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Anticancer and Anti-Angiogenic Potentials of Probiotics and Their Bioactive Metabolites Against Colorectal Cancer and its Tumour Microenvironment: A Narrative Review

Umi Khalsom Mohd Bajuri, Kalavathy Ramasamy and Siong Meng Lim*

Collaborative Drug Discovery Research (CDDR) Group, Faculty of Pharmacy, Universiti Teknologi MARA (UiTM) Cawangan Selangor, Kampus Puncak Alam, 42300 Bandar Puncak Alam, Selangor Darul Ehsan, Malaysia.

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ABSTRACT

Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths globally. Unfortunately, current cytotoxic chemotherapy and targeted therapy against CRC are compromised by side effects and cancer resistance. This calls for alternative prevention strategies. Given the majority of CRC are sporadic in nature and associated with diet, probiotics (living microorganisms that when consumed in adequate amount will confer health benefits on the host) and their bioactive metabolites (postbiotics/functional compounds generated during fermentation) are deemed as viable options for chemoprevention against CRC. This narrative review highlights recent scientific evidence of the strain-dependent (single-/multi-strains) anticancer effects (action on CRC) as well as modulation of gut microbiota and angiogenesis (actions on tumour microenvironment) of probiotics and their bioactive metabolites. This review features the major mechanisms underlying the dual actions of probiotics and their bioactive metabolites against CRC. The anticancer effects of probiotics are related to reduced inflammation, cell apoptosis, suppressed tumour growth and viability, increased anti-oxidant activity and altered gut microbiota. The anti-angiogenic effects of probiotics are manifested through downregulation of pro-angiogenic VEGF and MMP2, MMP9, METTL3-related pathways, increased anti-angiogenic marker, decreased tumour microvascular density, tumour volume and tube formation as well as downregulation of angiogenesis markers. The most widely studied probiotic-derived bioactive metabolites (exopolysaccharides (EPS), macromolecules, short chain fatty acids (SCFA) and catalase) are also effective against CRC. EPS increased cell apoptosis, downregulated AKT-1, mTOR, JAK-1 pathways, inflammatory markers, cell proliferation, viability and tumour growth. Macromolecules increased Z0-1 protein level whilst reduced cell invasion and MMP-9 gene. SCFA induced cell apoptosis and pH-mediated switch between apoptosis and necrosis, whilst reduced cell viability, proliferation and inhibited Wnt-β/ catenin pathway. Catalase decreased hydrogen peroxide level and increased catalase activities. Generally, clinical evidence of probiotics and their bioactive metabolites against CRC remains limited and their strain-dependent effects pose challenges for clinical applications.

* Corresponding author. *E-mail address*: stevenlim79@yahoo.com, lim219@uitm.edu.my

INTRODUCTION

Colorectal cancer (CRC), which is characterised by abnormal growth of normal cells in the colon lining or rectum (American Cancer Society, 2024), is a threat to global public health. The vast majority of CRC cases are sporadic (Keum & Giovannucci, 2019) and associated with multi-factors such as high intake of red meat, being overweight, and the lack of fibre in the diet (Christodoulides et al., 2020; Vernia et al., 2021). CRC is highly influenced by dietary patterns, lifestyle factors and obesity to the point that it can be considered markers for socioeconomic development (Sawicki et al., 2021). The actual cause of CRC hitherto remains poorly understood. Nevertheless, imbalanced gut microbiota and intestinal metabolome are increasingly linked to CRC (Li et al., 2022; Peng et al., 2021). Dysbiosis, which involves perturbations in microbial populations, may cause

inflammation that alters gut microbes/ gut immune system interactions and eventually leads to CRC (Peng et al., 2021). There is growing evidence indicating that restoration of gut microbiota could potentially prevent CRC (Alrafas et al., 2020; Yang et al., 2020).

Although chemotherapy remains the main treatment modality against advanced CRC, the side effects of chemotherapy and the constant development of multidrug resistance by cancer have, however, become the stumbling block of effective treatment against CRC (Ashique et al., 2024) . Recently, targeted therapy (i.e., monoclonal antibodies and small molecule inhibitors) that targets tumour angiogenesis has emerged as a promising treatment modality against CRC (Battaglin et al., 2018; Ohishi et al., 2023). Unfortunately, an anti-angiogenic agent like bevacizumab is also associated with side effects (Minhajat et al., 2023). The development of cancer resistance also poses great challenges against the effective use of targeted therapy (Ohishi et al., 2023).

The limitations of chemotherapy and targeted therapy have called for alternative prevention strategies against CRC through diet modifications (Kim et al., 2022). In this regard, probiotics and their bioactive metabolites are increasingly recognised for their role in preventing CRC strain-dependently (Eslami et al., 2019; Yue et al., 2020b). Probiotics have been recommended as an alternative for managing CRC due to their reported safety profiles and absence of adverse effects on humans (Ayivi et al., 2020). Probiotics have a long history of safe usage and are categorised as "Generally Recognised as Safe" (GRAS) microorganisms (Kvakova et al., 2022). Probiotics reportedly had fewer side effects when tested in preclinical settings (Sanders et al., 2010). Elsewhere, probiotics supplementations have been used to reduce chemoresistance induced by *Fusobacterium nucleatum* and enhance the effectiveness of chemotherapy by inducing oxidative stress that caused cancer cell death (Ha et al., 2024).

Their health-promoting properties are linked to their ability to enhance gut microbiota and boost immune defenses, thus preventing CRC without pharmacological interventions (Anderson & Sears, 2023; Ha et al., 2024). There were already clinical trials (NCT04131803 and NCT03742596) that were performed to understand the association between gut microbiota modulation, SCFA and CRC (Hou et al., 2022). SCFA-producing probiotics may be used as a gut microbiota-based therapy to increase the abundance of SCFA and SCFA-producing bacteria to inhibit intestinal tumour development (Kang et al., 2023). Interestingly, SCFAs-guided modulation in mouse and human CRC models augmented their responses to chemotherapy and immunotherapy (Hou et al., 2022).).

Considering the increased importance of probiotics and their bioactive metabolites for prevention against cancer, the present narrative review aims at highlighting recent scientific evidence of the strain-dependent (single- and multi-strains) anticancer and anti-angiogenesis effects of probiotics and their bioactive metabolites against CRC and its tumour microenvironment. The growing preclinical evidence of the strain-dependent beneficial effects of probiotics and their bioactive metabolites against CRC warrants translation into clinical settings. More importantly, this review also features the major mechanisms underlying the actions of probiotics and their bioactive metabolites against CRC and its tumour microenvironment. This will certainly provide important insights into clinical applications of superior probiotic strains and/ or their bioactive metabolites against CRC and its tumor microenvironment and further validation of their specific mechanisms of action.

Probiotics

Probiotics are *live microorganisms that, when administered in adequate amounts, confer a health benefit on the host* (FAO/WHO, 2002; Hill et al., 2014). The characteristics of probiotics include being non-pathogenic, of human origin, resistant to processing, low pH condition, high tolerance to gastric juice, able to produce antimicrobial substances such as bacteriocins, and able to colonise the gastrointestinal tract (GIT) (Fijan, 2023). In general, probiotics are made up of bacteria, mold, and yeast. The most common microorganisms used as probiotics are lactic acid bacteria (LAB) and bifidobacteria (Taye et al., 2021). The LAB, which are gram-positive bacteria, carry out a fermentation process, producing lactic acid as the by-product from carbohydrates via heterofermentative or homofermentative pathways (Ayivi et al., 2020; Fijan, 2023). LAB like the lactobacilli are widely used against pathological conditions as they elicit better adherence and colonisation of the human GIT, hence promoting and maintaining a better immune system (Ayivi et al., 2020; Singh et al., 2011). Bifidobacteria, on the other hand, are key components of human gut microbiota and amongst the early microbial colonisers of the intestines of newborns (Ali et al., 2024). Some *Bifidobacterium* strains are probiotics that modulate the human immune system and exert anti-cancer effects against colon tumors (Ali et al., 2024; Asadollahi et al., 2020). Generally, the benefits of probiotics can be categorised into three levels of action: i) interference of the growth of pathogenic microorganisms, ii) immunomodulation and iii) improvement of mucosal immune system and barrier function (George et al., 2018).

