angiotensin I (Ang I), an inactive decapeptide. ACE (EC 3.4.15.1), a transmembrane metallopeptidase, then cleaves a dipeptide from the C-terminal of angiotensin I to produce angiotensin II (Ang II) [11]. Ang II then binds to angiotensin type 1 (AT_1) receptors to cause vasoconstriction in vascular smooth muscle cells (VSMC) or to angiotensin type 2 receptors (AT_2) in endothelial and VSMC to cause vasodilation by triggering the release of nitric oxide (NO), a vasodilator [10]. In disease conditions, the activity of renin and/or ACE may increase to cause an increase in BP. Also, pathologic conditions may upregulate AT_1 to reduce NO production leading to elevated BP [12]. Alternatively, ACE inactivates bradykinin (a nanopeptide vasodilator) by cleaving a dipeptide from the C-terminal [13]. Active bradykinin binds to its receptors (B1 and B2) to induce NO generation. Over the years, several studies have demonstrated the role of gut microbiota in the maintenance of physiological homeostasis such as BP [14]. Different studies have shown that imbalances in the richness, the reciprocal abundance, and the presence and/or localization of normal gut bacteria species are associated with hypertension. The changes result in a decrease in acetate- and butyrate-producing bacteria and a marked increase in Firmicutes/Bacteroidetes ratio [14, 15]. This has raised scientific interest about the use of dietary intervention to correct gut microbiota disturbances and to control high BP. Probiotics are known to exert health effects when administered in adequate quantities. Studies on the ability of probiotic bacteria alone (e.g., probiotic capsules) [6, 16, 17] and the ability of probiotics in combination with their fermented products to control high BP [18] have shown positive effects. Recent studies have discovered new functional properties of probiotics that affect BP. Probiotic *Lactobacillus rhamnosus* GG, *Lactobacillus helveticus*, *Lactobacillus gasseri*, *Lactobacillus reuteri*, and *Bifidobacterium* have been found to induce NO production in microphages when the bacteria are present in adequate quantities [19, 20]. They therefore enhance vasodilation and could reduce high BP. Other probiotics such as VSL#3 (a cocktail of *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subsp. *Bulgaricus*, *Bifidobacterium longum*, *Lactobacillus Bifidobacterium breve*, *Bifidobacterium infantis*, and *Streptococcus salivarius* subsp. *Thermophilus*) and *L. breves* have been found to reduce the levels of polyamines in tissues [21, 22]. Reduction of polyamines in the vasculature is known to reduce BP. Another mechanism by which probiotic bacteria may decrease BP is by their antioxidant abilities. *Lactobacillus fermentum* E-3 and E-18, *L. gasseri*, and *S. thermophilus* produce superoxide dismutase [23–25] while *S. thermophilus* 821, *B. longum* 15708, *L. plantarum* KCTC 3099, *L. helveticus* CD6, and *L. rhamnosus* GG have strong metal chelating abilities [26, 27]. These properties may enable probiotics to regulate vascular contraction and relaxation. Added to these, it has been reported that probiotics enhance dietary calcium ion absorption in the gut. They produce short chain fatty acids and lactic acid which reduce gut pH and increase dietary calcium solubility and absorption [28]. Other probiotics such as *L. breves* enhance dietary transepithelial calcium transport by increasing TRPV6 (a membrane calcium channel) expression in the gut [29]. In hypertensive patients, dietary calcium absorption leads to a calcium-induced suppression of renin and inhibition of extracellular calcium uptake resulting in a lowered BP [30].

Since controlling the risk factors associated with primary hypertension is critical in preventing and/managing the disease, the following section discusses our current knowledge on the roles of probiotics and their fermented products in controlling cholesterol levels, inflammation, and the renin-angiotensin system in the effort to reduce hypertension. The potential of recombinant antihypertensive probiotics to reduce BP has also been discussed.

