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SYSTEMATIC REVIEW – OPEN ACCESS

Review of the Effects of Probiotics and Their Metabolites in the Treatment of Liver Cancer: An Update on Probiotics as a New Treatment

Sheyda Bouzari[1], Ronak Bakhtiari[2], Hatef Ajoudanifar[1], Seyed Ataollah Sadat Shandiz[3]

ABSTRACT

Objective: Concerns are growing about the prevalence of liver cancer as one of the most common health-threatening cancers due to the numerous risk factors for this type of cancer such as hepatitis, obesity, diabetes and fatty liver. This review article was done to investigate the protective role of probiotics and their metabolites against liver cancer, where probiotics showed various useful effects for treatment of disease especially cancer.

Materials and Methods: For this study, searches were carried out using keywords including probiotic, Bacteria, *Lactobacillus*, *Bifidobacterium*, Liver Cancer and Hepatocellular Carcinoma from reliable databases such as Google Scholar, PubMed, Science Direct, and Wiley.

Results: This review revealed that, where products of probiotic bacteria had also therapeutic effects such as OK-432, Nisin, Bovicin HC5 (as important products), probiotic bacteria of the genus *Lactobacillus* and *Bifidobacterium* such as *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus* strains were the most useful probiotic bacteira against liver cancer.

Conclusion: The use of probiotics due to their natural source and their secretory metabolites can be a good way to develop new therapies for various diseasesm with this systematic review showing that probiotics have shown therapeutic effects on liver cancer by having a wide range of properties such as anti-inflammatory and anti-apoptotic properties.

Keywords: Probiotics, bacteria, neoplasms, liver neoplasms, carcinoma, hepatocellular

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[1]Department of Biology, Damghan Branch, Islamic Azad University, Damghan, Iran
[2]Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[3]Department of Biology, Central Tehran Branch, Islamic Azad University, Tehran, Iran

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Correspondence
Ronak Bakhtiari & Hatef Ajoudanifar,
Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
Department of Biology, Damghan Branch, Islamic Azad University, Damghan, Iran
Phone: +00989119182429
+00989119182429
e-mail:
dr.bakhtiarir@gmail.com
hajoudanifar@ymail.com

INTRODUCTION

The liver, as the body's main detoxifying organ, is constantly exposed to harmful toxins such as pathogens, alcohol, and fat which increases the risk of a wide range of liver diseases like hepatitis, liver fibrosis, cirrhosis and cancer (1). The incidence of various cancers has increased significantly in recent years. Cancer is the main leading cause of death (2). Hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA) are recognized as the most common types of cancer of liver (LC). HCC is a dangerous type of cancer, which originates from epithelial cells. HCC or primary liver cancer which has a diverse geographical distribution, is one of the most common malignant cancers of the liver originating from epithelial cells and it is also the third leading cause of cancer death worldwide (3). Countries or regions where the disease is high are China, Taiwan, Korea and other Southeast Asian countries. Numerous epidemiological studies have shown that the main risk factors for HCC are age, sex and cirrhosis with any etiology. Therefore, the main cause of HCC is excessive alcohol consumption and/or chronic infection with hepatitis B virus or hepatitis C virus (HCV) and exposure to aflatoxin (4). Common treatments for HCC include a combination of surgery, chemotherapy and radiotherapy.

Recently, admirable steps have been taken to create new and effective treatments especially with natural compounds for treatment of different diseases. Probiotics are one of these treatments which is used in several studies to treat HCC (5). Probiotics, having long been used to make a variety of foods, are microorganisms (bacteria and yeast) that settle in different parts of the body (mainly the intestine; as the natural flora), and cause health-promoting properties for the host by their biological action. The most important and common probiotics belong to the genus *Lactobacillus* and *Bifidobacterium*. With this particular systematic review being written to investigate the effect of probiotics and their metabolites in the remedies of LCm there are several studies on the health properties of probiotics. Anti-inflammatory, anti-cancer, anti- mutagenicity, inhibiting harmful bacteria, stimulating and strengthening the immune system, anti-infection and cholesterol lowering are some of these useful properties (6).

MATERIALS and METHODS

This study was carried out as a systematic review. All records containing keywords such as probiotic, Bacteria, *Lactobacillus*, *Bifidobacterium*, Liver Cancer and Hepatocellular Carcinoma were searched from reliable databases including Google Scholar, PubMed, WOS, Science Direct, and Wiley (Last 20 years). The abstracts of all articles were studied at the beginning. Chosen articles were carefully studied to obtain information as the next step. Taking together, one hundred forty-four articles were obtained from databases but finally forty-two related articles were selected to write a systematic review (Fig.1).