Both single and multi-strain probiotics have been observed to yield beneficial effects. However, there is no significant evidence that showed multi-strain probiotics could exert the desired effects better than single strain or otherwise. In most cases, single strain probiotics exert equivalent effects as multi-strain probiotics. As such, the choice of probiotics should not be based on the number of strains but rather on evidence-based trials (McFarland, 2021).On another note, the use of probiotics to stimulate production SCFA by increasing butyrate-producing bacteria could also be beneficial for gut microbiota restoration and immunomodulation (Rivière et al., 2016). The beneficial effects of probiotics are also strain-dependent. Some probiotic strain may exert multiple mechanisms against CRC. For example, *L. rhamnosus* were found to reduce the inflammatory proteins, NFκB-p65 and TNFα, in DMH-induced rat model (Gamallat et al., 2016; Huang et al., 2019). Elsewhere, *L. rhamnosus* were also found to induce cell apoptosis through upregulation of caspase 3, caspase 9 and Bax as well as downregulation of Bcl2 in both *in vitro* (Dehghani et al., 2021) and *in vivo* models (Gamallat et al., 2016; Gamallat et al., 2019). Different strains of probiotics may exert similar mechanisms of action but at varying degree. For example, different substrains of *L. paracasei* (Huang et al., 2016), *L. plantarum* (Adiyoga et al., 2022; Yue et al., 2020b) *and L. rhamnosus* (Pahumunto & Teanpaisan, 2023; Taherian-Esfahani et al., 2016) reduced CRC cell growths and proliferations. Regarding anti-angiogenic effect, both *L. rhamnosus* (Gamallat et al., 2016; Huang et al., 2019) and *L. plantarum* reduced VEGF expressions (Fareez et al., 2024; Yue et al., 2020b). More specially, probiotic supernatants from *P. acidilactici*, *L. helveticus* and *L. rhamnosus*, for instance, showed an increase in the anti-inflammatory cytokines, IL-10. *P. acidilactici* increased IL-10 by two-fold against HT26 (Barigela & Bhukya, 2021) whereas *L. helveticus* increased IL-10 by three-fold in AOM/ DSS-induced mouse colon (Rong et al., 2018). Elsewhere, *L. rhamnosus* GG increased IL-10 by two-fold in DMH-induced ACF rat model (Gamallat et al., 2019). Besides, *L. plantarum* LAB12 downregulated COX-2 by 57% in CT26-ortotopic nude mouse model (Fareez et al., 2024) whereas *L. acidophilus* 314 reduced 71% of COX-2 level in C57BL/6J-Apc^{Min}/J mouse model (Urbanska et al., 2010).

Probiotics as viable alternatives with anticancer effects against CRC

Given the main treatments against CRC are associated with many health complications which could, in turn, affect the quality of life of the patients, probiotics have been identified as one of the potential alternatives to address these issues (Dikeocha et al., 2021). It was suggested that LAB may exhibit strain-dependent targeting of anticancer pathway or modulation of the immune system (Deng et al., 2023; Zhong et al., 2014). Additionally, the protective role of probiotics against CRC is also increasingly based on the hypothesis that dysbiosis could be the main cause of the disease (Marmol et al., 2017). Recent evidence has started to link CRC to gut microbiota which is modulated by diet and other environmental factors (Conlon & Bird, 2015; David et al., 2014; DeWeerdt, 2015; Zackular et al., 2016). Studies which compared the composition of gut bacteria in stool samples of CRC patients and healthy individuals found significant decrease of good bacteria such as bifidobacteria in the diseased group (Sobhani et al., 2013; Wang et al., 2012). An imbalanced gut environment or dysbiosis may induce inflammation or alter immune system, leading to gene instability (Ciernikova et al., 2015; Gao et al., 2017; Meng et al., 2018; Yamamoto & Matsumoto, 2016). Dysbiosis may be driven by *Bacteroides fragilis* toxin (BFT) and the polyketide synthase (pks)-expressing clade of *Escherichia coli*, which may have also either interfered with the host DNA repair capabilities or overexpressed specific pro-inflammatory pathways (Drewes et al., 2016). Probiotics could help to maintain the balance of microbiota environment and yield positive impact towards anticancer activity (Butel, 2014; Chen & Khismatullin, 2014; Chong, 2014). LAB with their bioactive metabolites could reportedly alter microbial populations in the colon and epigenetics (Kumar et al., 2013).

There is mounting evidence that supports the use of probiotics against CRC. Conventionally, probiotics have been found to exert beneficial effects like protection of DNA from oxidative damage, alteration in carcinogen metabolism, reduction of inflammation, suppression of microbiota that are responsible for mutagen production, increment of the number of beneficial bacteria in the gut, increased production of anticancer substances, reduction of carcinogens and enhancement of immune response**.** The anti-inflammatory effects of probiotics and their bioactive metabolites, in particular, may be related to multiple pathways. For instance, a probiotic mixture of VSL#3 elicited anti-inflammation activity through decreased levels of TNF-α and IL-6, inactivation of NF-Kb, and improved colonic inflammation (Kumar et al., 2017; Wang et al., 2018). In yet another study, *L. helveticus* S8 decreased the expression of NF-κB and upregulated IL-10 cytokine production (Rong et al., 2018). In addition, exopolysaccharides (EPS) from *L. acidophilus* 20079 showed anti-inflammatory pathways in colon cancer by modulating apoptotic and nuclear factor-κB (NF-κB) inflammatory pathways (El-Deeb et al., 2018). Besides, the Wingless and Int (Wnt) signaling pathway is also commonly affected by probiotics against CRC. One of the common branches of Wnt pathway that is associated with CRC is the Wnt/ β-catenin pathway. *C. butyricum* decreased proliferation and increased apoptosis through Wnt signalling pathway (Chen et al., 2020). *L. fermentum*

ZS09 inhibited epithelial-mesenchymal transition through the Wnt/β-catenin signaling pathway (Liu et al., 2021). In addition, *L. rhamnosus* GG was also found to regulate mTOR and Wnt signalling pathways (Taherian-Esfahani et al., 2016). Apart from that, the Janus kinase (JAK)/ signal transducer and activator of transcription (STAT) signaling pathways were also associated with mechanisms of probiotics against CRC. *L. paracasei* downregulated antiproliferative and apoptotic JAK-1 genes (Mousavi Jam et al., 2021). In addition, EPS from probiotic yeast, *Kluyveromyces marxianus* and *Pichia kudriavzevii* hindered AKT-1, mTOR, and JAK-1 pathways, and induced apoptosis in CRC cells (Saadat et al., 2020). *Lactobacillus casei* Zhang (LCZ) also showed antiproliferation potential by decreasing the production of host inflammatory cytokines as well as inhibiting the phosphorylation of signal transducer and activator of transcription 3 (stat3) that may be related to the JAK/STAT signaling pathway. Regarding angiogenesis, probiotics act as anti-angiogenic agents through mechanisms such as the VEGF signaling pathway. The VEGF receptors contribute to cell proliferation and survival. VEGF immunoexpression was also upregulated with cancer progression and interconnected with other receptors that may promote cancer cells progression (Zhou et al., 2018). Probiotics such as *Saccharomyces boulardii* reduce cancer cell growth by downregulating hypoxia-inducible factor (HIF) 1 and 2, which are linked to VEGF expression and may induce inflammation and tumor growth (Zhou et al., 2018).

Fig. 2.1 illustrates the anticancer mechanisms of probiotics against CRC and its tumour microenvironment based on recent preclinical evidence. In general, the beneficial effects of probiotics against CRC are mediated through anti-inflammation activity, regulation of immune responses, apoptosis, suppression of cancer cell/ tumour growth, migration and invasion, anti-oxidant activity and regulation of the gut microbiota. Table 2.1 summarises the recent *in vitro* and *in vivo* evidence of mechanisms underlying the probiotic (including their components/ fermented supernatant)-induced strain-dependent anticancer effects and modulation of gut microbiota against CRC.

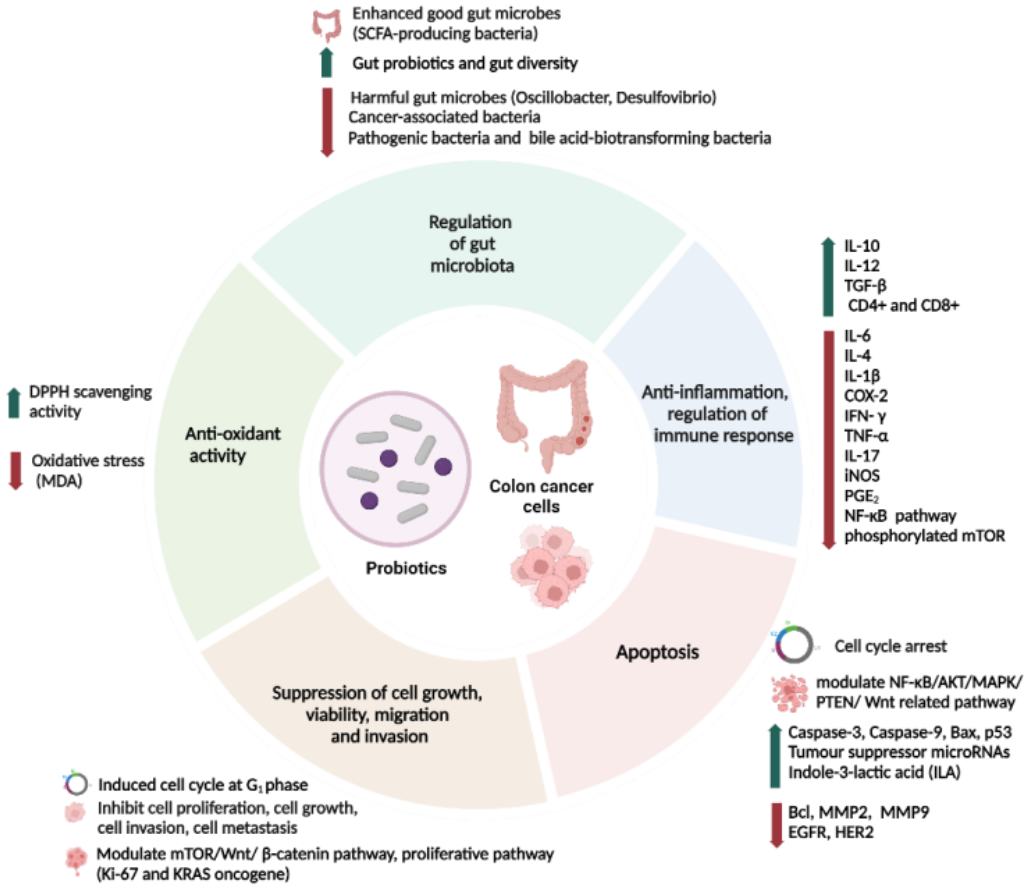

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Table 2.1 *In vitro* and *in vivo* evidence of mechanisms underlying the probiotic (including their components/ fermented supernatant)-induced strain-dependent anti-cancer effects against CRC

