Review

Potential effects of probiotics on atherosclerosis

Gerrit A. Stuivenberg[1,2] ID **, Annabel Poon[1,2], Jeremy P. Burton[1,2], J. David Spence[3]** ID

[1]Microbiology and Immunology, Western University, London N6A 3K7, Canada.
[2]The Canadian Centre for Human Microbiome and Probiotic Research, Lawson Health Research Institute, London N6A 4V2, Canada.
[3]Stroke Prevention and Atherosclerosis Research Centre, Robarts Research Institute, Western University, London N6G 2V4, Canada.

Correspondence to: Prof. J. David Spence, Stroke Prevention and Atherosclerosis Research Centre, Robarts Research Institute, Western University, 1400 Western Road, London N6G 2V4, Canada. E-mail: dspence@robarts.ca

Abstract

The rising global incidence of atherosclerosis (AS) highlights the inadequacies in our understanding of the pathophysiology and treatment of the disease. Increasing evidence outlines the importance of the intestinal microbiome in AS, wherein gut-derived uremic toxins (GDUTs) may be of concern. Plasma levels of the GDUTs trimethylamine n-oxide (TMAO), *p*-cresyl sulfate, and indoxyl sulfate are associated with accelerated renal function decline and increased cardiovascular risk. Thus, reducing the amount of GDUTs in circulation is expected to benefit patients with AS. Because some beneficial bacteria can clear GDUTs *in vitro* and *in vivo*, orally administered probiotics targeting the intestinal tract represent a promising way to bring about these changes. As such, this perspective reviews the potential use of probiotics to treat AS, particularly in patients with non-traditional risk factors and/or impaired renal function.

Keywords: Probiotics, atherosclerosis, uremic toxins, gut microbiota

INTRODUCTION

Atherosclerosis (AS) is a chronic inflammatory disease characterized by the thickening of arteries due to plaque accumulation, and narrowing of arteries due to plaque rupture and thrombosis. AS progression is strongly dependent on dyslipidemia, hypertension, and inflammatory responses, and thus, traditional risk

factors such as age, sex, diabetes, blood pressure, and smoking are thought to account for most cases. Many readers may be surprised that a probiotic could be leveraged to improve AS outcomes. However, as shown in Table 1, traditional risk factors account for only a small proportion of AS, and toxic metabolites formed by the gut microbiota emerged as strong predictors of AS severity[1], measured as carotid total plaque area (TPA)[2].

There is increasing evidence to support the role of gut microbiota in both the progression and prevention of AS. Jie *et al.* showed that the gut microbiota of AS patients distinguished them from healthy controls and could predict AS risk[3]. Microbial metabolites produced in the gastrointestinal tract, such as trimethylamine N-oxide (TMAO)[4] and the short-chain fatty acid (SCFA) propionate[5], have been linked to AS severity and protection, respectively. *Lactobacillus rhamnosus* is enriched in stable atherosclerotic plaques, suggesting it may have a role in regulating plaque stability[6]. Together, these findings highlight the ability of resident microbes to influence plaque growth and stability.

Orally delivered probiotics are an effective way of altering the metabolic flux of the gut microbiota to influence patient outcomes, because the distribution of metabolites produced by the network of gut microbes will change. For established risk factors of AS, probiotics could help equilibrate lipid metabolism, vascular function, and macrophage polarization, which would benefit patients. Thus, probiotics have promise in managing the newly emerging risk factors of AS by altering gut microbiota composition[7,8] and clearing harmful gut-derived uremic toxins (GDUTs)[9-11]. The aim of this perspective is to assess whether probiotics may improve AS, with a focus on emerging risk factors. We also provide recommendations on how to identify novel probiotic candidates.

EXTREMES OF AS HIGHLIGHT THE IMPORTANCE OF THE GUT MICROBIOTA

Carotid plaque burden is strongly associated with cardiovascular risk[12,13]. Among patients in whom TPA and levels of traditional risk factors have been measured, three phenotypes can be distinguished: Explained AS, in which plaque burden is explained by traditional risk factors; Unexplained AS, wherein AS is severe despite little risk; and Protected individuals, who have high levels of traditional risk factors, but little or no carotid plaque. Figure 1 shows the distribution of these phenotypes among 316 of 3,056 patients who attended the Stroke Prevention & Atherosclerosis Research Centre, Robarts Research Institute, London, Canada.

In that study, metabolic products of the gut microbiota that typically accumulate in individuals with renal failure, GDUTs, were assayed in plasma by ultra-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. Plasma levels of TMAO, *p*-cresyl sulfate, *p*-cresyl glucuronide, and phenylacetylglutamine were significantly lower among patients with the protected phenotype, and higher in those with the unexplained phenotype, despite no significant differences in renal function or in dietary intake of nutrient precursors of GDUT. As shown in Table 1, both TMAO and *p*-cresyl sulfate were significant predictors of TPA in a model that excluded diabetes, sex and systolic blood pressure, i.e., these intestinal metabolites were stronger predictors of TPA than those three traditional risk factors. While our study did not show a significant effect on TPA by other GDUTs, such as indoxyl sulfate, some have been implicated to promote AS, and will be discussed later in the review.

USING PROBIOTICS TO REDUCE GUT MICROBIOTA METABOLITES RELEVANT TO AS

The AS-relevant probiotic studies conducted on humans and mice are summarized in Tables 2 and 3, respectively.

Table 1. Backward linear regression of total plaque area (cube root transform)

Model summary

Model	R		R square	Adjusted R square	Std. error of the estimate
12	0.517		0.267	0.245	2.66330

Coefficients

Model	Unstandardized coefficients		Standardized coefficients	t	Sig.
	B	Std. error	Beta		
12 (Constant)	0.047	1.566		0.030	0.976
Age	0.074	0.019	0.239	3.877	< 0.001
Serum high-density lipoprotein	-1.169	0.511	-0.155	-2.288	0.023
P-cresyl sulfate	0.017	0.006	0.179	2.809	0.005
Pack-years smoking	0.041	0.009	0.280	4.569	< 0.001
Serum triglycerides	-0.497	0.185	-0.183	-2.691	0.008
TMAO	0.069	0.030	0.149	2.307	0.022

Dependent variable: cube root transform of TPA mm^2.

Excluded variables

Model	Beta in	t	Sig.	Partial correlation	Collinearity statistics
					Tolerance
12 Total choline (mg)	-0.062	-1.003	0.317	-0.071	0.955
Phenyl sulfate	-0.005	-0.085	0.933	-0.006	0.883
Diabetes mellitus	-0.005	-0.081	0.936	-0.006	0.873
Hippuric acid	0.027	0.425	0.672	0.030	0.899
Indoxyl sulfate	-0.027	-0.295	0.768	-0.021	0.450
Diastolic blood pressure	0.023	0.374	0.709	0.026	0.960
P-cresyl glucuronide	-0.001	-0.016	0.988	-0.001	0.600
Phenylacetylglutamine	0.048	0.545	0.587	0.038	0.463
Total choline, no supplements (mg)	-0.063	-1.016	0.311	-0.071	0.951
Systolic blood pressure	0.055	0.889	0.375	0.063	0.956
Sex	-0.065	-0.977	0.330	-0.069	0.809

Reproduced by permission of Elsevier from[1]. TMAO: Trimethylamine n-oxide.

TMAO

In the context of AS, perhaps the best-known intestinal metabolite is TMAO. Phosphatidylcholine[14] and carnitine[15], which have high concentrations in animal-based protein sources, are converted to trimethylamine by gut bacteria, and then oxidized in the liver to TMAO[16]. Among patients referred for coronary angiography, TMAO levels in the top quartile were associated with a 2.5-fold increase in the risk of myocardial infarction, stroke, or vascular death over 3 years[17]. The harmful effects of TMAO include thrombogenesis[18], accelerated renal function decline, and increased mortality[19]. Figure 2 shows that levels of TMAO and other intestinal metabolites are increased with even moderate impairment of renal function[20], reaching levels that are normal in older patients. As such, elevated TMAO explains, in part, the elevated risk of developing AS in patients with impaired renal function[21].