Probiotics, Hypercholesterolemia, and Hypertension

There is a large pool of evidence to show that high BP and high total cholesterol are linked [31–33]. In fact, a positive cross-sectional relation has been found between dietary cholesterol intake and systolic BP (SBP) as well as diastolic BP (DBP) [31]. Hypertension is more prevalent in hypercholesterolemic subjects relative to normolipid subjects. Also, high BP has been found to be induced when the total blood cholesterol level exceeds 6.4 mmol/l [8]. Excess blood cholesterol is deposited on the arterial walls making them hardened and narrowed with cholesterol plaque [34]. The heart therefore strains to pump blood through the narrowed arteries and BP becomes abnormally high. In a study to investigate the ability of serum cholesterol to independently affect BP levels, Ferrara et al. [35] examined 73 patients with sustained newly discovered and never-treated hypertension. After grouping the patients according to their serum cholesterol levels, they observed that BP at rest and during 24-h monitoring was similar in the three groups but increased with increasing serum cholesterol during sympathetic stimulation. Also, intima-media layer of the carotid arteries was significantly thickened in the groups with higher cholesterol levels relative to those of lower cholesterol levels. These results indicate that cholesterol levels alone can influence BP.

Over the years, metabolic disease has been shown to have a direct link with a shift in the balance of the microbiota [36–38]. Many studies have demonstrated the cholesterol-lowering effects of probiotic consumptions in humans [39–42] which may consequently lower the risk of high BP. In a randomized controlled trial, administration of *L. reuteri* NCIMB 30242 capsules to 127 volunteers decreased LDL-cholesterol by 11.64% ($P < 0.001$), total cholesterol by

9.14%, ($P < 0.001$), non-HDL-cholesterol by 11.30% ($P < 0.001$), and apoB-100 by 8.41% ($P = 0.002$) relative to placebo [43]. Also in a double double-blind, placebo-controlled, randomized, parallel-arm, multi-center study, a yogurt formulation of *L. reuteri* NCIMB 30242 reduced LDL-cholesterol by 8.92% ($P = 0.016$), total cholesterol by 4.81% ($P = 0.031$), and non-HDL-cholesterol by 6.01% ($P = 0.029$) over placebo [44]. Though the actual mechanism by which probiotics reduce cholesterol is not clear, a few hypotheses have been proposed. Probiotics from the genera *Lactobacillus*, *Lactococcus*, and *Bifidobacterium* have been found to express bile salt hydroxylases (BSH) which may reduce serum cholesterol levels in humans [45, 46]. BSH hydrolyzes conjugated bile acids to liberate free primary bile acids which are excreted in feces [47]. Probiotics with active BSH may therefore reduce cholesterol levels by increasing free bile salt production from cholesterol in their colonized area thus reducing cholesterol-associated problems. However, since overproduction of bile salts can lead to bile acid malabsorption, gastrointestinal problems, and gallstones [48], extensive in vivo studies are required to ascertain the safety of probiotic BSH cholesterol reduction. Other probiotics such as *L. acidophilus*, *L. bulgaricus*, and *L. casei* ATCC 393 possess both intracellular and extracellular cholesterol reductase with which they reduce cholesterol into coprostanol [49]. Probiotics also metabolize prebiotics (nondigestible food ingredients that selectively stimulate the growth and/or activity of one or a limited number of resident colonic bacterial species) to produce short chain fatty acids (SCFAs) which play an important role in cholesterol reduction. For instance, Marcil et al. [50] reported that butyrate may inhibit cholesterol biosynthesis by inhibiting DL-3-hydroxy-3-methyl-glutaryl-CoA reductase activity. Ooi et al. [51] in a randomized, double-blind, placebo-controlled, and parallel-designed study observed that 12 weeks consumption of *L. gasseri* CHO-220 and inulin reduced total cholesterol by 7.84% and low-density lipoprotein cholesterol by 9.27% in 32 hypercholesterolemic men and women. The probiotics and the SCFAs produced from inulin may have played vital roles in cholesterol reduction in the patients. Several other hypotheses such as binding of cholesterol to probiotic cellular surface and subsequent incorporation into their cell membrane to influence their membrane fluidity [52] and coprecipitation of cholesterol with deconjugated bile have been proposed as possible mechanisms by which probiotics reduce cholesterol and prevent cholesterol-associated diseases such as high BP [45]. Since reduction in total and low-density lipoprotein cholesterol reduce BP [53, 54], the administration of cholesterol-lowering probiotics may be effective in protecting against or reducing high BP. However, it is important that the mechanism by which these probiotics reduce cholesterol levels in humans be established in light of the host-microbial crosstalk and other biochemical networks that underlie BP.