Anticancer mechanisms		**Probiotics**	**Experimental model**	**Findings**	**References**
Anti-inflammation and regulation of immune responses	*In vitro*				
	Single-strain	*P. acidilactici* TMAB26 (culture supernatant)	HT-29 and Caco-2	Reduced mRNA levels of TNF-α, IL-6 but increased mRNA levels of IL-10	(Barigela & Bhukya, 2021)
		L. plantarum OCO1 (cell free supernatant)	HCT116 and HT-29	Downregulated pro-growth and pro-migratory activity of IL-6. These effects were associated with inhibition of the ERK and of the mTOR/p70S6k pathways and with the inhibition of the E- to N-Cadherin switch.	(Vallino et al., 2023)
	In vivo				
	Single-strain	*L. casei* Zhang	AOM/ DSS-induced mouse model	Inhibited CRC-risk microbes and enhanced adiponectin secretion, but triggered different anti-inflammatory and anti-oncogenic pathways	(Zhang et al., 2017)
		L. helveticus NS8	AOM/ DSS-induced mouse model	Inactivation of the proinflammatory NF-κB pathway and upregulation of the anti-inflammatory IL-10 cytokines as well as marked downregulation of IL-17-producing T cells	(Rong et al., 2018)
		Bacillus subtilis	AOM/ DSS-induced mouse model	The mRNA expression levels of IL-6 and IL-17a were lower while those of IL-10 and TGF-β1 were higher	(Wu et al., 2019)
		S. cerevisiae SC-2201	AOM/ DSS-induced mouse model	Suppressed the expression of proinflammatory mediators which included interleukin-1β, interleukin-6, cyclooxygenase-2, nucleotide-binding domain, leucine-rich repeat and pyrin domain-containing protein 3	(Wang et al., 2024)
		L. rhamnosus AFY06	AOM/ DSS-induced mouse model	Mitigated the intestinal inflammatory process by regulating the NF-κB pathway	(Zhang et al., 2024)
		L. acidophilus	AOM-induced mouse model.	Increased level of CD4+ and CD8+ as well as IL-10 and IFN- γ serum levels	(Agah et al., 2018)
		Candida albicans	AOM-induced CRC rat model	Decrease the serum level IFN-γ, IL-4 and TGF-β	(Shams et al., 2021)
		L.plantarum			
		L. acidophilus	AOM-induced mouse model.	Increased level of CD4+ and CD8+ as well as IL-10 and IFN- γ serum levels	(Agah et al., 2018)
		L. rhamnosus GG	DMH-induced aberrant crypt foci (ACF) rat model	Reduced the inflammatory proteins NFκB-p65, COX-2 and TNFα	(Gamallat et al., 2016)
		L. rhamnosus GG	DMH-induced ACF rat model	Elevated serum IL-2, IL-6, and IFN-γ, but attenuated serum level of IL-10.	(Gamallat et al., 2019)
		L. rhamnosus	DMH-induced rat model	Downregulated protein expressions of iNOS,	(Huang et al., 2019)

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			TNF-α, NF-kB, COX-2	
	L. plantarum LAB12	CT26-ortotopic nude mouse model	Downregulated COX-2	(Fareez et al., 2024)
	L. acidophilus 314	C57BL/6J-Apc^{Min}/J mouse model	Increased IL-12 serum level but reduced plasma C-reactive protein and downregulation of COX-2 expression	(Urbanska et al., 2010)
Multi-strain	Bifico: *B. longum* *Enterococcus faecalis* *L. acidophilus*	AOM/ DSS-induced mouse model	Downregulated *Tnfα*, *Il1β*, *Il6* and *Ptgs1* as well as reduced the proinflammatory PGE_2	(Song et al., 2018)
	Probiotics VSL#3: *B. breve* *B. infantis* *B. longum* *L. acidophilus* *L. casei* *L. delbrueckii subp. Bulgaricus* *L. plantarum* *Streptococcus salivarius*	AOM/ DSS-induced mouse model	Decreased the levels of TNF-α and IL-6 in the colon tissue	(Wa
	B. bifidum (Bb-02TM) *B. lactis* (BR-04TM) *B. lactis* (Bi-07TM) *L. acidophilus* (NCFM®) *L. paracasei* (Lpc 37T M)	1,2- dimethylhydeazine (DMH) mouse model	Stimulated the production of TGF-β and IL-10	(Reis et al., 2022)
	B. longum *B. bifidum* *L. acidophilus* *L. plantarum*	Mouse model bearing CT26 tumour	Infiltration of immune cells in the tumour tissues and an increased number of CD8+ cells in the tumour and spleen tissues	(Shang et al., 2020)
	L. acidophilus *L. fermentum* *L. gasseri* 52b *L. plantarum*	Mouse model bearing CT26 tumour	Lowered the production of IL-4 and TGF-β but increased production of IFN-γ and IL-12	(Hatami et al., 2023)
	B. breve, *B. lactis* *L. acidophilus* *L. plantarum* *L. reuteri* *L. rhamnosus* *S. boulardii*	HCT-116 ectopic xenograft mouse model	Suppressed ROS and decreased inflammatory cytokines as well as inhibited phosphorylated mTOR	(Geagea et al., 2019)
Apoptosis	*In vitro*			
Single-strain	Kefir (cell-free fractions)	HT-29 and Caco-2	Induced apoptosis and cell cycle arrest at G1 phase	(Khoury et al., 2014)

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	Leuconostoc mesenteroides (conditioned-medium)	HT-29	Induced apoptosis by modulating NF-κB/AKT/PTEN/MAPK pathways	(Vahed et al., 2017)
	L. acidophilus (cell-free extracts)	HT-29	Overexpression of caspase 3, caspase 9 and increased Bax/ Bcl-2 ratio	(Baghbani-Arani et al., 2020)
	L. delbrueckii (cell-free extracts)			
	L. salivarius Ren	HT-29	Suppressed cell proliferation and induced cell apoptosis through suppressing AKT signalling pathway	(Dong et al., 2020)
	L. rhamnosus GG (supernatant)	HT-29	Induced cell apoptosis by upregulation of caspase 3, caspase 9 and Bax as well as downregulation of Bcl2	(Dehghani et al., 2021)
	Lentilactobacillus buchneri (supernatant)	HT-29	Inhibited cell proliferation and upregulated Bax, caspase 3 and caspase 9	(Abedi et al., 2024)
	L. acidophilus (supernatant)	HT-29 and SW480	Upregulated BAX, CASP3, and CASP9 and downregulated BCl-2, MMP2, and MMP9 genes as well as increased the expression of tumour suppressor microRNAs	(Saffar et al., 2024)
	S. cerevisiae (supernatant)			
	L. paracasei K5	Caco-2	Induced apoptosis via modulation of expression of specific Bcl-2 family proteins	(Chondrou et al., 2018)
	L. casei (live, heat killed and cell free supernatant)	Caco-2	Induced cell apoptosis	(Elham et al., 2022)
	S. cerevisiae (heat killed)	SW480	Induced cell apoptosis via the Akt/NF-κb signalling pathway	(Shamekhi et al., 2019)
	L. gallinarum (culture-supernatant)	HCT116 and LoVo	Promoted cell apoptosis by producing indole-3-lactic acid (ILA)	(Naoki et al., 2022)
Multi-strain	*B. bifium* *B. breve* *B. logum*	LS174T	Induced cell apoptosis and downregulating EGFR and HER2	(Asadollahi et al., 2020)
In vivo				
Single-strain	*L. rhamnosus* GG	DMH-induced rat model	Increased Bax, p53, and caspase-3 proteins expression	(Gamallat et al., 2016; Gamallat et al., 2019)
	S. cerevisiae	DMH-induced rat model	Altered the function of P53, Bcl2	(Abedi et al., 2018)
	L. rhamnosus	DMH-induced rat model	Downregulated protein expression of bcl-2.	(Huang et al., 2019)
	L. salivarius Ren	DMH-induced rat model	Suppressed cell proliferation and induced cell apoptosis through suppressing AKT signalling pathway	(Dong et al., 2020)
	L. rhamnosus AFY06	AOM/ DSS-induced mouse model	Inhibited intestinal tumour development by regulating the apoptosis pathway	(Zhang et al., 2024)