Indeed, dietary and pharmacological treatments to lower TMAO have been developed; for instance, switching from red meat to white meat or vegetarian/vegan meals significantly reduced plasma TMAO over one month[22]. Additionally, two inhibitors are currently under development: one targets choline
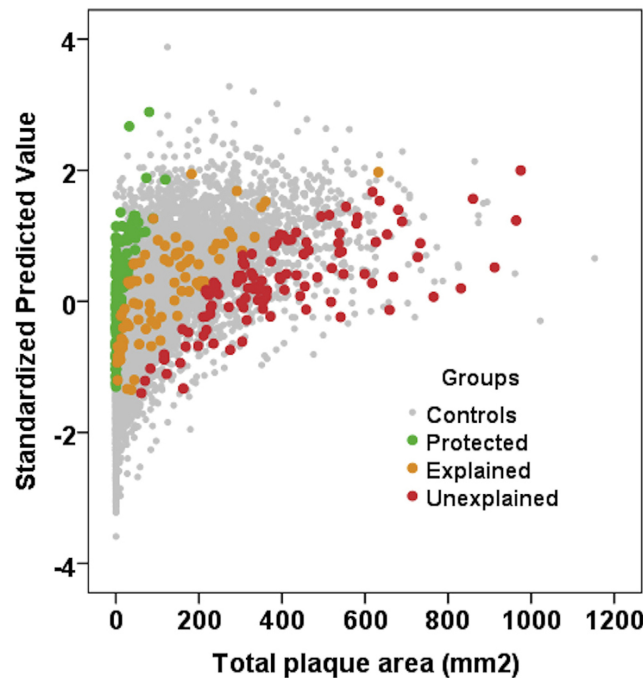
Figure 1. Distribution of the study participants among the clinic population of 3,056 patients in the linear regression model. Measured TPA is plotted against the standardized predicted value of plaque area based on the risk factors in the regression model: age, sex, diabetes, smoking status, serum creatinine, systolic and diastolic blood pressures, total cholesterol, triglycerides, LDL-C, and HDL-C. Control patients are shown as grey dots. The red dots represent study participants with residual scores > 2, i.e., they are the 5% extreme cases with unexplained AS, with more plaque than predicted by risk factors by 2 standard deviations or more. Green dots represent protected patients; these are the 5% extremes with much less plaque than predicted, with residual scores < -2; orange dots represent patients with explained AS, whose plaque burden is predicted by the risk factors, with residual scores between -2 and 2. Reproduced by permission of Elsevier from[1]. TPA: Total plaque area; LDL-C: low-density lipoprotein-C; HDL-C: high-density lipoprotein-cholesterol; AS: atherosclerosis.

trimethylamine lyase[23], a bacterial enzyme involved in generating trimethylamine from nutrients such as phosphatidylcholine and carnitine, while the other inhibits flavin-containing monooxygenase 3[24], a hepatic enzyme that converts trimethylamine into TMAO. Given the microbial origins of the metabolite, the potential for probiotics to reduce plasma TMAO is also a subject of inquiry.

It is well established that the gut microbiota composition of AS patients drastically differs from that of healthy controls. Typically, these patients present an increased abundance of *Enterobacteriaceae* and *Streptococcus* species[3]. However, additional studies have also linked subclinical markers of AS to increased levels of *Collinsella*, where the healthy controls had more *Roseburia* and *Eubacterium*[25]. In addition, bacterial nucleic acids originating from the genera *Chryseomonas*, *Veillonella*, and *Streptococcus* were detected in AS plaques, with several of these phylotypes also being identified in the gut[25,26]. Furthermore, a metagenome-wide association study revealed that AS patients differ from healthy controls not only in gut microbiome composition, but also in its functional and metabolic capacity[3]. This is particularly true for the metabolism and transport of molecules crucial for cardiovascular health, such as TMAO, taurine, and sphingolipids[3,25]. Ultimately, these microbial and functional alterations may contribute to the development and progression of AS through various mechanisms[3,25,26], which are described in greater detail throughout this perspective. Considering that one method of action of probiotics is to increase the relative abundance of certain beneficial microbes to shift the microbiome toward a composition more similar to that of healthy control[27], it is not surprising that probiotics are being considered therapeutic for AS.

Table 2. Summary of human studies using probiotics relevant to AS

Article	Strain(s)	Summary/main findings
The effect of yoghurt and its probiotics on blood pressure and serum lipid profile; a randomised controlled trial	● *Lactobacillus acidophilus* La5 ● *Bifidobacterium animalis* subsp. lactis Bb12	● 156 overweight men and women over 55 years old ○ 4 intervention groups: ■ Probiotic yogurt + probiotic capsules ■ Probiotic yogurt + placebo capsules ■ Control milk + probiotic capsules ■ Control milk + placebo capsules ■ Probiotic yogurt did not significantly change blood pressure, heart rate, or serum lipid concentrations ■ Probiotic capsules did not change blood pressure or concentrations of total cholesterol or triglycerides
Lactobacillus plantarum 299v supplementation improves vascular endothelial function and reduces inflammatory biomarkers in men with stable coronary artery disease	● Lp299v	● 20 men with stable coronary artery disease ○ Consumed drink containing Lp299v daily for 6 weeks ● Improved brachial flow-mediated dilation, endothelium-dependent vasodilation in resistance arteries ● Supplementation decreased circulating levels of IL-8, leptin ● The genus *Lactobacillus* was enriched in post-probiotic stool samples without other changes ● Lp299v improved vascular endothelial function and decreased systemic inflammation in men with coronary artery disease ○ Proposed circulating gut-derived metabolites responsible
Multispecies probiotic supplementation favorably affects vascular function and reduces arterial stiffness in obese postmenopausal women - a 12-week placebo-controlled and randomized clinical study	● *Bifidobacterium bifidum* W23 ● *Bifidobacterium lactis* W51 ● *Bifidobacterium lactis* W52 ● *Lactobacillus acidophilus* W37 ● *Lactobacillus brevis* W63 ● *Lactobacillus casei* W56 ● *Lactobacillus salivarius* W24 ● *Lactococcus lactis* W19 ● *Lactococcus lactis* W58	● 81 obese postmenopausal women aged 45-80 years ○ 3 interventions: ■ Placebo ■ Low probiotic dose ■ Original probiotic dose ● High dose intervention decreased: ○ Systolic blood pressure ○ Vascular endothelial growth factor ○ Pulse wave analysis systolic pressure, augmentation index and velocity ○ IL-6, TNFa, and thrombomodulin ● Low dose intervention decreased: ○ Systolic blood pressure ○ IL-6 ● Supplementation favorably modifies functional and biochemical markers of vascular dysfunction in obese postmenopausal women
Short Communication: effect of supplementation with *Lactobacillus casei* shirota on insulin sensitivity, β-cell function, and markers of endothelial function and inflammation in subjects with metabolic syndrome - a pilot study	● LcS	● 30 subjects supplemented with LcS for 12 weeks ● sVCAM-1 levels reduced in serum ● Did not improve parameters of insulin sensitivity, β-cell function, endothelial function, low-grade inflammation
Synbiotics easing renal failure by improving gut microbiology (SYNERGY) - a randomized trial	● Not specified (prebiotics and probiotics combined)	● Stage III and IV CKD patients ● Synbiotics administered for 6 weeks ● Reduced serum PCS but not IS ● Enriched *Bifidobacterium* in gut microbiota ● Increased albuminuria in the synbiotic arm
The influence of prebiotic arabinoxylan oligosaccharides on microbiota derived uremic retention solutes in patients with chronic kidney disease: a randomized controlled trial	● Arabinoxylan oligosaccharides	● Patients with stage III and IV CKD ● 4-week intervention with washout ● No significant changes in serum IS, PCS, or insulin resistance ● Slight reduction in TMAO levels
Probiotics-supplemented low-protein diet for microbiota modulation in patients with advanced chronic kidney disease (ProLowCKD): results from a placebo-controlled randomized trial)	● *Bifidobacterium longum* ● *Lactobacillus reuteri*	● Patients with stage III and IV CKD ● 3-month supplementation following a low-protein diet ● Probiotic group showed trends in reduced uremic toxins and proatherogenic markers ● Potential reduction in antihypertensive/diuretic use

Effects of probiotic supplementation on inflammatory biomarkers and uremic toxins in non-dialysis chronic kidney patients: a double-blind, randomized, placebo-controlled trial	● *Streptococcus thermophilus* ● *Lactobacillus acidophilus* ● *Bifidobacterium longum*	● Stage III and IV CKD patients ● Daily probiotics for 3 months ● Increased IL-6 levels
Randomized controlled trial of strain-specific probiotic formulation (Renadyl) in dialysis patients	● *Lactobacillus, Streptococcus* ● *Bifidobacterium species*	● Patients undergoing maintenance hemodialysis ● Two 2-month treatment periods with washout ● No significant efficacy confirmed due to the small sample size
Effect of short-term synbiotic treatment on plasma *p*-cresol levels in patients with chronic renal failure: a randomized clinical trial	● Not specified (synbiotic formulation)	● Patients with CKD stages 3-4 ● Synbiotic or placebo for 4 weeks ● Synbiotic lowered plasma *p*-cresol but did not affect gastrointestinal symptoms
An innovative synbiotic formulation decreases free serum indoxyl sulfate, small intestine permeability and ameliorates gastrointestinal symptoms in a randomized pilot trial in stage IIIb-IV CKD patients	● *Lactobacillus plantarum* ● *Lactobacillus acidophilus* ● *Bifidobacterium longum* ● *Bifidobacterium lactis* ● *Streptococcus thermophilus*	● CKD stage IIIb-IV patients ● Synbiotics administered for 2 months ● Reduced serum indoxyl sulfate and intestinal permeability ● Improved gastrointestinal symptoms
Effects of probiotics, prebiotics, and synbiotics on uremic toxins, inflammation, and oxidative stress in hemodialysis patients: a systematic review and meta-analysis of randomized controlled trials	● Various strains (review/meta-analysis of RCTs)	● Systematic review and meta-analysis of 23 RCTs on probiotics, prebiotics, and synbiotics in hemodialysis patients ● Decreased PCS, endotoxins, and inflammatory markers ● Improved antioxidant capacity
A randomized double-blind placebo-controlled trial of probiotics in post-surgical colorectal cancer	● *Lactobacillus acidophilus* BCMC 12,130 ● *Lactobacillus lactis* BCMC 12,451 ● *Lactobacillus casei* BCMC 12,313 ● *Bifidobacterium longum* BCMC 02120 ● *Bifidobacterium bifidum* BCMC 02290 ● *Bifidobacterium infantis* BCMC 02129	● 52 post-surgical colorectal cancer patients ● Randomized to receive placebo or probiotics for 6 months ● Reduced pro-inflammatory cytokines (TNF-α, IL-6, IL-10, IL-12, IL-17A, IL-17C, IL-22) in the probiotic group ● No significant effect on IFN-γ
Vitamin D and probiotic co-supplementation affects mental health, hormonal, inflammatory and oxidative stress parameters in women with polycystic ovary syndrome	● *Lactobacillus acidophilus* ● *Bifidobacterium bifidum* ● *Lactobacillus reuteri* ● *Lactobacillus fermentum*	● 60 women with polycystic ovary syndrome ● Randomized to receive Vitamin D + probiotics or placebo for 12 weeks ● Significant improvements in mental health (BDI, GHQ, DASS) ● Reduction in total testosterone, hirsutism, hs-CRP, MDA ● Improvement in TAC and GSH levels in the treatment group