Probiotics, Inflammation, and Hypertension

Several studies have suggested the role of inflammation in the pathophysiology of hypertension in both human and experimental animal models [55–57]. Even though the relationship between inflammatory cytokines and hypertension is inconstant among different ethnic groups [58, 59], there is still reason to believe that inflammation and hypertension may be linked. Sessol et al. [60] followed a study involving 20,525 females (≥45 years) for a median of 7.8 years and observed that 5365 of the participants developed hypertension and these were those who had high levels of C-reactive protein (CRP), an inflammatory cytokine. It was also observed that CRP was significantly linked with a high risk of developing hypertension even in individuals with very low levels of baseline BP (<140/<90 mmHg) and no traditional CVD risk factors. Also, Niskanen et al. [61] after following 379 middle-aged men with no evidence of diabetes or hypertension at baseline for 11 years reported that subjects with CRP levels ≥3 mg/l were 3.6 times more likely to develop hypertension than men with levels ≤1.0 mg/l (P 0.001). Many other markers of vascular inflammation and thrombosis such as IL-6, TNF-α, endothelin-1, and ICAM-1 have been shown to have a positive correlation with hypertension [62]. A potential mechanism by which inflammation may promote hypertension is by causing endothelial dysfunction. Endothelial dysfunction can lead to an increase in systemic vascular resistance and reduce nitric oxide availability [63] resulting in increased BP. In another study to find any association between inflammation and hypertension, Guzik et al. [64] found that mice lacking T and B cells (RAG-1 deficient mice) do not develop hypertension after Ang II and desoxycorticosterone acetate salt infusion. However, adoptive transfer of T cells but not B cells restored the hypertensive effect in the mice. By mRNA analysis, T and natural killer (NK) cells have been shown to express renin, the renin receptor, angiotensinogen, and ACE. T cells also express AT_1 and AT_2 receptors [65] and hence, a direct relationship may exist between inflammation and high BP. Trott et al. [66] have suggested how inflammation and high BP may be related. They hypothesized that the RAS, oxidative stress, salt, and other hypertensive stimuli may cause protein modification and the modified proteins may serve as neoantigens. The neoantigens cause the activation of T cells and T cell-derived signals to promote the entry of inflammatory cells into blood vessels and the kidneys resulting in the release of cytokines. Together with water and salt retention in the kidney, T cells promote vasoconstriction in the blood vessels and this can elevate BP. Many different studies have however reported a relationship between gut dysbiosis and inflammation [67, 68]. An increase in the levels of Veillonellaceae, Enterobacteriaceae, Pasteurellacaea, and Fusobacteriaceae and a decrease in Erysipelotrichales, Bacteroidales, and Clostridiales levels have been shown to be strongly linked