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		L. plantarum LAB12	CT26-ortotopic nude mouse model	Upregulated p53 and Caspace-3	(Fareez et al., 2024)
		C. butyricum	$Apc^{min/+}$ mouse model	Decreased proliferation and increased apoptosis through Wnt signalling pathway	(Chen et al., 2020)
Suppression of cell/ tumour viability, proliferation, growth, migration and invasion	*In vitro*				
	Single-strain	*L. paracasei* subp. *paracasei* X12	HT-29	Induced cell cycle at G_1 phase by inhibiting cyclin E1, meanwhile enhancing p27, which were mediated by mTOR/4EBP12 signalling pathway	(Huan Table continued overleaf
		L. crispatus SJ-3C-US (culture supernatant)	HT-29	Modulated expression of mTOR and Wnt/ β-catenin pathways genes	(Taherian-Esfahani et al., 2016)
		L. rhamnosus GG (culture supernatant)			
		L. plantarum YYC-3 (cell free supernatant)	HT-29 and Caco-2	Inhibited cell growth, invasion and migration	(Yue et al., 2020a)
		Faecalibacterium prausnitzii (cell-free supernatant)	HCT116	Inhibited cell proliferation	(Dikeocha et al., 2022)
		Propionibacterium freudenrichii (cell-free supernatant)	HCT116	Inhibited cell proliferation	(Dikeocha et al., 2023)
		L. acidophilus IIA2B4 (intracellular and extracellular extract)	WiDr	Inhibited cell growth	(Adiyoga et al., 2022)
		L. plantarum IIA-1A5 (intracellular and extracellular extract)			
		L. paracasei SD1 (cell-free supernatant)	Caco-2	Inhibited cell growth	(Pahumunto & Teanpaisan, 2023)
		L. rhamnosus SD11 (cell-free supernatant)			
	Multi-strain	*B. longum* *B. bifidum* *L. acidophilus* *L. plantarum*	CT26	Inhibited proliferation, invasion and migration of cells	(Shang et al., 2020)
	In vivo				
	Single-strain	*S. cerevisiae*	DMH-induced rat model	Reduction in size and number of ACF	(A Table continued overleaf
		L. rhamnosus GG	DMH-induced aberrant crypt foci rat model	Reduced formation of ACF	(G
		L. fermentum ZS09	AOM/ DSS-induced mouse model	Inhibited epithelial–mesenchymal transition through the Wnt/ β-catenin signalling pathway	(Liu et al., 2021)

		L. gallinarum	$Apc^{Min/+}$ mouse model AOM/ DSS mouse model	Inhibited colorectal tumourigenesis	(Naoki et al., 2022)
		Faecalibacterium prausnitzii	AOM-induced rat model	Reduced formation of ACF	(Dikeocha et al., 2022)
		Propionibacterium freudenrichii	AOM-induced rat model	Reduced formation of ACF	(Dikeocha et al., 2023)
		C. butyricum (supernatant)	HCT116 xenograft nude mouse mode	Suppressed tumour development and metastasis	(Zhang et al., 2023a)
	Multi-strain	*B. bifidum* (Bb-02TM) *B. lactis* (BR-04TM) *B. lactis* (Bi-07TM) *L. acidophilus* (NCFM®) *L. paracasei* (Lpc 37T M)	DMH-induced mouse model	Attenuated the proliferative pathway (Ki-67 and KRAS oncogene)	(Reis et al., 2022)
Antioxidant activity	*In vitro*				
	Single-strain	*Propionibacterium freudenrichii* (cell-free supernatant)	HCT116	Reduced oxidative stress (MDA)	(Dikeocha et al., 2023)
		L. acidophilus (cell free extracts)	HT-29	DPPH scavenging activity	(Baghbani-Arani et al., 2020)
		L. delbrueckii (cell free extracts)			
Regulation of gut microbiota	*In vivo*				
	Single-strain	*L. casei* Zhang	AOM/ DSS-induced mouse model	Increased some specific gut microbes	(Zh
		L. helveticus NS8	AOM/ DSS-induced mouse model	Promoted beneficial commensal microbes while suppressed cancer-associated microbes	(Rong et al., 2018)
		Bacillus subtilis	AOM/ DSS-induced mouse model	Increased the number of probiotics and inhibited the reproduction of harmful bacteria	(Wu et al., 2019)
		S. cerevisiae SC-2201	AOM/ DSS-induced mouse model	Alleviated the decreased *Bacteroidota* and *Campylobacterota*, increased *Proteobacteria* as well as increased *Basidiomycota, Apiosordaria, Naganishia,* and *Taphrina* genera in the colorectal cancer group. However, the levels of *Xenoramularia, Entoloma,* and *Keissleriella* were significantly increased.	(Wang et al., 2024)
		C. butyricum	$Apc^{min/+}$mouse model	Modulated the gut microbiota composition, as demonstrated by decreases in some pathogenic bacteria and bile acid (BA)-biotransforming bacteria and increases in some beneficial bacteria, including SCFA- producing bacteria	(Chen et al., 2020)
		L. gallinarum	$Apc^{Min/+}$ mouse model	Increased abundance of gut probiotics and depleted potential gut pathogens	(Naoki et al., 2022)

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	Faecalibacterium prausnitzii	AOM-induced rat model	Modulated the gut microbiota of the rats and enhanced its diversity	(Dikeocha et al., 2022)
	Propionibacterium freudenrichii	AOM-induced rat model	Enhanced the diversity of gut microbiota	(Dikeocha et al., 2023)
	L. rhamnosus GG	DMH-induced ACF rat model	Altered the gut microbiome structure, composition and functions at phylum, family and genus level	(Gamallat et al., 2019)
Multi-strain	Bifico	AOM/ DSS-induced mouse model	Decreased the abundance of genera *Desulfovibrio*, *Mucispirillum*, and *Odoribacter*, and increased the genus *Lactobacillus*	(Song et al., 2018)
	Probiotics VSL#3	AOM/ DSS-induced mouse model	Increased *Bacillus* and *Lactococcus* and decreased *Oscillibacter* and *Lachnoclostridium*	(Wang et al., 2018)
	Probiotics VSL#3	Trinitrobenzene sulfonic acid (TNBS)-induced rat model	A positive correlation between proximal colon dysplasia score and proximal colon tissue microbial richness or diversity.	(Appleyard et al., 2011)

Anti-inflammation and regulation of immune responses

The strain-dependent anti-inflammatory effects of either single or multi-strain probiotics against CRC appear to be widely studied. A previous *in vitro* study found *P. acidilactici* (culture supernatant) to reduce mRNA levels of TNF-α and IL-6 but increased mRNA levels of IL-10 in HT-29 and Caco-2 cells (Barigela & Bhukya, 2021). Another *in vitro* study of *L. plantarum* OCO1 (cell free supernatant) reported downregulation of pro-growth and pro-migratory activities of IL-6 on HCT116 and HT-29 cells which was associated with inhibition of the ERK and mTOR/p70S6k pathways as well as E-to N-Cadherin switch. (Vallino et al., 2023).

In terms of *in vivo* studies, which had predominantly adopted the AOM/ DSS-induced mouse model, *L. casei* Zhang, for instance, inhibited CRC-risk microbes and enhanced adiponectin secretion but triggered different anti-inflammatory and anti-oncogenic pathways (Zhang et al., 2017). *L. helveticus* NS8, another example, inactivated the proinflammatory NF-κB pathway and upregulated the anti-inflammatory IL-10 cytokines and downregulated IL-17-producing T cells (Rong et al., 2018). Elsewhere, *Bacillus subtilis* increased mRNA expressions of IL-10 and TGF-β1 but reduced mRNA expressions of IL-6 and IL-17a (Wu et al., 2019). In addition, *S. cerevisiae* SC-2201 suppressed the expression of proinflammatory mediators, including IL-1β, IL-6, COX-2, nucleotide-binding domain, leucine-rich repeat, and pyrin domain-containing protein 3 (Wang et al., 2024). Furthermore, *L. rhamnosus* AFY06 mitigated the intestinal inflammatory process by regulating the NF-κB pathway (Zhang et al., 2024). Interestingly, the multi-strain Bifico downregulated TNF-α, Il1β, Il6, and PTGS1, reducing the proinflammatory PGE2 (Song et al., 2018). Elsewhere, the multi-strain VSL#3 decreased the levels of TNF-α and IL-6 in the colon tissue (Wang et al., 2018).

In terms of AOM-induced rodent model, *L. acidophilus*, for example, increased level of CD4+ and CD8+ as well as IL-10 and IFN- γ serum levels (Agah et al., 2018). Meanwhile, *Candida albican*s and *L. plantarum* decreased IFN-γ, IL-4, and TGF-β (Shams et al., 2021)levels. In terms of DMH-induced aberrant crypt foci rat model, *L. rhamnosus* GG reduced the inflammatory proteins NFκB-p65, COX-2, and TNFα (Gamallat et al., 2016) but elevated serum IL-2, IL-6, and IFN-γ as well as an attenuated serum level of IL-10 (Gamallat et al., 2019). *L. rhamnosus*, on the other hand, downregulated protein expressions of iNOS, TNF-α, NF-kB, and COX-2 (Huang et al., 2019). Elsewhere, multi-strain probiotics stimulated the production of TGF-β and IL-10 (Reis et al., 2022).

Regarding rodents bearing CT-26 tumour, *L. plantarum* LAB12 downregulated COX-2 (Fareez et al., 2024). Elsewhere, various combinations of multi-strain probiotics resulted in the infiltration of immune cells in the tumor tissues and an increased number of CD8+ cells in the tumor and spleen tissues (Shang et al., 2020), lowered production of IL-4 and TGF-β but increased production of IFN-γand IL-12 (Hatami et al., 2023). Regarding rodents bearing HCT-116 tumour, multi-strain probiotics suppressed ROS, decreased inflammatory cytokines, and inhibited phosphorylated mTOR (Geagea et al., 2019). Regarding the C57BL/6J-Apc^{Min}/J mouse model, *L. acidophilus* 314 increased IL-12 serum level but reduced plasma C-reactive protein and downregulated COX-2 expression (Urbanska et al., 2010).