AS: Atherosclerosis; Lp299v: *Lactobacillus plantarum* 299v; LcS: *Lactobacillus casei* Shirota; CKD: chronic kidney disease; PCS: *p*-cresyl sulfate; IS: indoxyl sulfate; TMAO: trimethylamine n-oxide; RCTs: randomized controlled trials; BCMC: registered probiotic trademark name from B-Crobes Laboratories Sdn. Bhd., Malaysia; BDI: beck depression inventory; GHQ-28: general health questionnaire-28; DASS: depression anxiety and stress scale; CRP: C-reactive protein; MDA: malondialdehyde; TAC: antioxidant capacity; GSH: glutathione.

Strains of lactobacilli and bifidobacteria are common in probiotic formulations and tend to be the first bacteria investigated for new microbial therapies, including those for AS. Treatment with oral *Lactobacillus acidophilus* NCFM or *L. acidophilus* BG2F04 decreased dimethylamines, potential precursors for trimethylamine production[28]. There is also evidence that some lactobacilli can directly mitigate the harmful effects of TMAO directly as it relates to AS. In mice, the oral supplementation of *Lactiplantibacillus plantarum* CGMCC 8198 slowed AS progression by reducing inflammation and oxidative stress caused by TMAO. There is also evidence to support *Lactocasebacillus rhamnosus* GG as an efficient TMAO-reducing strain in both humans and animal models[29]. Interestingly, the effect of *L. rhamnosus* may extend beyond the gut. While present in both stable and unstable (or actively growing) atherosclerotic plaques, *L. rhamnosus* DNA had greater enrichment in stabilized plaques[6]. Indeed, the details of how nucleic acid components of probiotics are enriched in plaques and how they may slow AS progression are not clear. Certainly, the DNA of other bacterial species is present in these plaques, and thus, stability may rely on

Table 3. Summary of rodent studies using probiotics relevant to AS

Article	Strain(s)	Summary/main findings
The anti-cholesterolaemic effect of a consortium of probiotics: an acute study in C57BL/6J mice	● *Lactobacillus acidophilus* CUL21, CUL60 ● *Bifidobacterium bifidum* CUL20 ● *Bifidobacterium animalis* CUL34 ● *Lactobacillus plantarum* CUL66	● C57BL/6J mice ● High-fat diet + probiotics for 2 weeks ● Reduced plasma cholesterol ● Increased fecal bile acids ● No effect on LDL/HDL ratio
Preventive effect and molecular mechanism of *Lactobacillus rhamnosus* JL1 on food-borne obesity in mice	● *Lactobacillus rhamnosus* JL1	● C57BL/6J mice ● High-fat diet + probiotics for 10 weeks ● Lower body weight ● Reduced liver index ● Improved lipid metabolism via the AMPK pathway
Lactobacillus acidophilus ATCC 4356 prevents atherosclerosis via inhibition of intestinal cholesterol absorption in apolipoprotein E-knockout mice	● *Lactobacillus acidophilus* ATCC 4356	● ApoE-/- mice ● Western diet + probiotics for 16 weeks ● Lower plasma cholesterol ● Reduced intestinal cholesterol absorption and AS
The Lab4P consortium of probiotics attenuates atherosclerosis in LDL receptor deficient mice fed a high fat diet and causes plaque stabilization by inhibiting inflammation and several pro-atherogenic processes	● *Lactobacillus acidophilus* CUL21, CUL60 ● *Bifidobacterium bifidum* CUL20 ● *Bifidobacterium animalis* CUL34 ● *Lactobacillus plantarum* CUL66	● LDL receptor-deficient mice ● High-fat diet + probiotics for 12 weeks ● Reduced plaque burden ● Increased HDL ● Decreased LDL and plaque stabilization
Lactobacillus mucosae and *Bifidobacterium longum* synergistically alleviate immobilization stress-induced anxiety/depression in mice by suppressing gut dysbiosis	● *Lactobacillus mucosae* NK41 ● *Bifidobacterium longum* NK46	● C57BL/6 mice ● Immobilization stress + probiotics for 3 weeks ● Alleviated anxiety-like behaviors ● Reduced gut dysbiosis and inflammation
Probiotics alleviate oxidative stress in H_2O_2-exposed hepatocytes and t-BHP-induced C57BL/6 mice	● *Lactococcus lactis* MG5125 ● *Bifidobacterium bifidum* MG731 ● *Bifidobacterium animalis* MG741	● C57BL/6 mice ● Oxidative stress induced by t-BHP + probiotics ● Improved oxidative stress markers and hepatic enzyme activity
Antioxidant activity of *Lactobacillus plantarum* NJAU-01 in an animal model of aging	● *Lactobacillus plantarum* NJAU-01	● Kun Ming mice ● D-galactose-induced aging + probiotics ● Increased antioxidant enzymatic activities ● Reduced lipid oxidation
The probiotic Lactobacillus coryniformis CECT5711 reduces the vascular pro-oxidant and pro-inflammatory status in obese mice	● *Lactobacillus coryniformis* CECT5711	● C57BL/6J ● High-fat diet + probiotics for 12 weeks ● Improved endothelial function ● Reduced oxidative stress and inflammation

AS: Atherosclerosis; LDL: low-density lipoprotein; HDL: high-density lipoprotein; AMPK: AMP-activated protein kinase.
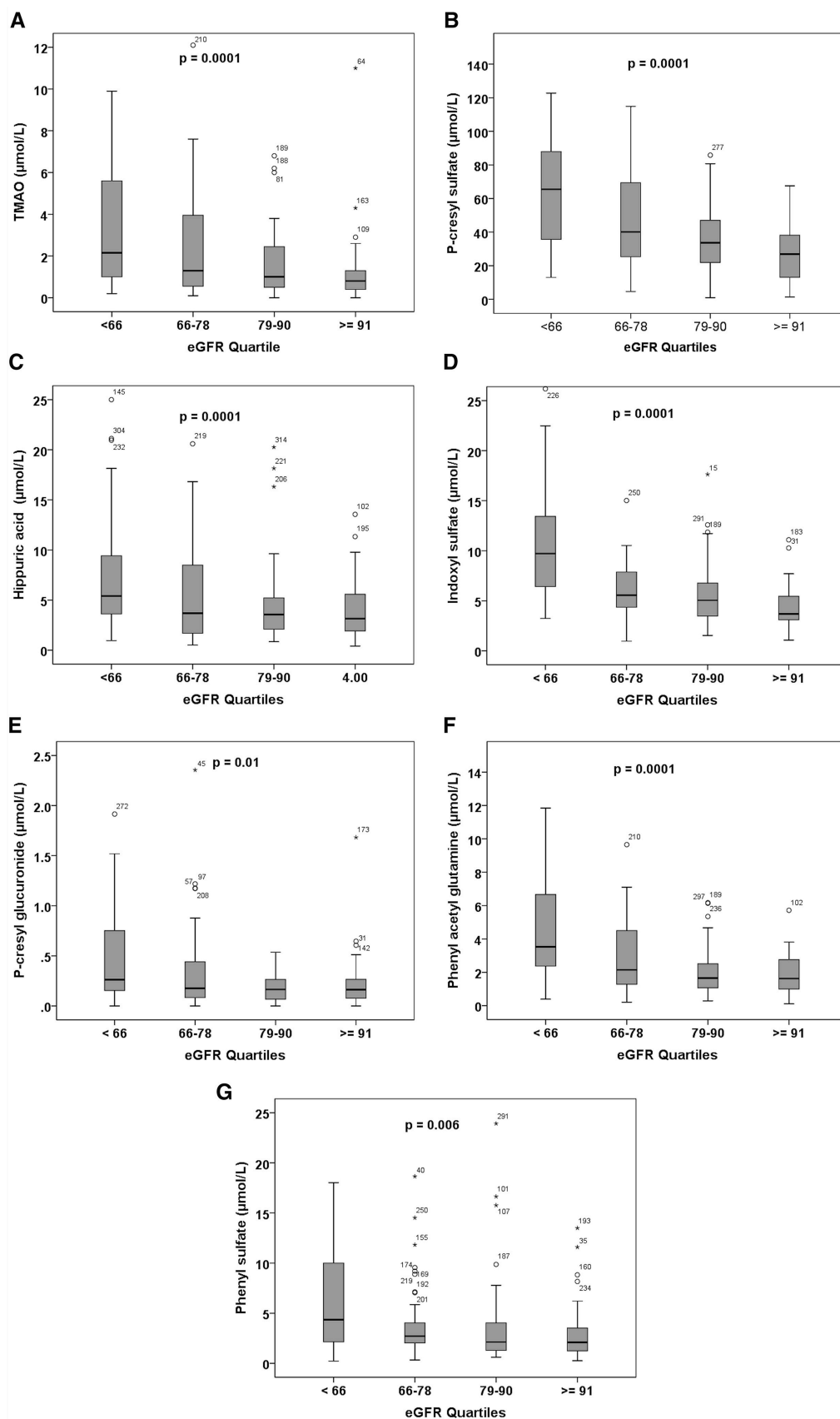
Figure 2. Plasma levels of GDUT by quartile of eGFR. Boxplots show plasma levels of GDUT in mmol/L by quartile of eGFR (calculated using the CKD-EPI equations). eGFR quartiles: Q1, ,66 mL/min per 1.73 m^2; Q2, 66-78; Q3, 79-90; Q4, $ 91. (A) TMAO; (B) P-cresyl sulfate; (C) hippuric acid; (D) indoxyl sulfate; (E) P-cresyl glucuronide; (F) phenylacetyl glutamine; (G) phenyl sulfate. Plasma levels of all the metabolites were significantly higher in lower quartiles of eGFR; P values were computed for differences in median values by the Kruskal-Wallis test because the distribution of plasma levels was not normally distributed. Reproduced by permission of Elsevier from[20].GDUT: Gut-derived uremic toxin; eGFR: estimated glomerular filtration rate; CKD: chronic kidney disease; TMAO: trimethylamine N-oxide.