with inflammation [69, 70]. Therefore, many studies have conducted to show the potential of probiotics to mitigate the condition. Probiotics such as *B. infantis* 35624 are known for their ability to induce T regulatory (T_{REG}) cells [71]. T_{REG} cells maintain tolerance to self-antigens and their depletion can result in inflammation and autoimmune diseases [72, 73]. Probiotic *B. infantis* promotes an increased production of $CD25^{+}Foxp3^{+}$ lymphocytes in murine models which protect against lipopolysaccharide or pathogen-induced NFκB activation [74]. *B. infantis* also stimulates human dendritic cells and selectively enhances the upregulation of Foxp3 expression in naïve lymphocytes [74]. It has also been reported that *B. infantis* induced the production of high levels of $Foxp3^{+}$ T_{REG} cells and interleukin-10 (IL-10) within peripheral blood of human volunteers who consumed *B. infantis* [75] and hence may reduce inflammations. A combination of *L. casei*, *Bifidobacterium breve*, and galactooligosaccharides [76] and *B. longum* (alone) [77] have also been shown to reduced serum CRP levels and improve the overall clinical appearance of patients with chronic inflammation. Administration of *L. acidophilus* ATCC 4356 [78], *L. helveticus* NS8 [79], and *L. rhamnosus* (LGG) [80] has also been reported to increase the production of IL-10 while inhibiting the production of proinflammatory cytokines in murine models, and this may play a role in inhibiting the onset of hypertension. Recently, Gomez-Guzman et al. [6] observed that administration of probiotics (*L. fermentum* CECT5716 (LC40) or *Lactobacillus coryniformis* CECT5711 (K8) plus *L. gasseri* CECT5714 (LC9) (1:1)) for 5 weeks reduced vascular reactive oxygen species levels in spontaneous hypertensive rats (SHR) by reducing NADPH oxidase activity. The probiotic treatment also significantly improved endothelial relaxation induced by acetylcholine in SHR rats and resulted in a reduction in SBP (13.4 ± 1.9 and 14.7 ± 1.9%) by LC40 and K8/LC9, respectively, with no significant changes in the heart rate. They also observed significant increase in the levels of *Lactobacillus* sp. and reduced the numbers of *Bacteroides* and *Clostridium* sp. relative to the control group indicating the ability of the probiotics to promote an increase in the levels of certain beneficial bacteria required for lowering BP. Though many probiotics are known to have immunomodulatory effects, the effects may be strain specific [81]. Therefore, it is important that probiotics that trigger specific immune response involved in BP regulation be identified and developed for managing hypertension.

Probiotics, RAS, and Hypertension

The health benefits and clinical effects associated with probiotic fermented foods have been known for ages. Most studies on the ability of probiotics to reducing BP have been elucidated through fermentation of food products in order to release bioactive peptides, such as the ACE inhibitory peptides that play a crucial role in inhibiting the RAS (Table 1). Probiotic fermented foods tend to be more effective in significantly reducing SBP or DBP compared to probiotics alone [17]. This is probably because some of the biopeptides released through fermentation are also active against high BP [18, 83, 84] and hence the combined effect is higher than that observed from probiotics alone. Nevertheless, even a small reduction in high BP can have significant health implications and cardiovascular consequences [85]. The fermentation method exploits the proteolytic systems of probiotic bacteria to hydrolyze food proteins and to release bioactive peptides (Fig. 1). Food-derived antihypertensive peptides may be safe [86] and have no side effects as those caused by synthetic drugs. Synthetic antihypertensive drugs are known to cause dysgeusia, dizziness, headache, angioedema, and cough [87, 88].

Lactic Acid Bacteria Proteolytic Systems

Lactic acid bacteria (LAB) possess cell-envelope proteinase (CEP) with which they initiate milk protein hydrolysis into oligopeptides [89]. CEPs are serine proteases and belong to the subtilisin family. They are anchored to the cell wall via sortase A (SrtA). The type of CEP in LAB may be strain and specie dependent. However, the most abundant CEP in LAB is *prtH*3 and is present in over 80% of LAB strains followed by *prtH* and *prtH4* [90]. The CEP genes in lactobacilli are genome encoded while those in lactococci are either genome or plasmid encoded. CEPs are synthesized as preproteins of about 2000 residues with several functional domains: a prepro (PP) domain, A, B, helix (H), S domains, a catalytic serine protease domain (PR), and a cell wall spacer domain (W) [91, 92]. CEP activation requires the maturation of PrtM (PrtM1 and PrtM2). PrtM1 is required for PrtH activation while PrtM2 is involved in the activation of other CEPs [90].

Peptides produced by CEP hydrolysis are transported into LAB cells for further hydrolysis (Fig. 1). Dipeptides are transported by Opp transport systems, tripeptides, and tetrapeptides (containing hydrophobic branched-chain amino acids) by Dpp transport systems while oligopeptides (hydrophilic and charged) are transported by the DtpT transport system [93]. Only one peptide transporter (DtpT) has however been identified in *L. reuteri* [94]. Various peptidases in LAB cells hydrolyze the absorbed peptides to release essential amino acids.