RESULTS

This review identified the effects of different probiotics and their metabolite against liver cancer. According to the Appendix 1, probiotic bacteria of the genus *Lactobacillus* and *Bifidobacterium* such as *L. plantarum*, *L. casei*, *L. acidophilus*, *L. rhamnosus* strains, in addition to Saccharomyces Cerevisiae which was also used as a yeast against cancer, were the most useful probiotic bacteria against liver cancer especially HCC. Products of probiotic bacteria had also therapeutic effects such as OK-432, Nisin, Bovicin HC5 (as important products) against liver cancer. The bacteria used in these studies were extracted from various sources such as human breast milk, fermented milk, fermented soybean, fermented barley, and new born infant feces. However, most of these probiotics were obtained from ATCC. Most of these studies were *in vitro*, animal and clinical (n=10) studies, respectively. The information of the studies are shown in detail in Appendix 1 and Appendix 2.

DISCUSSION

Liver cancer is a cancer that affects many patients. The increasing prevalence of LC due to its close relationship with lifestyle and the prevalence of diet-related diseases such as fatty liver has caused concern among researchers (3). Among the different types of liver cancer, HCC accounts for 75%–85% of cases and is the main type of liver cancer. While cirrhosis of the liver plays an important role in the progression of HCC so that 80%-90% of patients with HCC suffer from liver cirrhosis, HCC is a complex cancer in which various factors play role in its pathogenesis. Viruses of hepatitis type B, C, and D, NAFLD, NASH, alcohol abuse, obesity and diabetes mellitus, exposure to aflatoxin (mycotoxins) are among the factors involved in the development of HCC (4). Hepatitis B virus, which increases the risk of HCC through production of oncogenic proteins, oxidative stress, damage to endoplasmic reticulum, DNA damage, and increased expressions of nuclear factor-κB (NF-κB) and cyclooxygenase-2 (COX-2) (43, 44), is an important risk factor which is associated with more than 50% of HCC cases. Hepatitis B could. HCV, as another risk factor which could increase 15 to 20 times the risk of HCC participates in development of HCC via induction of oxidative stress in hepatocytes, promotion of liver fibrosis and induction of the liver inflammation by affecting CD8+ T cells, Th17 and regulatory T cells (Treg) and elevation in the production of pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6 (45, 46). NAFLD/NASH is becoming a dangerous risk factor for the development of HCC. In a process that could lead to oxidative stress, mitochondrial dysfunction, and ER stress, NAFLD/NASH play a role in induction of steatosis and toxicity caused by lipids (47). On the other hand, steatosis

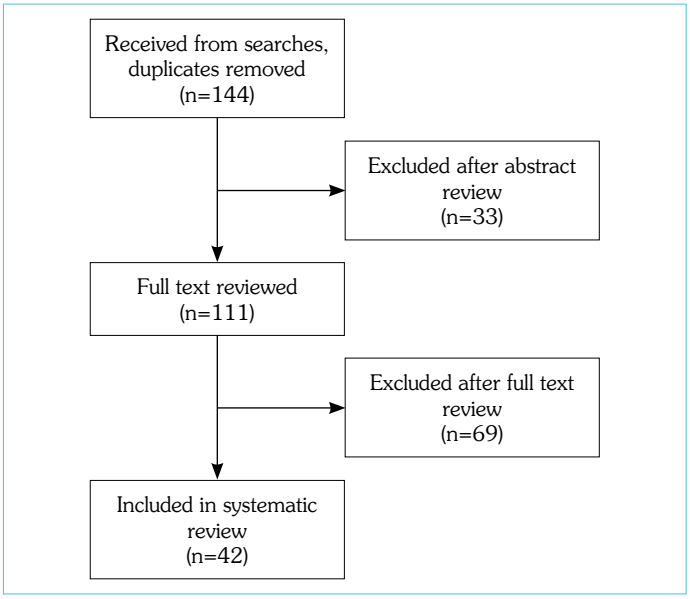

Figure 1. Flow diagram of the study

involved in induction of liver inflammation through upregulation of NF-κB and production of the pro-inflammatory cytokines including TNF-α, IL-1β and IL-6 (48). Moreover, in addition to NASH provoking inflammatory responses via induction of damage-associated molecular patterns and ultimately activation of immune cells such as Kupffer cells (43), NAFLD/NASH induces activation of natural killer T cells (NKT) together with CD8+ T cells which promotes liver injury.

As mentioned above, important processes such as oxidative stress and inflammation and other mentioned processes play an important role in the pathogenesis of liver cancer (49). Hence, the use of natural compounds such as medicinal plants and probiotics with proven therapeutic properties that can reduce these harmful processes can be a good way to treat various disease. Probiotics, shown by researchers to have effectiveness on various types of cancers, are beneficial microorganisms where their effects on human health and the prevention of various diseases have further been proven. Researchers have probiotics shows their anti-cancer effects through different mechanisms (50). Binding to carcinogens and adsorption of them and neutralizing the poisons is one of these mechanisms. This mechanism prevents damage to genes (5). El-Nezami et al. (51) revealed that probiotic supplementation including *Lactobacillus rhamnosus* LC705 and *Propionibacterium freudenreichii subsp. shermanii* strains could attenuate biologically effective dose of aflatoxin exposure as a carcinogen factor for liver. In another similar study, N. Nduti et al. (52) concluded that probiotic yogurt including *Streptococcus thermophilus*, *Lactobacillus rhamnosus* GR-1 and *Weissella cibaria* NN20 plays a role in reduction of aflatoxin metabolite 1 concentration. While probiotics affect the liver by improving gut microbiota as another anti-cancer mechanism (5), gut microbiota are closely related to the liver through the production of beneficial metabolites. Researchers understood that elevation in accumulation of LPS plays a role in growth of the HCC pathogenesis by stimulating the production of pro-inflammatory cytokines in the liver (53). It has been shown that probiotics can have anti-cancer effects by producing beneficial metabolites such as short chain fatty acids including acetate, propionate and butyrate (54). For chronic