community dynamics as opposed to a single species or strain. *Enterobacter aerogenes* ZDY01 was also inquired for its probiotic potential and was shown to attenuate choline-induced AS in mice by decreasing cecal TMA and promoting reverse cholesterol transport[30]. While these results highlight the real possibility that probiotics could reduce TMAO burden, most data come from animal studies or small-cohort clinical trials. Further testing of these probiotics in larger populations of human AS patients is required to establish efficacy and the potential for any associated risks.

Indoxyl sulfate and *p*-cresyl sulfate
As shown in Table 1, *p*-cresyl sulfate was a stronger predictor of TPA than TMAO. It would be desirable, therefore, to develop therapies to reduce not only the plasma levels of TMAO, but also *p*-cresyl sulfate and other toxic GDUTs, such as indoxyl sulfate, which have been shown to promote AS. Indoxyl sulfate and *p*-cresyl sulfate are produced by the gut microbiota through the fermentation of tryptophan and tyrosine, respectively. These toxins are commonly associated with chronic kidney disease, where their accumulation occurs due to impaired renal function[31-33]. However, indoxyl sulfate and *p*-cresyl sulfate are also closely linked to cardiovascular diseases and mortality[34-36]. In AS patients, these toxins are elevated in the plasma, but this observation cannot be explained by kidney function or the intake of dietary precursors[1]. This suggests the gut microbiota of AS patients has a heightened ability to produce GDUTs, leaving them at risk of the deleterious effects of these toxins. Furthermore, there are very few strategies available for their elimination, and most of these strategies impart an incomplete effect[37].

To date, bifidobacteria have demonstrated the greatest success in preventing the build-up of *p*-cresyl sulfate and its microbial precursor, *p*-cresol. Probiotic formulations containing at least one strain of *Bifidobacterium longum* have been shown to reduce plasma *p*-cresol in two independent studies after 3 months of supplementation[38,39]. Another study that used a microbial mixture of *Bifidobacterium infantis*, *Lactobacillus acidophilus*, and *Enterococcus faecalis* showed oral supplementation of these microbes reduced fecal and serum *p*-cresol. A synbiotic therapy (probiotic + prebiotic) containing bifidobacteria was also shown to significantly reduce *p*-cresyl sulfate in a placebo-controlled trial[40]. These findings indicate the strong potential to use probiotics as a treatment to reduce GDUT. However, due to the existence of a handful of studies reporting probiotic mixtures did not reduce levels of *p*-cresol or *p*-cresyl sulfate, the efficacy of probiotics for this purpose remains unclear[41-44]. It is of interest to note, however, that the trials where no decrease in GDUTs was observed utilized multi-strain probiotics and not an individual bacterium[40-45]. Delivering bacteria in different combinations alters their activity and potentially blocks their beneficial functions. Highlighting this point, the oral delivery of five different combinations of beneficial bacteria to nephrectomized rats showed that only two could reduce blood urea nitrogen and serum creatinine despite the similar consortia[46]. While the use of multi-strain probiotics certainly has merit in some cases, care should be taken to ensure that the beneficial properties are maintained when delivered alongside other strains.

When considering individual strains, both *Bifidobacterium breve* and *Bifidobacterium longum* can reduce toxin burden and enhance SCFA production[47-51], both of which would be beneficial to AS patients. While

the mechanism of toxin clearance is often unknown, both *B. breve* and *B. longum* have been shown to clear *p*-cresol directly from bacteriological growth media[10] and a colonic environment[52,53]. Taken together, these studies suggest that oral supplementation of *B. breve* or *B. longum* could offer protection against gut microbiota metabolites relevant to AS. These studies also point toward the importance of proper strain selection. In the future, there should be an emphasis on testing individual probiotic strains for the desired function, whether toxin clearance or any other, and ensuring that these activities are retained when the strains are delivered in combination.

There have been fewer studies focusing on the clearance of indoxyl sulfate using probiotics; however, they are still worth considering. In cohorts of hemodialysis patients, who often have a high burden of uremic toxins, three studies assessed probiotics using different strains of *B. longum*. Two of these studies showed a clear decrease in indoxyl sulfate[48,50] after 5 weeks of oral administration, while another indicated decreased indoxyl glucuronide without reaching traditional measures of significance[41]. Beyond probiotics, AST-120, an oral activated carbon supplement, reduces the serum and urine levels of indoxyl sulfate in patients with uremia by adsorbing indole in the intestines, thereby increasing its excretion into feces[54]. Unfortunately, AST-120 often requires the patient to consume 30 or more capsules daily, not considering any other medications they may be taking[54,55]. It would, therefore, be beneficial to identify probiotic strains that could effectively reduce indole in the gut, similar to what has been done for *p*-cresol, the microbial precursor of *p*-cresyl sulfate.

PROBIOTICS FOR TRADITIONAL RISK FACTORS OF AS

Beyond reducing metabolites produced by the intestinal microbiota, probiotics have also shown great potential in protecting against traditional AS risk factors such as lipid metabolism, endothelial dysfunction, and inflammation.

Cholesterol

Maintaining a well-controlled cholesterol equilibrium is paramount in slowing AS progression[56-59]. Regrettably, numerous inquiries into antiatherosclerotic drugs have yielded disappointing results[60-62]. Alternatively, there have been various reports of probiotics having favorable impacts on lipid metabolism, suggesting their potential utility in AS patients.

Several strains of lactobacilli have demonstrated robust hypocholesterolemic effects *in vivo*[56-59]. Among these, the Lab4 probiotic consortium with *Lactobacillus plantarum* CUL66 stood out because it caused a notable reduction in total plasma cholesterol levels while also mitigating diet-induced weight gain[63]. It must be noted that these studies were conducted in mice, necessitating further validation in human cohorts before any assertions can be made regarding their efficacy against AS. The use of probiotics to reduce serum cholesterol remains blurred. For instance, a clinical trial utilizing *Lactobacillus acidophilus* and *Bifidobacterium animalis* via yogurt or capsules did not improve AS risk factors in overweight individuals[64]. The lack of success in this study does not negate the potential effectiveness of probiotics. Researchers often rely on commercially available strains lacking documented activity against the disease. Therefore, future clinical trials should focus on utilizing strains with established cholesterol-lowering properties, such as those identified in the murine investigations[63,65-67].

Inflammation

The orchestration of the inflammatory response is a pivotal determinant of AS progression and stability[68]. Within this complex milieu, cytokines, notably TNF-α, CRP, and IL-6, emerge as influential players in AS development. During the nascent stages of AS, these cytokines act as catalysts, inciting endothelial cell

activation, amplifying the synthesis of adhesion molecules, and leading to the migration of immune cells[69]. As AS advances to later stages, the presence of proinflammatory cytokines manifests as plaque rupture and thrombosis. Thus, promoting an anti-inflammatory environment raises hope for AS treatment, which has been corroborated by compelling clinical trials[70,71].