Four main LAB endopeptidases have been characterized namely PepO, PepF, PepG, and PepE [93]. PepO is a monomeric metalloprotease that can hydrolyze peptides from 5 up to 35 amino acid residues. Three paralogous genes encode PepO namely *pepO*, *pepO2*, and *pepO3*. PepF hydrolyzes peptides containing 7~17 amino acids and has a broad

Table. 1 In vivo studies on the effects of probiotic fermented foods on blood pressure

Product	Subjects	Study design	ACE inhibitory peptides[a]	Blood pressure reduction	Reference
L. helveticus fermented milk, 150 ml/day for 21 weeks	39 mild hypertensive; age 30–62 years	Randomized, placebo-controlled	VPP, IPP, TP, AGSAP, IPAVP, APLRV, AHKAL	SBP: 6.7 mmHg, DBP: 3.6 mmHg	[109, 110]
S. thermophiles fermented yogurt, 450 ml/day	50 hypertensive females, 18–55 years	Randomized, placebo-controlled trials	NR	SBP: 8.0 ± 2.3 mmHg, DBP: 4.0 ± 2.3 mmHg	[111]
L. helveticus fermented sour milk, 150 ml/day; first period 8–10 weeks, washout period 3–4 weeks, second period 5–7 weeks	60 (first period)/39 (second period) mild hypertension; age not available	Two-cross over trial periods with a washout period in between	VPP, IPP, TP, AGSAP, IPAVP, APLRV, AHKAL	First period: SBP: 16 mmHg, second period: DBP: 11 mmHg	[110, 112]
L. helveticus in tablets containing powdered fermented milk, 6 tablets/day for 4 weeks	40 high-normal blood pressure, 40 mild hypertension; age not available	Randomized, double-blind, placebo-controlled	VPP, IPP, TP, AGSAP, IPAVP, APLRV, AHKAL	SBP: 5.0 mmHg, DBP: 11.2 mmHg	[110, 113]
L. helveticus fermented milk, 160 g/day for 4 weeks	46 borderline hypertensive; age 23–59 years	Randomized, double-blind, placebo-controlled	VPP, IPP, TP, AGSAP, IPAVP, APLRV, AHKAL	SBP: 5.2 mmHg	[110, 114]
L. casei strain Shirota and *L. lactis* YIT 2027, 100 ml of fermented milk containing GABA daily 12-week period	39 mildly hypertensive patients (16 women and 23 men) aged 28–81 years (mean, 54.2 years)	Randomized, placebo-controlled, single-blind trial	DKIHPF, NVPGEIV, KVLPVPE, VIGSPPEN, SPPEIN	SBP: 17.4 ± 4.3 mmHg, DBP: 7.2 ± 5.7 mmHg	[115]
L. plantarum A7, 200 ml/day of probiotic soy milk	40 hypertensive patients, 35–68 years	Randomized, double-blind, controlled clinical trial	DVWY, FDART, FQ, VAE, VVG, WTFR	SBP: 14.7 ± 0.48 mmHg, DBP: 10 ± 0.7 mmHg	[82, 116]
L. helveticus and *S.cerevisiae* fermented milk, 95 ml daily for 8 weeks	30 elderly hypertensive patients. Years not reported	Randomized, placebo-controlled	VPP, IPP, TP, AGSAP, IPAVP, APLRV, AHKAL	SBP: 14.1 ± 3.1 mmHg, DBP: 6.9 ± 2.2 mmHg	[117, 118]
L. helveticus fermented milk, 150 ml twice daily for 10 weeks after a 4-week run-in period	94 hypertensive patients, 51–55 years	Randomized, double blinded placebo-controlled parallel group study	VPP, IPP, TP, AGSAP, IPAVP, APLRV, AHKAL	SBP: 4.1 ± 0.9 mmHg, DBP: 1.8 ± 0.7 mmHg	[110, 119]
L. casei TMC0409 fermented milk supplemented with whey protein concentrate. 200 ml of fermented milk, twice daily for 8 weeks *S. thermophilus* TMC 1543 fermented milk supplemented with whey protein concentrate 200 ml of fermented milk twice daily for 8 weeks	20 healthy adult men between 30 and 51 years (mean 40.1 years)	NR	NR	Significant reduction in SBP ($P < 0.05$)	[120]
L. plantarum TENSIA™, 50 g of probiotic cheese per day for 3 weeks	40 hypertensive subjects, 30–69 years	Randomized blinded controlled parallel three-arm study	DVWY, FDART, FQ, VAE, VVG, WTFR	Morning SBP: 12.2 ± 1.5 mmHg, DBP: 4.0 ± 0.9 mmHg; evening SBP: 8.8 ± 0.9 mmHg, DBP: 1.6 ± 1.2 mmHg	[121]