Apoptosis

Single or multi-strain probiotics could induce strain-dependent apoptosis against CRC by modulating key apoptosis regulators. In terms of *in vitro* studies, which had predominantly adopted the HT-29 cell line, Kefir (cell-free fractions), for instance, induced apoptosis and cell cycle arrest at the G1 phase (Khoury et al., 2014). *Leuconostoc mesenteroides* (conditioned-medium), for example, induced cell apoptosis by modulating NF-κB signalling pathway (Vahed et al., 2017). Elsewhere, *L. acidophilus* and *L. delbrueckii* (cell-free extracts) resulted in overexpression of caspase 3, caspase 9 and increased Bax/ Bcl-2 ratio (Baghbani-Arani et al., 2020). Additionally, *L. salivarius* Ren suppressed cell proliferation and induced cell apoptosis through suppressing AKT signalling pathway (Dong et al., 2020). Furthermore, *L. rhamnosus* GG (supernatant) induced cell apoptosis by upregulation of caspase 3, caspase 9 and Bax as well as downregulation of Bcl2 (Dehghani et al., 2021). Moreover, *Lentilactobacillus buchneri* (supernatant) inhibited cell proliferation and upregulated Bax, caspase 3 and caspase 9 (Abedi et al., 2024). Also, *L. acidophilus* and *S. cerevisiae* (supernatant) upregulated Bax, Casp3, and Casp9 and downregulated Bcl2, MMP2, and MMP9 genes and increased the expression of tumor suppressor microRNAs (Saffar et al., 2024).

In terms of Caco-2 cell line, *L. paracasei* K5 induced apoptosis via modulation of expression of specific Bcl-2 family proteins *in vitro* (Chondrou et al., 2018). *L. casei* (live, heat-killed, and cell-free supernatant) also induced cell apoptosis (Elham et al., 2022). As for the other cell lines, *S. cerevisiae* (heat-killed), a probiotic yeast,

induced cell apoptosis against SW480 via the Akt/NF-κb signaling pathway (Shamekhi et al., 2019). Elsewhere, *L. gallinarum* (culture-supernatant) promoted cell apoptosis against HCT116 and LoVo by producing indole-3-lactic acid (ILA) (Naoki et al., 2022). Interestingly, multi-strain probiotics induced cell apoptosis against LS174T by downregulating EGFR and HER2 (Asadollahi et al., 2020).

In terms of *in vivo* studies, which had predominantly adopted the DMH-induced rat model, *L. rhamnosus* GG increased Bax, p53, and Caspase-3 proteins expression (Gamallat et al., 2016; Gamallat et al., 2019). *S. cerevisiae*, on the other hand, altered the function of P53, Bcl2 (Abedi et al., 2018). Furthermore, *L. rhamnosus* downregulated protein expression of bcl-2 (Huang et al., 2019). Moreover, *L. salivarius* Ren suppressed cell proliferation and induced cell apoptosis by suppressing the AKT signaling pathway (Dong et al., 2020). Regarding the AOM/ DSS-induced mouse model, *L. rhamnosus* AFY06 inhibited intestinal tumour development by regulating the apoptosis pathway (Zhang et al., 2024). Regarding rodents bearing CT-26 tumour, *L. plantarum* LAB12 upregulated p53 and Caspace-3 (Fareez et al., 2024). In terms of $Apc^{min/+}$ mouse model, *Clostridium butyricum* decreased proliferation and increased apoptosis through Wnt signalling pathway (Chen et al., 2020)

Suppression of CRC growth, migration, and invasion

Single or multi-strain probiotics could induce strain-dependent suppression of CRC growth, migration and invasion. In *vitro* studies have predominantly adopted the HT-29 cell line, *L. paracasei* subp. *paracasei* X12, for instance, induced cell cycle at G_1 phase by inhibiting cyclin E1 but enhancing p27, which were mediated by mTOR/ 4EBP12 signalling pathway (Huang et al., 2016). For example, *L. crispatus* SJ-3C-US and *L. rhamnosus* GG (culture supernatant) modulated expression of mTOR and Wnt/ β-catenin pathway genes (Taherian-Esfahani et al., 2016). *L. plantarum* YYC-3 (cell-free supernatant), another example, inhibited cell growth, invasion, and migration (Yue et al., 2020a). Regarding the HCT116 cell line, *Faecalibacterium prausnitzii* and *Propionibacterium freudenrichii* (cell-free supernatant) inhibited cell proliferation (Dikeocha et al., 2023; Dikeocha et al., 2022). As with the other cell lines, *L. acidophilus* IIA2B4 and *L. plantarum* IIA-1A5 (intracellular and extracellular extract), for instance, inhibited WiDr's cell growth (Adiyoga et al., 2022). Elsewhere, *L. paracasei* SD1 and *L. rhamnosus* SD11 (cell-free supernatant) inhibited cell growth of Caco-2 (Pahumunto & Teanpaisan, 2023). Interestingly, multi-strain probiotics inhibited proliferation, invasion and migration of CT26 (Shang et al., 2020).

In terms of *in vivo* studies, which had predominantly adopted the DMH-induced rodent model, *S. cerevisiae* and *L. rhamnosus* GG reduced the formation of ACF (Abedi et al., 2018; Gamallat et al., 2019). In addition, multi-strain probiotics attenuated the proliferative pathway (Ki-67 and KRAS oncogene) (Reis et al., 2022). In terms of the AOM/ DSS-induced mouse model, *L. fermentum* ZS09 inhibited epithelial-mesenchymal transition through the Wnt/ β-catenin signaling pathway (Liu et al., 2021). *L. gallinarum* inhibited colorectal tumourigenesis (Naoki et al., 2022). Regarding AOM-induced rat model, *Faecalibacterium prausnitzii* and *Propionibacterium freudenrichii* reduced formation of ACF (Dikeocha et al., 2023; Dikeocha et al., 2022). Regarding rodents bearing HCT116 tumour, *Clostridium butyricum* (supernatant) suppressed tumour development and metastasis (Zhang et al., 2023a). As for the $Apc^{Min/+}$ mouse model, *L. gallinarum* also inhibited colorectal tumourigenesis (Naoki et al., 2022).

Antioxidant activity

Single strain probiotics could induce strain-dependent antioxidant activity. *Propionibacterium freudenrichii* (cell-free supernatant), for example, reduced oxidative stress (MDA) against HCT116 cell line (Dikeocha et al., 2023). *L. acidophilus* and *L. delbrueckii* (cell free extract), on the other hand, produced DPPH scavenging activity against HT-29 cell line (Baghbani-Arani et al., 2020).

Regulation of gut microbiota

Single or multi-strain probiotics could induce strain-dependent regulation of gut microbiota. In terms of *in vivo* studies, which had predominantly adopted the AOM/ DSS-induced mouse model, *L. casei* Zhang, for instance, increased some specific gut microbes (Zhang et al., 2017). *L. helveticus* NS8, for example, promoted beneficial commensal microbes while suppressing cancer-associated microbes (Rong et al., 2018). *Bacillus subtilis*, another example, increased the number of probiotics and inhibited the reproduction of harmful bacteria (Wu et al., 2019). *S. cerevisiae* SC-2201, yet another example, alleviated the decreased Bacteroidota and Campylobacterota, increased Proteobacteria as well as increased Basidiomycota, Apiosordaria, Naganishia, and

Taphrina genera in the CRC group. However, Xenoramularia, Entoloma, and Keissleriella levels were significantly increased (Wang et al., 2024). Multi-strain probiotic Bifico, on the other hand, decreased the abundance of genera *Desulfovibrio*, *Mucispirillum*, and *Odoribacter*, and increased the genus *Lactobacillus* (Song et al., 2018). Elsewhere, multi-strain probiotic #VSL3 increased *Bacillus* and *Lactococcus* and decreased *Oscillibacter* and *Lachnoclostridium* (Wang et al., 2018). Regarding the $Apc^{Min/+}$ mouse model, *C. butyricum* decreased pathogenic bacteria, bile acid-biotransforming bacteria and increased beneficial bacteria, including short-chain fatty acid-producing bacteria (Chen et al., 2020). Elsewhere, *L. gallinarum* increased abundance of gut probiotics and depleted potential gut pathogens (Naoki et al., 2022). Regarding AOM-induced rat model, *Faecalibacterium prausnitzii* and *Propionibacterium freudenrichii* enhanced gut microbiota diversity (Dikeocha et al., 2023; Dikeocha et al., 2022). Regarding the DMH-induced ACF rat model, *L. rhamnosus* GG altered the gut microbiome structure, composition and functions at the phylum, family and genus level (Gamallat et al., 2019). Regarding trinitrobenzene sulfonic acid (TNBS)-induced rat model, multi-strain probiotics yielded a positive correlation between proximal colon dysplasia score and proximal colon tissue microbial richness or diversity (Appleyard et al., 2011).