The production of TNF-α within atherosclerotic plaques and elevated levels of the compound in the bloodstream correlate with AS progression[72]. Recent investigations have highlighted a consortium of probiotics such as *Lactobacillus mucosae* NK41, *Bifidobacterium longum* NK46, *Bifidobacterium breve* DSM 16604 and DSM 24706 that can reduce TNF-α *in vivo*[73,74]. While the mechanism behind this observation is still unclear, this nuanced influence of probiotics on the release of inflammatory mediators appears to be intertwined with altered miRNA expression within the gastrointestinal tract[75]. For example, a strain of *Lactobacillus plantarum* has been shown to reduce TNF-α mRNA expression over the span of three weeks, likely by regulating miRNA-450a expression[76]. In contrast, a clinical investigation of the same strain showed that there were no substantial shifts in TNF-α, IL-6, IL-1b, nor cortisol after a four-week period, compared to placebo[77]. The difference in outcomes underscores the need to tailor probiotic interventions to the unique attributes of study participants, because the second intervention was in patients with depression, not AS. Elevated IL-6 has also emerged as a biomarker for AS risk and progression[78,79]. Some probiotics have been shown to reduce the presence of such inflammatory cytokines. Indeed, a study involving colon cancer patients revealed that the postoperative administration of a probiotic blend containing multiple strains of lactobacilli and bifidobacteria for four weeks resulted in a marked reduction in proinflammatory cytokines TNF-α, IL-6, and IL-22 in peripheral blood[80]. These findings and others from animal studies are promising, but there is a need for randomized, double-blind human trials to authenticate the anti-inflammatory potential of probiotics in AS patients.

C-reactive protein (CRP) is another inflammatory marker produced in response to cytokines like IL-6, IL-1β, and TNF-α, and is associated with vascular endothelial cell impairment, thrombosis, and ultimately AS progression[81,82]. Detectable within early lesions, CRP levels also increase as AS advances to later stages[83]. To date, some studies investigating the impact of probiotics on CRP levels have been successful. An 8-week course of probiotic capsules containing *L. acidophilus*, *Lactobacillus casei*, and *Bifidobacterium bifidum* significantly reduced CRP[84]. Another study involving a probiotic capsule containing four strains, combined with vitamin D administration for 12 weeks, demonstrated decreased CRP in women with polycystic ovarian syndrome[85]. While direct evidence linking gut microbiota modulation of inflammatory factors to AS progression remains inconclusive, these studies offer valuable insights and avenues for exploring novel preventive and therapeutic strategies for AS.

While further research is needed to validate the efficacy of probiotics in modulating inflammatory factors and slowing AS advancement, these findings pave the way for innovative strategies in preventing and treating AS.

Endothelial function
Endothelial dysfunction is a key event in the early stages of AS development/progression and is caused by disrupted vascular homeostasis due to oxidative stress and inflammation[86,87]. Probiotics can reduce oxidative stress, quell vascular inflammation, and increase nitric oxide (NO) production, each of which could independently delay the progression of AS[88-90].

Oxidative stress promotes endothelial dysfunction by impairing the vascular endothelium, causing inflammation, and oxidizing low-density lipoprotein-C (LDL-C)[91-93]. Restoring the endothelial structure

could improve AS outcomes, and probiotics have shown some promise in this area. For instance, probiotic Kefir, a fermented beverage that can be prepared using either milk or water and kefir grains that are combined with a starter culture that contains a combination of yeast species and lactic acid bacteria, has been associated with enhanced endothelial function and vascular structure repair in animal studies[94]. Furthermore, specific probiotics such as *Lactococcus lactis* MG5125, *Bifidobacterium bifidum* MG731, and *Bifidobacterium animalis* MG741 have showcased antioxidant properties by bolstering total antioxidant capacity and mitigating oxidative stress[95-98]. *Lactobacillus plantarum* NJAU-01 and *Lactobacillus fermentum* DR9 have demonstrated the ability to decrease lipid oxidation levels and enhance antioxidant enzyme activity in rats[99,100]. These discoveries indicate that probiotics have potential as therapeutic interventions to prevent diseases associated with oxidative stress like AS but require further testing in human cohorts.

Reduced production and sensitivity of NO can also disrupt vascular equilibrium[101-103]. Using probiotics to enhance NO production and availability may be a new approach to combat AS. For instance, *Lactobacillus coryniformis* CECT5711 has been shown to inhibit certain inflammatory markers in obese mice and improve endothelial function by increasing NO levels[104]. Similarly, *Lactobacillus casei* boosts NO production in some human cells[105]. Nevertheless, the impact of probiotics on NO production in vascular endothelial cells or animal models has not been tested. Considering that endothelial cells are a primary source of NO via NO synthase expression, how probiotics regulate NO production in these cells should also be inquired[106]. Furthermore, *Lactobacillus fermentum* CECT5716 has been reported to counteract the endothelial dysfunction induced by certain medications by reducing oxidative stress and inflammation in the blood vessels[107]. These findings suggest that probiotics may help alleviate vascular manifestations by targeting and suppressing pathways related to oxidative stress.

While all the strains mentioned in this section so far have only been shown to have activity in animal or cell-based models, clinical studies have also reported that certain probiotics can improve endothelial dysfunction. In a 12-week trial involving 81 participants, a probiotic that contained *Lactobacillus*, *Bifidobacterium*, and *Lactococcus* reduced systolic blood pressure, inflammatory markers, and parameters linked to endothelial dysfunction[108]. However, not all probiotics exhibit these clinical benefits. For example, *Lactobacillus casei* Shirota (LcS) did not show significant improvements in low-grade inflammation or endothelial dysfunction in patients with metabolic syndrome[109]. These studies highlight the need for further research in AS patients and with larger sample sizes to better understand the impact of probiotics on endothelial function.

HOW TO FIND THE RIGHT PROBIOTIC FOR AS?

In general, selecting a probiotic strain for a target disease should be based on recommendations from in-depth reviews or organizations such as the Food and Drug Administration or the European Society for Paediatric Gastroenterology, Hepatology and Nutrition. In our opinion, the available pool of research seems insufficient to provide an accurate recommendation for an AS-specific probiotic. At present, there are no probiotic recommendations for AS within the Canadian or American websites of clinically documented probiotic strains and products (www.usprobioticguide.com, accessed on 28 February 2024; www.probioticchart.ca, accessed on 28 February 2024). A major concern we have is that most of the studies mentioned in this perspective measured the effect of probiotics on factors relevant to AS, such as inflammation, but were not performed in AS patients. Therefore, while these strains show promise in managing some of the factors associated with AS, they must be tested in an appropriate cohort. As such, our current suggestions will focus on how to design new studies to identify and assess useful probiotic strains to treat AS.

Finding new strains

In an ideal situation, performing *in vitro* experiments before any human or animal studies would assist in identifying beneficial strains with specific target activities related to AS. However, as we have mentioned, studies often rely on commercially available strains rather than those recognized for their specific traits.

We believe that the identification of strains relevant to AS, or any other disease should follow a series of simple steps. First, strains should be sequenced to identify useful genes harbored within their genetic content. The utility of these genes should then be proven by demonstrating the activity *in vitro* (e.g., reduction in uremic toxins or serum cholesterol). Strains with sufficient activity can then be assessed using relevant cell culture and animal models. The final step would then be to perform a well-designed clinical trial in an appropriate population of humans (i.e., individuals with a certain disease or condition). Certainly, this concise set of steps represents a more appropriate selection process than choosing bacterial strains based solely on availability. However, it is crucial to highlight that this method of strain selection overlooks the essential need to assess each strain for stability and safety before introducing it to a group of at-risk individuals (i.e., AS patients). The discussion on how to conduct these evaluations on probiotic strains is omitted here as it has been comprehensively addressed elsewhere[110-113].

HOW SHOULD STRAINS BE ASSESSED CLINICALLY?

When evaluating strains in clinical settings, researchers should utilize randomized, double-blind placebo-controlled studies to reduce bias. Trials should ideally be carried out in target populations, in this case, AS patients, alongside standard treatments to avoid interfering with patients' medical care. To date, only a handful of clinical trials have investigated how probiotics directly influence AS patients, with most of the research being conducted in mouse or rat models. While these studies demonstrate the feasibility of the approach, they do not provide concrete evidence to support the widespread adoption of a specific strain or product.

After identifying beneficial strains with proven clinical efficacy against AS, the challenge of determining how to assess the results of various strains emerges. Should the focus be on probiotics that alleviate inflammation or promote cholesterol clearance? Is a combination of strains more effective than one alone? The answer is not straightforward, and in fact, the existence of a "one-size-fits-all" probiotic for AS is unlikely. Individuals respond differently to the physiological challenges of AS, as demonstrated by the existence of a protected phenotype [Figure 1], and thus, patients may also have similar responses to treatment wherein one patient might benefit more from a cholesterol-reducing probiotic while another might respond better to that with anti-inflammatory action. By assembling a diverse range of strains with various activities against AS symptoms and risk factors, this potential issue could be overcome.

CONCLUSIONS

This review focused on highlighting the potential for probiotics to protect against both the traditional and non-traditional risk factors of AS. Probiotics show promise as therapies for AS, with the possibility of plaque stabilization and a reduced risk of cardiovascular events. The microbial supplements mainly consisted of lactobacilli or bifidobacteria and were often tested using animal models. The limited number of quality clinical trials in AS patients and conflicting findings make strain recommendation for AS difficult. Therefore, more studies are needed to identify new strains with specific metabolic functions against AS and to test them in the appropriate human populations. Long-term studies that also address how probiotic administration improves not only symptoms and quality of life but also additional clinical parameters such as plaque burden, cardiovascular events, and survival are needed.