A alanine, *R* arginine, *N* asparagine, *D* aspartic acid, *C* cysteine, *E* glutamic acid, *Q* glutamine, *G* glycine, *H* histidine, *I* isoleucine, *L* leucine, *K* lysine, *M* methionine, *F* phenylalanine, *P* proline, *S* serine, *T* threonine, *W* tryptophan, *Y* tyrosine, *V* valine, *NR* not reported

[a] The ACE inhibitory peptides were identified in separate studies using the same probiotic strain species

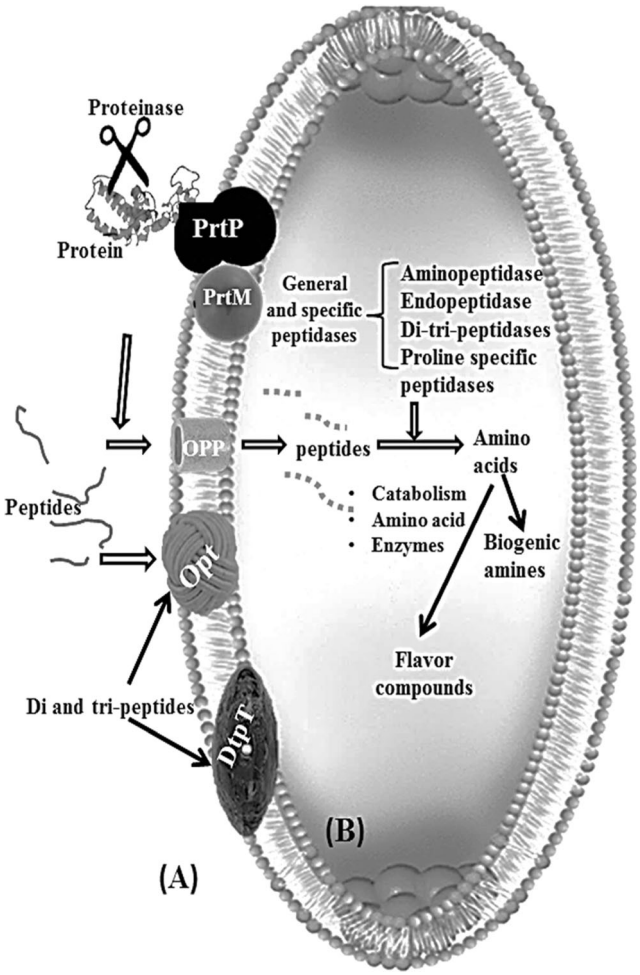

Fig. 1 The LAB proteolytic systems. (*A*) Extracellular components: *PrtP* (a cellular envelope proteinase) requires *PrtM*, (proteinase maturation protein) for maturation; *Opp* (an oligopeptide permease) transports oligopeptides into the cell; *DtpT* (an ion linked transporter for di- and tripeptides), and *Opt* (an ABC transporter for peptides). (*B*) Intracellular peptidases: general (PepN, PepC) and specific (PepX, PepQ) peptidases and amino acid catabolic enzymes (carboxylase, aminotransferases, etc.)