Probiotics as anti-angiogenic agents

CRC relies on the formation of new blood vessels through tumor angiogenesis for the supply of nutrients and oxygen (Bickel et al., 2014). Tumor angiogenesis also serves as the channel for the metastasis of CRC (Angelucci et al., 2018). Tumor angiogenesis is initiated by an "angiogenic switch," whereby pro-angiogenic and anti-angiogenic molecules derangement in favor of tumor neovascularization. Environment hypoxia, associated with enhanced expression and activation of transcription factor hypoxia-inducible-factor-1 (HIF-1) pathway or HIF-1-independent pathways, is thought to be the primary trigger of the angiogenic switch (Lin et al., 2016). The "angiogenic switch" is coordinated directly and indirectly by the signalling molecules such as the vascular endothelial growth factor (VEGF) that act as a pro-angiogenic molecule and thrombospondin 1 (TSP-1) that plays the role of a negative regulator of angiogenesis (Bickel et al., 2014). Disrupted balance of the pro-angiogenic and anti-angiogenic factors can promote the transition from avascular colonic tumor to angiogenic phenotypes (Mabeta et al., 2022). Fig. 2.2 illustrates the anti-angiogenic mechanisms of probiotics against CRC based on recent scientific evidence. In general, the anti-angiogenic effects of probiotics are mainly manifested through downregulation of the pro-angiogenic VEGF as well as MMP2, MMP9, METTL3-related pathways, increased anti-angiogenic marker (angiostatin), decreased tumor microvascular density, tumour volume and tube formation as well as downregulation of other angiogenesis markers (CCDNI, CD31, PECAM1, Gal-3). Table 2.2 summarises the *in vitro*, *in vivo*, and clinical evidence of probiotics (including their fermented supernatant)-induced

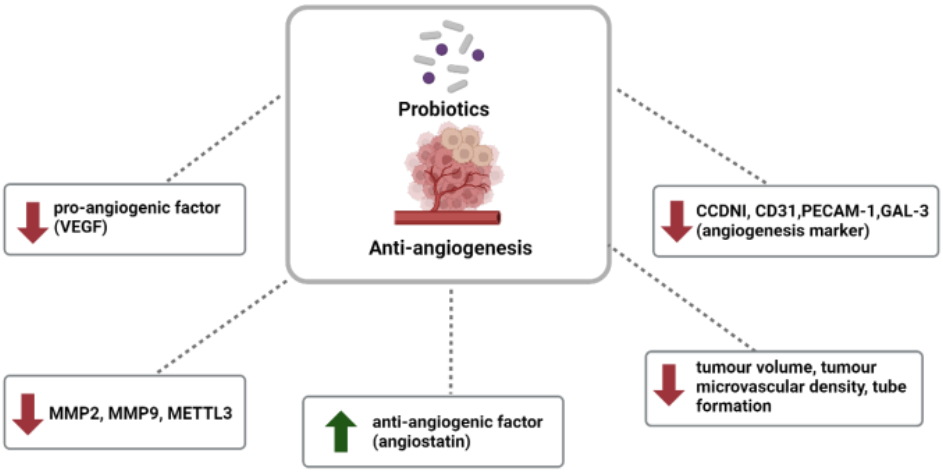

Fig. e mediated throu ays, (3) inc d tube formation, (5) downregulation of other angiogenesis markers (CCDNI, CD31, PECAM1, Gal-3). Remark: Part of this image was created with BioRender (BioRender.com).

Table 2.2 *In vitro, in vivo* and clinical evidence of probiotic (including their fermented supernatant)-induced strain-dependent anti-angiogenic effect against CRC.

	Probiotics	**Experimental model**	**Findings**	**References**
In vitro				
Single-strain	*L. crispatus* SJ-3C-US (culture supernatant)	HT-29	Decreased CCND1, an angiogenesis marker	(Taherian-Esfahani et al., 2016)
	L. rhamnosus GG (culture supernatant)			
	L. plantarum YYC-3 (cell free supernatant)	HT-29 and Caco-2	Inhibited MMP2, MMP9, and VEGFA gene and protein secretion	(Yue et al., 2020a)
	L. rhamnosus GG (supernatant)	HT-29 and HCT116 HUVEC	Produced pro-resolving, antiangiogenic (reduce VEGF-A release and lower number of tube structures) and homeostatic functions that were dependent on FPR1 expression and on the subsequent MAPK signalling activation	(Liotti et al., 2022)
	Clostridium butyricum (supernatant)	HCT116 and Caco-2	Downregulated METTL3 expression and decreased the expression of vimentin and VEGFR2 to reduce epithelial–mesenchymal transition and vasculogenic mimicry formation	(Zhang et al., 2023a)
In vivo				
Single-strain	*L. rhamnosus* GG	DMH-induced ACF rat model	Decreased VEGFα expression	(Gamallat et al., 2016)
	L. rhamnosus	DMH-induced rat model	Downregulated protein expressions of VEGF-α	(Huang et al., 2019)
	S. cerevisiae	DMH-induced rat model	Decreased expression of CD31	(Abedi et al., 2018)
	L. fermentum ZS09	AOM/ DSS- induced mouse model	Downregulated VEGF	(Liu et al., 2021)
	S. cerevisiae SC-2201	AOM/ DSS-induced mouse model	Suppressed the expression of VEGF	(Wang et al., 2024)
	L. acidophilus ATCC 4356	AOM-induced mouse model	Inhibited GAL-3 and VEGF immunoexpression	(Odun-Ayo et al., 2015)
	C. butyricum (supernatant)	HCT116 xenograft nude mouse model	Reduced METTL3 with depleted vimentin protein and strong E-cadherin staining as well as less tube formation	(Zhang et al., 2023b)
	L. plantarum LAB12	CT26-ortotopic nude mouse model	Downregulated VEGF and PECAM-1	(Fareez et al., 2024)
Multi -strain	Probiotics VSL#3	TNBS)-induced rat model	Increased the antiangiogenic factor angiostatin	(Appleyard et al., 2011)
	B. bifidum W23 *B. lactis* W52 *L. acidophilus* W37 *L. brevis* W63 *L. casei* W56 *L. lactis* W19 *L. lactis* W58	CC531 transplanted rat model	Reduced tumour volume was achieved by inhibiting angiogenesis, as tumour microvascular density was significantly lower	(Jakubauskas et al., 2022)

Table continued overleaf

	L. salivarius W24			
			Clinical trial	
	L. acidophilus	Rectal cancer patients	A significant expression increases of the selected tumour suppressor miRs (miR-20a related to angiogenesis), lncRNAs, and genes and a substantial expression decrease of the selected oncomiRs, onco-lncRNAs and oncogenes	(Khodaii et al., 2022)

The anti-angiogenic effect induced by single or multi-strain probiotics appears to be mediated predominantly through the VEGF-signalling pathway. In terms of in *vitro* studies, *L. crispatus* SJ-3C-US and *L. rhamnosus* GG (culture supernatant), for instance, decreased the angiogenesis marker, CCND1, in HT-29 cells (Taherian-Esfahani et al., 2016). *L. plantarum* YYC-3 (cell free supernatant), another example, reduced MMP2, MMP9 and VEGFA gene and protein secretion (Yue et al., 2020a). *L. rhamnosus* GG (supernatant), yet another example, produced pro-resolving, anti-angiogenic (reduced VEGF-A release and lower number of tube structures) and homeostatic functions that were dependent on FPR1 expression and on the subsequent MAPK signaling activation (Liotti et al., 2022). Elsewhere, *Clostridium butyricum* (supernatant) downregulated METTL3 expression and decreased the expression of vimentin and VEGFR2 to reduce epithelial-mesenchymal transition and vasculogenic mimicry formation in HCT116 and Caco-2 cell lines (Zhang et al., 2023a).

In terms of *in vivo* studies, which had predominantly adopted the DMH-induced rat model, *L. rhamnosus* GG decreased VEGFα expression (Gamallat et al., 2016; Huang et al., 2019) . *S. cerevisiae*, on the other hand, decreased the expression of CD31 (Abedi et al., 2018). Regarding the AOM/DSS-induced mouse model, *L. fermentum* ZS09 and *S. cerevisiae* SC-2201 downregulated VEGF (Liu et al., 2021; Wang et al., 2024). Regarding the AOM-induced mouse model, *L. acidophilus* ATCC 4356 inhibited GAL-3 and VEGF immunoexpression (Odun-Ayo et al., 2015). Regarding the TNBS-induced rat model, multi-strain probiotic VSL#3 increased the anti-angiogenic factor, angiostatin (Appleyard et al., 2011). Regarding rodents bearing HCT116 tumour, *Clostridium butyricum* (supernatant) reduced METTL3 with depleted vimentin protein and strong E-cadherin staining and less tube formation (Zhang et al., 2023b). Regarding rodents bearing CT-26 tumor, *L. plantarum* LAB12 downregulated VEGF and PECAM-1 (Fareez et al., 2024). Regarding rodents bearing CC531 tumours, multi-strain probiotics reduced tumor volume by inhibiting angiogenesis, whereby tumor microvascular density was significantly lower (Jakubauskas et al., 2022).

In terms of clinical trials that involved rectal cancer patients, *L. acidophilus* resulted in a significant expression increase of the selected tumor suppressor miRs (miR-20a related to angiogenesis), lncRNAs, and genes and a substantial expression decrease of the selected oncomiRs, once-lncRNAs, and oncogenes (Khodaii et al., 2022). The overexpression of miR-20a in CRC is linked to a reduction in TGF-βR2 protein levels, suggesting that TGF-βR2 is a direct target of the miR-17/20a cluster and this interaction inhibits downstream mediators, leading to suppression of angiogenesis (Dews et al., 2010). The modulation of miR expression by *L. acidophilus* may offer a novel approach against CRC whereby miR-20a may serve as a potential therapeutic target that could lead to personalized treatment strategies, optimizing patient-specific interventions against CRC. This could guide the development of probiotics and clinical guidelines that leverage their role in modulating miRNA expression. This also aligns with previous findings that support using probiotics as adjunct therapies, targeting molecular mechanisms specific to cancer subtypes, improving efficacy, and personalizing treatment strategies (Wang et al., 2021). That said, a critical evaluation of the clinical trial outcomes is needed to bridge the gap between preclinical and clinical relevance. In particular, selecting the right strains, evaluating potential risks, ensuring the standard quality of probiotics, refining delivery routes, and considering the variability of patients' gut microbial baseline must be properly performed before full clinical applications (Ha et al., 2024).