DECLARATIONS

Authors' contributions
Accepted the invitation to write the paper and wrote the first draft: Spence JD
Wrote the expanded manuscript; Stuivenberg GA
Made substantial contributions to the conception and design of the study and performed data analysis and interpretation: Stuivenberg GA, Spence JD, Burton JP
Performed literature search, as well as providing administrative, technical, and material support: Stuivenberg GA, Poon A, Burton JP, Spence JD
Edited the manuscript: Stuivenberg GA, Poon A, Burton JP, Spence JD

Availability of data and materials
Not applicable.

Conflicts of interest
All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

REFERENCES

1. Bogiatzi C, Gloor G, Allen-Vercoe E, et al. Metabolic products of the intestinal microbiome and extremes of atherosclerosis. *Atherosclerosis* 2018;273:91-7. DOI
2. Spence JD, Eliasziw M, DiCicco M, Hackam DG, Galil R, Lohmann T. Carotid plaque area: a tool for targeting and evaluating vascular preventive therapy. *Stroke* 2002;33:2916-22. DOI PubMed
3. Jie Z, Xia H, Zhong SL, et al. The gut microbiome in atherosclerotic cardiovascular disease. *Nat Commun* 2017;8:845. DOI PubMed PMC
4. Trøseid M, Ueland T, Hov JR, et al. Microbiota-dependent metabolite trimethylamine-N-oxide is associated with disease severity and survival of patients with chronic heart failure. *J Intern Med* 2015;277:717-26. DOI PubMed
5. Bartolomaeus H, Balogh A, Yakoub M, et al. Short-chain fatty acid propionate protects from hypertensive cardiovascular damage. *Circulation* 2019;139:1407-21. DOI PubMed PMC
6. Mitra S, Drautz-Moses DI, Alhede M, et al. In silico analyses of metagenomes from human atherosclerotic plaque samples. *Microbiome* 2015;3:38. DOI PubMed PMC
7. Korpela K, Salonen A, Vepsäläinen O, et al. Probiotic supplementation restores normal microbiota composition and function in antibiotic-treated and in caesarean-born infants. *Microbiome* 2018;6:182. DOI PubMed PMC
8. Hemarajata P, Versalovic J. Effects of probiotics on gut microbiota: mechanisms of intestinal immunomodulation and neuromodulation. *Therap Adv Gastroenterol* 2013;6:39-51. DOI PubMed PMC
9. Nguyen TTU, Kim HW, Kim W. Effects of probiotics, prebiotics, and synbiotics on uremic toxins, inflammation, and oxidative stress in hemodialysis patients: a systematic review and meta-analysis of randomized controlled trials. *J Clin Med* 2021;10:4456. DOI PubMed PMC
10. Stuivenberg GA, Chmiel JA, Akouris PP, Burton JP, Reid G. Probiotic bifidobacteria mitigate the deleterious effects of *para*-cresol in a drosophila melanogaster toxicity model. *mSphere* 2022;7:e0044622. DOI PubMed PMC
11. Din AU, Hassan A, Zhu Y, Yin T, Gregersen H, Wang G. Amelioration of TMAO through probiotics and its potential role in atherosclerosis. *Appl Microbiol Biotechnol* 2019;103:9217-28. DOI PubMed
12. Spence JD, Solo K. Resistant atherosclerosis: the need for monitoring of plaque burden. *Stroke* 2017;48:1624-9. DOI PubMed

13.   Baber U, Mehran R, Sartori S, et al. Prevalence, impact, and predictive value of detecting subclinical coronary and carotid atherosclerosis in asymptomatic adults: the BioImage study. *J Am Coll Cardiol* 2015;65:1065-74. DOI PubMed

14.   Wang Z, Klipfell E, Bennett BJ, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature* 2011;472:57-63. DOI PubMed PMC

15.   Koeth RA, Wang Z, Levison BS, et al. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nat Med* 2013;19:576-85. DOI PubMed PMC

16.   Zhen J, Zhou Z, He M, et al. The gut microbial metabolite trimethylamine N-oxide and cardiovascular diseases. *Front Endocrinol* 2023;14:1085041. DOI PubMed PMC

17.   Tang WH, Wang Z, Levison BS, et al. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *N Engl J Med* 2013;368:1575-84. DOI PubMed PMC

18.   Zhu W, Gregory JC, Org E, et al. Gut microbial metabolite TMAO enhances platelet hyperreactivity and thrombosis risk. *Cell* 2016;165:111-24. DOI PubMed PMC

19.   Tang WHW, Wang Z, Kennedy DJ, et al. Gut microbiota-dependent trimethylamine N-oxide (TMAO) pathway contributes to both development of renal insufficiency and mortality risk in chronic kidney disease. *Circ Res* 2015;116:448-55. DOI PubMed PMC

20.   Pignanelli M, Bogiatzi C, Gloor G, et al. Moderate renal impairment and toxic metabolites produced by the intestinal microbiome: dietary implications. *J Ren Nutr* 2019;29:55-64. DOI

21.   Olechnowicz-Tietz S, Gluba A, Paradowska A, Banach M, Rysz J. The risk of atherosclerosis in patients with chronic kidney disease. *Int Urol Nephrol* 2013;45:1605-12. DOI PubMed PMC

22.   Wang Z, Bergeron N, Levison BS, et al. Impact of chronic dietary red meat, white meat, or non-meat protein on trimethylamine N-oxide metabolism and renal excretion in healthy men and women. *Eur Heart J* 2019;40:583-94. DOI PubMed PMC

23.   Pathak P, Helsley RN, Brown AL, et al. Small molecule inhibition of gut microbial choline trimethylamine lyase activity alters host cholesterol and bile acid metabolism. *Am J Physiol Heart Circ Physiol* 2020;318:H1474-86. DOI PubMed PMC

24.   Liu H, Chen X, Hu X, et al. Alterations in the gut microbiome and metabolism with coronary artery disease severity. *Microbiome* 2019;7:68. DOI PubMed PMC

25.   Verhaar BJH, Prodan A, Nieuwdorp M, Muller M. Gut microbiota in hypertension and atherosclerosis: a review. *Nutrients* 2020;12:2982. DOI PubMed PMC

26.   Shen X, Li L, Sun Z, et al. Gut microbiota and atherosclerosis-focusing on the plaque stability. *Front Cardiovasc Med* 2021;8:668532. DOI PubMed PMC

27.   Plaza-Diaz J, Ruiz-Ojeda FJ, Gil-Campos M, Gil A. Mechanisms of action of probiotics. *Adv Nutr* 2019;10:S49-66. DOI PubMed PMC

28.   Melamed ML, Plantinga L, Shafi T, et al. Retained organic solutes, patient characteristics and all-cause and cardiovascular mortality in hemodialysis: results from the retained organic solutes and clinical outcomes (ROSCO) investigators. *BMC Nephrol* 2013;14:134. DOI PubMed PMC

29.   Cantero MA, Guedes MRA, Fernandes R, Lollo PCB. Trimethylamine N-oxide reduction is related to probiotic strain specificity: a systematic review. *Nutr Res* 2022;104:29-35. DOI PubMed

30.   Tang J, Qin M, Tang L, et al. Enterobacter aerogenes ZDY01 inhibits choline-induced atherosclerosis through CDCA-FXR-FGF15 axis. *Food Funct* 2021;12:9932-46. DOI PubMed

31.   Gryp T, Vanholder R, Vaneechoutte M, Glorieux G. p-Cresyl sulfate. *Toxins* 2017;9:52. DOI PubMed PMC

32.   Kwiatkowska I, Hermanowicz JM, Mysliwiec M, Pawlak D. Oxidative storm induced by tryptophan metabolites: missing link between atherosclerosis and chronic kidney disease. *Oxid Med Cell Longev* 2020;2020:6656033. DOI PubMed PMC

33.   Ramezani A, Massy ZA, Meijers B, Evenepoel P, Vanholder R, Raj DS. Role of the gut microbiome in uremia: a potential therapeutic target. *Am J Kidney Dis* 2016;67:483-98. DOI PubMed PMC

34.   Meijers BK, Claes K, Bammens B, et al. p-Cresol and cardiovascular risk in mild-to-moderate kidney disease. *Clin J Am Soc Nephrol* 2010;5:1182-9. DOI PubMed PMC

35.   Nakano T, Katsuki S, Chen M, et al. Uremic toxin indoxyl sulfate promotes proinflammatory macrophage activation via the interplay of OATP2B1 and Dll4-notch signaling. *Circulation* 2019;139:78-96. DOI PubMed PMC

36.   Opdebeeck B, Maudsley S, Azmi A, et al. Indoxyl sulfate and p-cresyl sulfate promote vascular calcification and associate with glucose intolerance. *J Am Soc Nephrol* 2019;30:751-66. DOI PubMed PMC

37.   Dobre M, Meyer TW, Hostetter TH. Searching for uremic toxins. *Clin J Am Soc Nephrol* 2013;8:322-7. DOI PubMed PMC

38.   Cosola C, Rocchetti MT, di Bari I, et al. An innovative synbiotic formulation decreases free serum indoxyl sulfate, small intestine permeability and ameliorates gastrointestinal symptoms in a randomized pilot trial in stage IIIb-IV CKD patients. *Toxins* 2021;13:334. DOI PubMed PMC

39.   Guida B, Germanò R, Trio R, et al. Effect of short-term synbiotic treatment on plasma p-cresol levels in patients with chronic renal failure: a randomized clinical trial. *Nutr Metab Cardiovasc Dis* 2014;24:1043-9. DOI