specificity. It is encoded by pepF, pepF1, and pepF2. Two paralogous genes have been reported for PepE in lactobacilli (*pepE* and *pepE2*). PepG and PepE are however absent in lactococci and streptococci [90]. Four LAB exopeptidases have been identified based on their specificities. They are aminopeptidases, dipeptides, tripeptidases, and proline-specific proteases. There are three classes of aminopeptidases based on their specificities (broad specificity, specific aminopeptidases for acidic or basic amino acids, and those specific for hydrophobic or aromatic residues). PepC and PepN are aminopeptidases with broad specificity and are present in all genomes. PepC, a member of the C1 family of cysteine peptidases, is specific for basic, acidic, hydrophobic/uncharged, and aromatic residues. Some studies have shown that antihypertensive peptides containing aromatic amino acids at the C-terminus and those with hydrophobic side chains have enhanced effects [95]; therefore, overexpressing the *pepC* gene in lactic acid bacteria could yield large amounts of ACE inhibitory peptides when used to ferment high protein foods. PepN on the other hand preferentially hydrolyzes basic residues followed by hydrophobic or uncharged residues [93].

The aminopeptidase PepS preferentially hydrolyzes aromatic residues and has been identified in *Pediococcus pentosaceus*, *S. thermophilus*, *Leuconostoc mesenteroides*, *L. casei*, and *Lactobacillus sakei*. PepA (glutamyl aminopeptidases) prefers to hydrolyze Glu and Asp residues. PepA has been identified in *Lactobacillus*, *Streptococci*, and *Lactococcus* but absent in *Pediococcus* and *Oenococcus* strains [89]. LAB tripeptidases usually have broad specificities for tripeptides but preferentially hydrolyze those containing hydrophobic amino acids. They however do not cleave tripeptides with proline residues. The tripeptidase PepT is found in all LAB strains and the *pepT* gene may occur as two paralogous genes in some strains such as *L. acidophilus*, *L. gasseri*, *Lactobacillus johnsonii*, and *Lactobacillus sanfranciscensis* [96]. Good quantities of isoleucyl-prolyl-proline (IPP) and valine-prolyl-proline (VPP) (the most popular anti-ACE peptides) may therefore be obtained in food fermented with LAB with overexpressed *pepT* genes or by treating the substrates with PepT tripeptidases. IPP and VPP have been shown to be resistant to gastrointestinal digestion and significantly reduce BP in both animal and humans [97]. The rigid structure of proline has been described to lock the carboxyl group into a conformation favorable for interaction with the positively charged residue at the active site of ACE to cause inhibition [98]. Therefore, overexpression of PepT in LAB could also yield other short peptides with proline at their C-terminus with anti-ACE activities. LAB also possess dipeptidases that cleave only dipeptides into amino acids as shown in Fig. 1. These peptidases may be highly specific or have broad specificity. Peptidases in the PepD and PepV families hydrolyze a large variety of dipeptides. *PepD* genes are heterogeneously distributed in LAB genomes. The PepL dipeptidase however is highly specific for Leu and Ala residues and has only been identified in *L. delbrueckii* [94].

Proline-specific peptidases hydrolyze proline residues from the N-terminal of peptides. PepR is a broad spectrum prolinase with a broad specificity for dipeptides including Met-Ala, Leu-Leu, and Leu-Gly-Gly [99] while the proline iminopeptidase (PepI) preferentially cleaves proline residues at the N-terminal of tripeptides [100]. PepP cleaves N-terminal amino acids that are directly linked to proline residues in oligopeptides. One *pepP* gene is ubiquitous in LAB genomes except in *L. sakei* and *P. pentosaceus*. PepQ is also present in all LAB strains as a copy per genome except for *L. delbrueckii* subsp. *Bulgaricus* which have two *pepQ* paralogs. While one paralogue is located in a separate cluster, the other is clustered with other orthologues. The proline-specific endopeptidase PepX is also ubiquitous in all LAB

as one gene per genome [101, 102]. However, LAB strains from dairy environments may have two PepX peptidase homologues [94].

Although probiotics and their fermented foods reduce high BP in SHRs, results in human studies are controversial. A meta-analysis of the effects of lactotripeptides IPP and VPP showed that the lactotripeptides could significantly reduce BP in Asians (Japanese) but only slightly in Caucasians [97, 103, 104]. In these studies, however, most of the individual studies were conducted on small populations making it difficult to statistically detect small effects [104]. Studies using larger populations are required to overcome such barriers. No study has yet reported the ability of live probiotics alone to reduce high BP in humans though such studies are required to establish the direct effects of probiotics in mitigating the condition.