Probiotic-derived bioactive metabolites with anticancer and anti-angiogenic effects against CRC and its tumour microenvironment

Postbiotics, also known as metabiotics, refer to the products of probiotics that produce physiological beneficial effects on the hosts, commonly the digestive tract (Teame et al., 2020). They are metabolites that can affect the human microbiome and signaling pathways by modulating the intestinal composition while regulating the cellular processes and metabolic pathways related to the activity of the host microbiota (Sharma & Shukla, 2016; Shenderov, 2013). As such, postbiotics are increasingly recommended for their safety dose parameters, long shelf life, and various signaling molecules that can positively affect cell function (Aguilar-Toalá et al., 2018).

It appears that different probiotic strains may produce different postbiotics (Ma et al., 2023). There are a few components in postbiotics, such as SCFA, peptides, proteins, polysaccharides, teichoic acids, and plasmalogens, that are thought to enhance the ionic balance and prevent the formation of pro-carcinogens from carcinogenic products by decreasing levels of beta-glucuronidase, beta-glucosidase, and nitroreductase (Verma & Shukla, 2013). Postbiotics may also help create a low pH environment in the gut lumen, thus inhibiting pro-carcinogenic conversion, ameliorating the tight junction proteins, maintaining the cell surface receptors, modulating the inflammatory cytokines, and enhancing cell apoptosis (Sharma & Shukla, 2016). More specifically, postbiotics like butyrate could induce apoptosis against CRC and inhibit the Wnt/β-catenin pathway

by inhibiting histone deacetylase (HDAC) and suppressing tumor-promoting genes (Forouzesh et al., 2020). SCFA could also enhance gut barrier integrity by upregulating tight junction proteins and reduce inflammation against CRC by inhibiting NF-κB activation (Mennigen et al., 2009). Additionally, SCFA could induce cell cycle arrest by upregulating VEGF expression through the upregulation of cyclin-dependent kinase inhibitors and exert anti-angiogenic effects (Deepak et al., 2016).

SCFA could act independently by directly modulating host signaling pathways, gene expression, and immune responses. Butyrate, for example, could decrease cancer cell viability (Doublier et al., 2022). SCFA could also function synergistically with probiotics, whereby the metabolites could amplify the probiotics' effects, such as improving gut barrier integrity and reducing inflammation (Ji et al., 2023). Whilst some probiotics could upregulate the host's production of anti-inflammatory cytokines (Zhang et al., 2024), their bioactive metabolites, like exopolysaccharides, could enhance immune modulation (Zahran et al., 2017). Other probiotics could enhance microbial balance, whereas their bioactive metabolites could mediate host-microbial interactions for optimal health benefits (Ma et al., 2023).

Based on recent preclinical evidence, Fig. 2.3 illustrates the anti-angiogenic and anticancer mechanisms against CRC and its tumor microenvironment. In general, exopolysaccharides increased cell apoptosis and its related genes (Bax, Caspase 3, Caspase 9, p21, TIMP-3, HIF-2α, HO-1, and PAI-1) as well as downregulated AKT-1, mTOR, JAK-1 pathways, inflammatory markers, cell proliferation, viability, tumor growth and its related genes (VEGF, HIF-1α and Bcl-2). Meanwhile, macromolecules helped to increase the Z0-1 protein level and reduced cell invasion and the MMP-9 gene. Interestingly, SCFA induced cell apoptosis and pH-mediated switch between apoptosis and necrosis whilst reducing cell viability, cell proliferation, and Wnt-β/ catenin pathway. Catalase, on the other hand, decreased hydrogen peroxide levels and increased catalase activities. Table 2.3 summarises the details of the *in vitro* and *in vivo* evidence of anticancer and anti-angiogenic effects of bioactive metabolites commonly produced by probiotics against CRC and its tumor microenvironment.

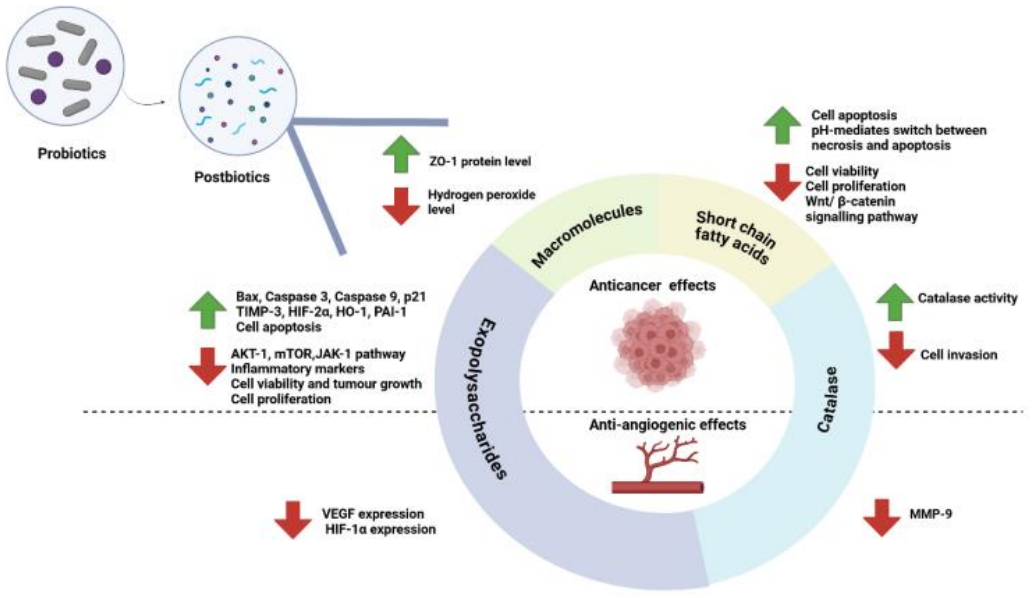

Fig. 2.3: Anti-angiogenic and anticancer postbiotic mechanisms against CRC and its tumor microenvironment based on recent preclinical evidence. The postbiotics are divided into four components: (1) exopolysaccharides (EPS), (2) macromolecules, (3) short-chain fatty acids, and (4) catalase. EPS increased cell apoptosis and its related genes (Bax, Caspase 3, Caspase 9, p21, TIMP-3, HIF-2α, HO-1, and PAI-1), downregulated AKT-1, mTOR, JAK-1 pathways, inflammatory markers, cell proliferation, viability, tumor growth and its related genes (VEGF, HIF-1α, and Bcl-2). Macromolecules helped to increase the Z0-1 protein level whilst reducing cell invasion and the MMP-9 gene. SCFA-induced cell apoptosis and pH-mediated switch between apoptosis and necrosis reduce cell viability and cell proliferation and inhibit the Wnt-β/ catenin pathway. Catalase decreased hydrogen peroxide levels and increased catalase activities. Remark: Part of this image was created with BioRender (BioRender.com).

Table 2.3 *In vitro* and *in vivo* evidence of anticancer and anti-angiogenic effects of bioactive metabolites commonly produced by probiotics against CRC

Bioactive metabolites	Source of postbiotics	Experimental model	Findings	References
			In vitro	
Exopolysaccharides	*L. brevis* LB63 *L. delbrueckii* ssp. *bulgaricus* B3 *L. plantarum* GD2 *L. rhamnosus* E9	HT-29	Induced apoptosis via increasing the expression of Bax, Caspase 3 and 9 while decreasing Bcl-2 and Survivin	(Tukenmez et al., 2019)
	Kluyveromyces marxianua *Pichia kudriavzevii*	HCT116, HT-29 and SW480	Hindered the AKT-1, mTOR, and JAK-1 pathways, and induced apoptosis	(Saadat et al., 2020)
	L. plantarum 12	HT-29	Inhibited cell proliferation, upregulated the expression of the pro-apoptotic proteins Bax, Cyt C, Caspase-3, Caspase-8 and Caspase-9 and decreased the expression of the anti-apoptosis protein Bcl-2	(Sun et al., 2021)
	Weissella confusa J4-1	HT-29	Inhibited cell proliferation by inducing G0/G1 phase cell cycle, upregulated p21 levels and downregulated mutant p53 and cyclin kinase 2 levels	(Liu et al., 2023)
	Pediococcus acidilactici NCDC 252	HCT116	Inhibited cell viability	(Kumar et al., 2020)
	L. acidophilus 20079	CaCo-2	Inhibited cell viability, increased ratio of the apoptotic cells in sub-G0/G1 cell cycle phase and up-regulated the expression of IKbα, P53 and TGF genes	(El-Deeb et al., 2018)
	L. acidophilus	HCT15 and Caco2	Downregulated the expression of VEGF, HIF-1α and upregulated the expression of TIMP-3, HIF-2α, HO-1 and PAI-1, suggesting anti-angiogenic and antioxidative properties	(Deepak et al., 2016)
Exopolysachharides (with 1Gy γ-R)	*L. rhamnosus* ATCC 7469	DMH-induced rat model	Ameliorated the oxidative and inflammatory biomarkers with modulated signalling molecular factors accompanied by improved histological structure	(Zahran et al., 2017)
Exopolysachharides	*Weissella confusa* J4-1	HT-29 xenograft model	Retarded tumour growth	(Liu et al., 2023)
Macromolecule such as a protein, nucleic acid, or a polysaccharide	*L. casei*	HCT116	Inhibited cell invasion by decreasing MMP-9 and increasing ZO-1 protein levels	(Escamilla et al., 2012)
	L. rhamnosus GG			
Butyrate, exopolysaccharides and	*L. plantarum* S2 *L. pentosus* S3	HT-29 and HT29-dx	Decreased cell viability	(Doublier et al., 2022)