40.   Rossi M, Johnson DW, Morrison M, et al. Synbiotics easing renal failure by improving gut microbiology (SYNERGY): a randomized trial. *Clin J Am Soc Nephrol* 2016;11:223-31. DOI PubMed PMC

41.   Natarajan R, Pechenyak B, Vyas U, et al. Randomized controlled trial of strain-specific probiotic formulation (Renadyl) in dialysis patients. *Biomed Res Int* 2014;2014:568571. DOI PubMed PMC

42.   de Faria Barros A, Borges NA, Nakao LS, et al. Effects of probiotic supplementation on inflammatory biomarkers and uremic toxins

in non-dialysis chronic kidney patients: a double-blind, randomized, placebo-controlled trial. *J Funct Foods* 2018;46:378-83. DOI

43.   Pavan M. Influence of prebiotic and probiotic supplementation on the progression of chronic kidney disease. *Minerva Urol Nefrol* 2016;68:222-6. PubMed

44.   De Mauri A, Carrera D, Bagnati M, et al. Probiotics-supplemented low-protein diet for microbiota modulation in patients with advanced chronic kidney disease (ProLowCKD): results from a placebo-controlled randomized trial. *Nutrients* 2022;14:1637. DOI PubMed PMC

45.   Poesen R, Evenepoel P, de Loor H, et al. The influence of prebiotic arabinoxylan oligosaccharides on microbiota derived uremic retention solutes in patients with chronic kidney disease: a randomized controlled trial. *PLoS One* 2016;11:e0153893. DOI PubMed PMC

46.   Ranganathan N, Patel B, Ranganathan P, et al. Probiotic amelioration of azotemia in 5/6th nephrectomized Sprague-Dawley rats. *ScientificWorldJournal* 2005;5:652-60. DOI PubMed PMC

47.   Nakabayashi I, Nakamura M, Kawakami K, et al. Effects of synbiotic treatment on serum level of p-cresol in haemodialysis patients: a preliminary study. *Nephrol Dial Transplant* 2011;26:1094-8. DOI

48.   Takayama F, Taki K, Niwa T. Bifidobacterium in gastro-resistant seamless capsule reduces serum levels of indoxyl sulfate in patients on hemodialysis. *Am J Kidney Dis* 2003;41:S142-5. DOI PubMed

49.   Andrade-Oliveira V, Amano MT, Correa-Costa M, et al. Gut bacteria products prevent AKI induced by ischemia-reperfusion. *J Am Soc Nephrol* 2015;26:1877-88. DOI PubMed PMC

50.   Taki K, Takayama F, Niwa T. Beneficial effects of Bifidobacteria in a gastroresistant seamless capsule on hyperhomocysteinemia in hemodialysis patients. *J Ren Nutr* 2005;15:77-80. DOI

51.   Ando Y, Miyata Y, Tanba K, et al. [Effect of oral intake of an enteric capsule preparation containing Bifidobacterium longum on the progression of chronic renal failure]. *Nihon Jinzo Gakkai Shi* 2003;45:759-64. PubMed

52.   Iwashita Y, Ohya M, Yashiro M, et al. Dietary changes involving *Bifidobacterium longum* and other nutrients delays chronic kidney disease progression. *Am J Nephrol* 2018;47:325-32. DOI PubMed

53.   Stuivenberg GA, Chmiel JA, Akouris PP, et al. Supplementing yogurt with probiotic Bifidobacteria to counter chronic kidney disease. *Fermentation* 2023;9:391. DOI

54.   Yamaguchi J, Tanaka T, Inagi R. Effect of AST-120 in chronic kidney disease treatment: still a controversy? *Nephron* 2017;135:201-6. DOI

55.   Schulman G, Vanholder R, Niwa T. AST-120 for the management of progression of chronic kidney disease. *Int J Nephrol Renovasc Dis* 2014;7:49-56. DOI PubMed PMC

56.   Noda H, Iso H, Irie F, Sairenchi T, Ohtaka E, Ohta H. Gender difference of association between LDL cholesterol concentrations and mortality from coronary heart disease amongst Japanese: the Ibaraki Prefectural Health Study. *J Intern Med* 2010;267:576-87. DOI PubMed

57.   Imamura T, Doi Y, Arima H, et al. LDL cholesterol and the development of stroke subtypes and coronary heart disease in a general Japanese population: the Hisayama study. *Stroke* 2009;40:382-8. DOI

58.   Tanabe N, Iso H, Okada K, et al; Japan Arteriosclerosis Longitudinal Study Group. Serum total and non-high-density lipoprotein cholesterol and the risk prediction of cardiovascular events - the JALS-ECC -. *Circ J* 2010;74:1346-56. DOI PubMed

59.   Sugiyama D, Okamura T, Watanabe M, et al; NIPPON DATA 80/90 Research Group. Risk of hypercholesterolemia for cardiovascular disease and the population attributable fraction in a 24-year Japanese cohort study. *J Atheroscler Thromb* 2015;22:95-107. DOI

60.   Russo MW, Hoofnagle JH, Gu J, et al. Spectrum of statin hepatotoxicity: experience of the drug-induced liver injury network. *Hepatology* 2014;60:679-86. DOI PubMed PMC

61.   Libby P. The forgotten majority: unfinished business in cardiovascular risk reduction. *J Am Coll Cardiol* 2005;46:1225-8. DOI PubMed

62.   Parker BA, Capizzi JA, Grimaldi AS, et al. Effect of statins on skeletal muscle function. *Circulation* 2013;127:96-103. DOI PubMed PMC

63.   Michael DR, Davies TS, Moss JWE, et al. The anti-cholesterolaemic effect of a consortium of probiotics: an acute study in C57BL/6J mice. *Sci Rep* 2017;7:2883. DOI PubMed PMC

64.   Ivey KL, Hodgson JM, Kerr DA, Thompson PL, Stojceski B, Prince RL. The effect of yoghurt and its probiotics on blood pressure and serum lipid profile; a randomised controlled trial. *Nutr Metab Cardiovasc Dis* 2015;25:46-51. DOI PubMed

65.   Yang M, Zheng J, Zong X, et al. Preventive effect and molecular mechanism of Lactobacillus rhamnosus JL1 on food-borne obesity in mice. *Nutrients* 2021;13:3989. DOI PubMed PMC

66.   Huang Y, Wang J, Quan G, Wang X, Yang L, Zhong L. Lactobacillus acidophilus ATCC 4356 prevents atherosclerosis via inhibition of intestinal cholesterol absorption in apolipoprotein E-knockout mice. *Appl Environ Microbiol* 2014;80:7496-504. DOI PubMed PMC

67.   O'Morain VL, Chan YH, Williams JO, et al. The Lab4P consortium of probiotics attenuates atherosclerosis in LDL receptor deficient mice fed a high fat diet and causes plaque stabilization by inhibiting inflammation and several pro-atherogenic processes. *Mol Nutr Food Res* 2021;65:e2100214. DOI PubMed PMC

68.   Sun Y, Wu Y, Jiang Y, Liu H. Aerobic exercise inhibits inflammatory response in atherosclerosis via Sestrin1 protein. *Exp Gerontol* 2021;155:111581. DOI PubMed

69. Kwaifa IK, Bahari H, Yong YK, Noor SM. Endothelial dysfunction in obesity-induced inflammation: molecular mechanisms and clinical implications. *Biomolecules* 2020;10:291. DOI PubMed PMC

70. Ridker PM, Devalaraja M, Baeres FMM, et al; RESCUE Investigators. IL-6 inhibition with ziltivekimab in patients at high atherosclerotic risk (RESCUE): a double-blind, randomised, placebo-controlled, phase 2 trial. *Lancet* 2021;397:2060-9. DOI

71. Bhasin S, Gill TM, Reuben DB, et al; STRIDE Trial Investigators. A randomized trial of a multifactorial strategy to prevent serious fall injuries. *N Engl J Med* 2020;383:129-40. DOI PubMed PMC

72. Fatkhullina AR, Peshkova IO, Koltsova EK. The role of cytokines in the development of atherosclerosis. *Biochemistry* 2016;81:1358-70. DOI PubMed PMC

73. Han SK, Kim DH. Lactobacillus mucosae and Bifidobacterium longum synergistically alleviate immobilization stress-induced anxiety/depression in mice by suppressing gut dysbiosis. *J Microbiol Biotechnol* 2019;29:1369-74. DOI PubMed

74. Primec M, Klemenak M, Di Gioia D, et al. Clinical intervention using *Bifidobacterium* strains in celiac disease children reveals novel microbial modulators of TNF-α and short-chain fatty acids. *Clin Nutr* 2019;38:1373-81. DOI

75. Rodríguez-Nogales A, Algieri F, Garrido-Mesa J, et al. Differential intestinal anti-inflammatory effects of *Lactobacillus fermentum* and *Lactobacillus salivarius* in DSS mouse colitis: impact on microRNAs expression and microbiota composition. *Mol Nutr Food Res* 2017;61:1700144. DOI PubMed

76. Wang Q, Sun Q, Wang J, Qiu X, Qi R, Huang J. Lactobacillus plantarum 299v changes miRNA expression in the intestines of piglets and leads to downregulation of LITAF by regulating ssc-miR-450a. *Probiotics Antimicro* 2021;13:1093-105. DOI