Recombinant Probiotics with Antihypertensive Effects

ACE inhibitory peptides released after probiotic fermentation are usually difficult to purify from the digested mixture. Also, capitalizing on the proteolytic ability of probiotics alone does not guarantee high quantities of ACE inhibitory peptides since the bacteria are living organisms and hence the type and quantity of the enzymes produced are difficult to control. This therefore makes the production of ACE inhibitory peptides by this method hardly reproducible. For these reasons, several studies have focused on producing recombinant probiotics that express ACE inhibitory peptides. Rao et al. [105] designed and expressed the antihypertensive peptide multimer AHPM by cloning the peptide sequence into the plasmid pGEX-3X and expressing it in *Escherichia coli* BL21. The recombinant AHPM fused with glutathione S-transferase (GST) and the proteins were expressed as inclusion bodies forming 35% of the total intracellular protein. A large quantity (399 mg/l) of the pure soluble GST-AHPM was obtained. They then cloned the ACE inhibitory peptide multimer AHPM-2 into pET32a and expressed it in *E. coli*. The expressed fusion Trx-AHPM-2 obtained after purification was subjected to simulated gastrointestinal digestion and the hydrolysate showed a strong ACE inhibitory activity (IC_{50} = 4.5 ± 0.3 μg/ml) [106]. Huang et al. [107] also used the plasmid pET-30a (+) bearing the anti-ACE peptide IYPR for protein expression in *E. coli* strains DH5a and BL21 (DE3). The recombinant protein accounted for 31% of the cellular protein with an IC_{50} value of 61 mg/l. The peptides reduced SBP significantly in SHRs after a single oral administration. These studies prove the possibility of producing large quantities of ACE inhibitory peptides for use as nutraceuticals using recombinant technology. Live recombinant probiotics have also been applied in BP lowering studies. In a quest to study the in vivo effect of recombinant antihypertensive probiotics, Yang et al. [16] transformed *L. plantarum* NC8 with pSIP409 plasmid-bearing ACE inhibitory peptides YFP and TFP originally obtained by chymotryptic hydrolysis of yellowfin sole (*Limanda aspera*) frame protein [108]. Rats fed with the recombinant probiotic strains had significantly reduced SBP relative to those who consumed *L. plantarum* (wild type) and PBS controls. The antihypertensive function of recombinant probiotic was maintained for at least 10 days (the SBP of the RLP-treated rats was 181.517 ± 2.312 mmHg, that of the *L. plantarum* treated rats was 195.876 ± 2.109 mmHg, and that of the PBS control rats was 197.376 ± 4.982 mmHg on the 24th day ($P < 0.05$)). Although recombinant probiotics could be effective in lowering BP, it is challenging to clone short peptides (especially di- and tripeptides). More studies are required to ascertain the possible safety concerns that may arise from consuming recombinant probiotics before they can be used in human studies.

Conclusion

The evidence that high BP is associated with gut dysbiosis makes it important to establish the ability of probiotics to reduce high BP in humans. However, though many probiotics and their fermented foods reduce high BP in SHRs, the results are conflicting in humans. Yet, the evidence that probiotics and their fermented products effectively reduce inflammation and hypercholesterolemia and affect the RAS (all of which have links with high BP) could support their application in improving cardiovascular health. Enhancing the proteolytic ability of probiotics by genetic engineering will be essential in increasing the levels of anti-ACE peptides in fermented foods. Recombinant probiotics could be a cheap and dependable source of antihypertensive peptides since the use of wild probiotics is tedious and may not be reproducible. Taken together, dietary interventions to correct gut dysbiosis and/or the consumption of fermented foods containing antihypertensive peptides could be novel nutritional therapeutic strategies for hypertension. As our knowledge about the hypotensive effects of probiotics grows, the mechanism by which they work is worth exploring. Also, a better understanding of the gut microbiota-host crosstalk and other networks underlying the control of BP will be critical in promoting the use of antihypertensive probiotics and their products.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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