Table continued overleaf

extracellular proteins	*L. rhamnosus* 14E4			
Acetate and propionate	*Propionibacterium acidipropionici* *P. freudenreichii*	Caco-2 and HT-29	Induced apoptosis with mitochondria and ANT involved in the cell death pathway	(Jan et al., 2002)
Acetate and propionate	*P. freudenreichii*	HT-29	Induced pH-mediated switch between apoptosis and necrosis	(Lan et al., 2007)
Short chain fatty acids	*C. butyricum*	$Apc^{min/+}$ mouse model	Decreased proliferation and increased apoptosis as well as suppressed the Wnt/ β-catenin signalling pathway	(Chen et al., 2020)
Catalase	*L. lactis*	DMH-induced mouse model	Increased catalase activities and reduced H_2O_2 levels with lesser extent of colonic damage and inflammation	(de Moreno de Leblanc et al., 2008)

Previous *in vitro* studies have predominantly investigated the anticancer and anti-angiogenic effects of probiotic-derived exopolysaccharides. Regarding HT-29 cell lines, probiotic-derived exopolysaccharides were widely reported for the induction of apoptosis. Exopolysaccharides from *L. brevis* LB63, *L. delbrueckii* ssp. *bulgaricus* B3, *L. plantarum* GD2 or *L. rhamnosus* E9 induced apoptosis via increasing the expression of Bax, Caspase 3 and 9 while decreasing Bcl-2 (Tukenmez et al., 2019). Exopolysaccharides from *Kluyveromyces marxianua* or *Pichia kudriavzevii*, on the other hand, hindered the AKT-1, mTOR, and JAK-1 pathways, and induced apoptosis (Saadat et al., 2020). Elsewhere, exopolysaccharides from *L. plantarum* 12 inhibited cell proliferation, upregulated the expression of the pro-apoptotic proteins Bax, Cyt C, Caspase-3, Caspase-8, and Caspase-9 and decreased the expression of the anti-apoptosis protein Bcl-2 (Sun et al., 2021). Furthermore, exopolysaccharides from *Weissella confusa* J4-1 inhibited cell proliferation by inducing the G0/G1 phase cell cycle, upregulated p21 levels, and downregulated mutant p53 and cyclin kinase 2 levels (Liu et al., 2023). In terms of other CRC cell lines, exopolysaccharides from *Pediococcus acidilactici* NCDC 252, for example, inhibited the cell viability of HCT116 (Kumar et al., 2020). Exopolysaccharides from *L. acidophilus* 20079, for instance, inhibited cell viability of the Caco2 cell line and increased the ratio of the apoptotic cells in the sub-G0/G1 cell cycle phase as well as up-regulated the expression of IKbα, P53, and TGF genes (El-Deeb et al., 2018) . Interestingly, exopolysaccharides from *L. acidophilus* downregulated the expression of VEGF and HIF-1α and upregulated the expression of TIMP-3, HIF-2α, HO-1, and PAI-1, suggesting anti-angiogenic and antioxidative properties against HCT15 and Caco2 cell lines (Deepak et al., 2016). In terms of *in vivo* studies, exopolysaccharides from *L. rhamnosus* ATCC 7469 (with 1Gy γ-R) ameliorated the oxidative and inflammatory biomarkers with modulated signaling molecular factors accompanied by improved histological structure against DMH-induced rat model (Zahran et al., 2017). Exopolysaccharides from *Weissella confusa* J4-1, on the other hand, retarded tumor growth in the HT-29 xenograft model (Liu et al., 2023).

Other bioactive metabolites like macromolecules from *L. casei* and *L. rhamnosus* GG inhibited cell invasion by decreasing MMP-9 and increasing ZO-1 protein levels in the HCT116 cell line (Escamilla et al., 2012). Short-chain fatty acid (butyrate), exopolysaccharides, and extracellular proteins from *L. plantarum* S2, *L. pentosus* S3, and *L. rhamnosus* 14E4 decreased cell viability HT-29 and HT29-dx cell lines (Doublier et al., 2022). Other short-chain fatty acids like acetate and propionate from *Propionibacterium acidipropionici* or *P. freudenreichii*, induced apoptosis in Caco-2 and HT29 with mitochondria and ANT involved in the cell death pathway (Jan et al., 2002) and induced pH-mediated switch between apoptosis and necrosis in HT-29 (Lan et al., 2007). In terms of *in vivo* evidence, SCFA, *C. butyricum* decreased proliferation and increased apoptosis and suppressed the Wnt/ β-catenin signaling pathway against the high-fat diet-induced $Apc^{min/+}$ mouse model (Chen et al., 2020). The antioxidant activity of probiotic-derived bioactive metabolites has also been reported. *L. lactis* increased catalase activities and reduced H_2O_2 levels with a lesser extent of colonic damage and inflammation in the DMH-induced mouse model (de Moreno de Leblanc et al., 2008).

CONCLUSION

Growing evidence supports the use of probiotics and their bioactive metabolites for the prevention of CRC. Their anticancer effects are mainly related to reducing cellular inflammation, inducing cell apoptosis, suppressing tumor growth and viability, and increasing anti-oxidant activity. Interestingly, probiotics and their bioactive metabolites are also effective against the tumour microenvironment of CRC through modulation of the host gut microbiota and tumour angiogenesis. The anti-angiogenic effects of probiotics and their bioactive metabolites could be particularly useful against CRC that are angiogenesis-dependent (Liotti et al., 2022). As such, probiotics and their bioactive metabolites, which are anti-angiogenic, should be essentially explored as a chemopreventive strategy against CRC (Fareez et al., 2024).

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In spite of the promising findings, it is essential to thoroughly assess the potential risks and challenges before translating these results to clinical settings. Also, given the strain-dependent effects of probiotics, extensive research on the characterization of specific strain(s) or bioactive metabolite(s), their specific health-beneficial effects, and their association with related pathways should be performed. The lack of strain comparison for application in clinical settings raises the need for extensive and in-depth preclinical screening of different probiotic strains against CRC to compare their anticancer activities and underlying mechanisms before proceeding to clinical trials (Xu et al., 2024). This is crucial because each probiotic strain has a different mechanism of action and efficacy depending on the host and diseases (Ghorbani et al., 2022). Besides, the differential efficacy between single- or multi-strain probiotics (and their bioactive metabolites) against CRC should be further investigated to determine as to which approach is better (McFarland, 2021). On another note, the potential translation of preclinical probiotic (and bioactive metabolites) research into clinical applications requires the fulfilment of several critical considerations. Firstly, the scalability of the probiotics is often influenced by manufacturing challenges like strain stability, viability as well as efficacy during production and storage (Gurram et al., 2021). This is because factors like high temperatures, humidity, pressure during manufacturing, and improper storage conditions could weaken the bacteria, making them less effective (Gurram et al., 2021). As such, ensuring the stability and viability of probiotics in their final form after the scale-up process is a delicate balance crucial for their beneficial effects. Secondly, the regulatory hurdles are significant, especially with variations in global frameworks that require compliance with stringent standards for live biotherapeutic products or food-grade probiotics (Siong & Sum, 2021). The standardization and harmonization of regulatory guidelines are vital to ensure the safe consumption of probiotics in general. More importantly, the regulations should clearly define the probiotic's characteristics, efficacy, safety requirements, and labeling claims (Arora & Baldi, 2015). Thirdly, the formulation of probiotics plays a vital role in ensuring the effect of probiotics can be conferred to the patients. Since probiotics can easily lose their viability under high temperatures or oxidative stress, stable formulations are needed to maximize their therapeutic potential and clinical applicability (Baral et al., 2021). In addition, emerging evidence supports the need for personalized approaches in clinical applications of probiotics and their bioactive metabolites. Future studies should essentially integrate modulation of gut microbiome (Chrysostomou et al., 2023) or even intra-tumoral microbes and cancer-related circulating microbial DNA (cmDNA) (You et al., 2022) with therapeutic goals. Besides, future studies should also consider using tumor molecular profiles with probiotic interventions to improve outcomes in metastatic CRC treatments by leveraging the gut microbiome (Addissouky et al., 2024). Microbiota regulation may help to modulate the tumor microenvironment through microbial signals, thus affecting the efficacy of immunotherapy (Jiang et al., 2023). More importantly, future probiotic studies should be directed towards long-term safety evaluations that align with existing regulatory standards for biologic therapies without imposing unnecessarily stringent requirements. It is essential to maintain rigor in collecting and reporting adverse event data to ensure comprehensive safety profiles are developed (Merenstein et al., 2023).

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AUTHOR'S CONTRIBUTION

Umi Khalsom Mohd Bajuri wrote the original draft. Kalavathy Ramasamy was involved in the supervision, review, and editing of the manuscript. Siong Meng Lim was involved in the conceptualization, supervision, review, and editing of the manuscript. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors affirm that there are no competing interests regarding the publication of this paper.

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