77. Rudzki L, Ostrowska L, Pawlak D, et al. Probiotic *Lactobacillus Plantarum* 299v decreases kynurenine concentration and improves cognitive functions in patients with major depression: a double-blind, randomized, placebo controlled study. *Psychoneuroendocrinology* 2019;100:213-22. DOI PubMed

78. Aker S, Bantis C, Reis P, et al. Influence of interleukin-6 G-174C gene polymorphism on coronary artery disease, cardiovascular complications and mortality in dialysis patients. *Nephrol Dial Transplant* 2009;24:2847-51. DOI

79. Han W, Wei Z, Zhang H, et al. The association between sortilin and inflammation in patients with coronary heart disease. *J Inflamm Res* 2020;13:71-9. DOI PubMed PMC

80. Zaharuddin L, Mokhtar NM, Muhammad Nawawi KN, Raja Ali RA. A randomized double-blind placebo-controlled trial of probiotics in post-surgical colorectal cancer. *BMC Gastroenterol* 2019;19:131. DOI PubMed PMC

81. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. *J Clin Invest* 2003;111:1805-12. DOI PubMed PMC

82. Peña E, de la Torre R, Arderiu G, Slevin M, Badimon L. mCRP triggers angiogenesis by inducing F3 transcription and TF signalling in microvascular endothelial cells. *Thromb Haemost* 2017;117:357-70. DOI

83. Li Y, Zhong X, Cheng G, et al. Hs-CRP and all-cause, cardiovascular, and cancer mortality risk: a meta-analysis. *Atherosclerosis* 2017;259:75-82. DOI

84. Akkasheh G, Kashani-Poor Z, Tajabadi-Ebrahimi M, et al. Clinical and metabolic response to probiotic administration in patients with major depressive disorder: a randomized, double-blind, placebo-controlled trial. *Nutrition* 2016;32:315-20. DOI

85. Ostadmohammadi V, Jamilian M, Bahmani F, Asemi Z. Vitamin D and probiotic co-supplementation affects mental health, hormonal, inflammatory and oxidative stress parameters in women with polycystic ovary syndrome. *J Ovarian Res* 2019;12:5. DOI PubMed PMC

86. Moss JWE, Williams JO, Ramji DP. Nutraceuticals as therapeutic agents for atherosclerosis. *Biochim Biophys Acta Mol Basis Dis* 2018;1864:1562-72. DOI PubMed PMC

87. Ooi BK, Chan KG, Goh BH, Yap WH. The role of natural products in targeting cardiovascular diseases via Nrf2 pathway: novel molecular mechanisms and therapeutic approaches. *Front Pharmacol* 2018;9:1308. DOI PubMed PMC

88. Rezazadeh L, Gargari BP, Jafarabadi MA, Alipour B. Effects of probiotic yogurt on glycemic indexes and endothelial dysfunction markers in patients with metabolic syndrome. *Nutrition* 2019;62:162-8. DOI PubMed

89. Malik M, Suboc TM, Tyagi S, et al. *Lactobacillus plantarum* 299v supplementation improves vascular endothelial function and reduces inflammatory biomarkers in men with stable coronary artery disease. *Circ Res* 2018;123:1091-102. DOI PubMed PMC

90. Aboulgheit A, Karbasiafshar C, Zhang Z, et al. *Lactobacillus plantarum* probiotic induces Nrf2-mediated antioxidant signaling and eNOS expression resulting in improvement of myocardial diastolic function. *Am J Physiol Heart Circ Physiol* 2021;321:H839-49. DOI PubMed PMC

91. Bian W, Jing X, Yang Z, et al. Downregulation of LncRNA NORAD promotes Ox-LDL-induced vascular endothelial cell injury and atherosclerosis. *Aging* 2020;12:6385-400. DOI PubMed PMC

92. Zhang N, Zhang Y, Wu B, et al. Deacetylation-dependent regulation of PARP1 by SIRT2 dictates ubiquitination of PARP1 in oxidative stress-induced vascular injury. *Redox Biol* 2021;47:102141. DOI PubMed PMC

93. Yan G, Zhang L, Feng C, et al. Blue light emitting diodes irradiation causes cell death in colorectal cancer by inducing ROS production and DNA damage. *Int J Biochem Cell Biol* 2018;103:81-8. DOI

94. Friques AG, Arpini CM, Kalil IC, et al. Chronic administration of the probiotic kefir improves the endothelial function in spontaneously hypertensive rats. *J Transl Med* 2015;13:390. DOI PubMed PMC

95. Lee JY, Kang CH. Probiotics alleviate oxidative stress in H_2O_2-exposed hepatocytes and t-BHP-induced C57BL/6 mice. *Microorganisms* 2022;10:234. DOI PubMed PMC

96. Herrington W, Lacey B, Sherliker P, Armitage J, Lewington S. Epidemiology of atherosclerosis and the potential to reduce the global burden of atherothrombotic disease. *Circ Res* 2016;118:535-46. DOI PubMed

97.   Bao MH, Li JM, Zhou QL, et al. Effects of miR-590 on oxLDL-induced endothelial cell apoptosis: roles of p53 and NF-κB. *Mol Med Rep* 2016;13:867-73. DOI PubMed

98.   Liu J, Li T, Pei W, et al. Lipidomics reveals the dysregulated ceramide metabolism in oxidized low-density lipoprotein-induced macrophage-derived foam cell. *Biomed Chromatogr* 2022;36:e5297. DOI PubMed

99.   Lew LC, Hor YY, Jaafar MH, et al. Lactobacilli modulated AMPK activity and prevented telomere shortening in ageing rats. *Benef Microbes* 2019;10:883-92. DOI

100.   Ge Q, Yang B, Liu R, et al. Antioxidant activity of *Lactobacillus plantarum* NJAU-01 in an animal model of aging. *BMC Microbiol* 2021;21:182. DOI PubMed PMC

101.   Meephat S, Prasatthong P, Potue P, Bunbupha S, Pakdeechote P, Maneesai P. Diosmetin ameliorates vascular dysfunction and remodeling by modulation of Nrf2/HO-1 and p-JNK/p-NF-κB expression in hypertensive rats. *Antioxidants* 2021;10:1487. DOI PubMed PMC

102.   Lv D, Luo M, Yan J, Yang X, Luo S. Protective effect of sirtuin 3 on CLP-induced endothelial dysfunction of early sepsis by inhibiting NF-κB and NLRP3 signaling pathways. *Inflammation* 2021;44:1782-92. DOI

103.   Gresele P, Momi S, Guglielmini G. Nitric oxide-enhancing or -releasing agents as antithrombotic drugs. *Biochem Pharmacol* 2019;166:300-12. DOI PubMed

104.   Toral M, Gómez-Guzmán M, Jiménez R, et al. The probiotic Lactobacillus coryniformis CECT5711 reduces the vascular pro-oxidant and pro-inflammatory status in obese mice. *Clin Sci* 2014;127:33-45. DOI PubMed

105.   Cizkova D, Cizek M, Maloveska M, Kmetova M, Kmet V, Bujnakova D. Cell-free *Lactobacillus casei* 21L10 modulates nitric oxide release and cell proliferation/cell death in lipopolysaccharide-challenged HT-29 cells. *Inflammation* 2021;44:2419-28. DOI PubMed

106.   Förstermann U, Closs EI, Pollock JS, et al. Nitric oxide synthase isozymes. Characterization, purification, molecular cloning, and functions. *Hypertension* 1994;23:1121-31. DOI

107.   Toral M, Romero M, Rodríguez-Nogales A, et al. *Lactobacillus fermentum* improves tacrolimus-induced hypertension by restoring vascular redox state and improving eNOS coupling. *Mol Nutr Food Res* 2018;62:e1800033. DOI PubMed

108.   Szulińska M, Łoniewski I, Skrypnik K, et al. Multispecies probiotic supplementation favorably affects vascular function and reduces arterial stiffness in obese postmenopausal women-A 12-week placebo-controlled and randomized clinical study. *Nutrients* 2018;10:1672. DOI PubMed PMC

109.   Tripolt NJ, Leber B, Blattl D, et al. Short communication: effect of supplementation with *Lactobacillus casei* Shirota on insulin sensitivity, β-cell function, and markers of endothelial function and inflammation in subjects with metabolic syndrome--a pilot study. *J Dairy Sci* 2013;96:89-95. DOI PubMed

110.   Reid G, Kim SO, Köhler GA. Selecting, testing and understanding probiotic microorganisms. *FEMS Immunol Med Microbiol* 2006;46:149-57. DOI PubMed

111.   Stuivenberg G, Daisley B, Akouris P, Reid G. In vitro assessment of histamine and lactate production by a multi-strain synbiotic. *J Food Sci Technol* 2022;59:3419-27. DOI PubMed PMC

112.   Lahtinen SJ, Boyle RJ, Margolles A, Frias R, Gueimonde M. Safety assessment of probiotics. In: Charalampopoulos D, Rastall RA, editors. Prebiotics and probiotics science and technology. New York: Springer; 2009. pp. 1193-235. DOI

113.   Sanders ME, Akkermans LM, Haller D, et al. Safety assessment of probiotics for human use. *Gut Microbes* 2010;1:164-85. DOI PubMed PMC