diseases, treatments using probiotics and natural and mineral products can be used (55, 56). Probiotics, having different mechanisms that can exert anti-cancer effects (57–59), exert anti-inflammatory properties which play role in reduction of the production of pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6 and IL-17 and decrease inflammatory cells including NK cells and Th17 cells, and ultimately play a role in reduction in inflammatory responses (5). With probiotics exhibiting anti-cancer effect via their anti-apoptosis properties and upregulation of Bax and downregulation of Bcl-2 and Caspases (22), Li et al. (34) further showed that administration of Prohep, as a probiotic mixture, could reduce tumor growth through the inhibiting angiogenesis, downregulation of IL-17 cytokine and Th17 cells, upregulation of anti-inflammatory cytokines and downregulation of IL-17, angiogenic factors, and receptors.

CONCLUSION

Recently, there is a growing tendency to use compounds of natural origin to treat diseases. Natural compounds can be a good option for treating various diseases due to the lack of side effects of chemical drugs, their availability and inexpensiveness. Probiotics have always been considered by researchers as beneficial microorganisms. Probiotics have shown therapeutic effects on liver cancer by having a wide range of properties such as anti-inflammatory and anti-apoptotic properties. Hence, the use of probiotics due to their natural source and their secretory metabolites can be a good way to develop new therapies for various diseases.

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Appendix 1. Effective probiotics on liver cancer

Probiotic bacterium/product	Source	Product/ bacterium	Study model	Participant	Concentration/ dose	Length of treatment	Result	Ref
Bacillus subtilis	Fermented soybean	–	*In vitro*	Human hepatocellular carcinoma Hep 3B cells and mouse hepatocellular (ML-1) cels	165.6 lg/ ml fermented soybean produced by 105 cells/ml of Bacillus subtilis	0–72 hours	Bacterial fermented soybean caused inhibition of the growth of tumor cells via induction of apoptosis, activation of caspase 3, 8 and 9, depolarization of mitochondrial membrane, and inhibition of the expressions of COX-2 and Ku70, increased nuclear DNA fragmentation factor 40 (DFF40), and cleavage form of poly(ADP-ribose) polymerase (PARP), increase in pro apoptotic (tBid, Bak and Bax) and decrease of anti-apoptotic (Bcl-2 and Bcl-xL) proteins, release of cytochrome c and Smac (second mitochondria-derived activator of caspase/direct IAP binding protein with low PI).	(7)
Lactobacillus rhamnosus GG bacteria (PTCC1637 strains)	ATCC		*In vitro*	HepG2 cells	50 mg,100 mg, 150 mg and 200 mg	24 h	Extracellular vesicles derived from Lactobacillus rhamnosus GG at a dose of 100 mg/ml showed significant cytotoxic effect on HepG2 cells. The bax/bcl2 expression ratio was significantly elevated after 50 and 100 mg/ml LDEVs.	(8)
Lactobacillus plantarum dy-1	Fermented Barley Extracts		*In vitro*	Human HepG2 Cells	50, 100, 200 µg/mL	24 h	Fermented Barley Extracts with Lactobacillus plantarum dy-1 at high concentration could modulate glucose consumption and reduce production of pro-inflammatory cytokines. It also diminishes Phosphorylation ofJNK and P38. Repression of DUSP9 and MiR-212 were also seen after treatment.	(9)
Lactobacillus acidophilus	ATCC		*In vivo*	Male Balb/c mice	$1x10^9$ cfu/gr Bla/016P/ M	Five months	Lactobacillus acidophilus increased miR-122 and PU.1 and down-regulated miR-221, miR-155, Bcl-w and KRAS. This probiotic can inhibit progression of cancer via delaying of metastasis, attenuating of inflammation and down and up-regulation of oncogenes/oncomirs and tumor suppressor genes/microRNAs.	(10)
Bifidobactrum bifidioum	ATCC		*In vivo*	Male Balb/c mice	$1x10^9$ cfu/gr Bla/016P/M	Five months	Bifidobactrum bifidioum increased miR-122 and PU.1 and down-regulated miR-221, miR-155, Bcl-w and KRAS. This probiotic can inhibit	(10)

Appendix 1 (cont). Effective probiotics on liver cancer

Probiotic bacterium/product	Source	Product/ bacterium	Study model	Participant	Concentration/ dose	Length of treatment	Result	Ref
							progression of cancer via delaying of metastasis, attenuating of inflammation and down and up-regulation of oncogenes/oncomirs and tumor suppressor genes/microRNAs.	
Lactobacillus plantarum WLPL09	Human breast milk	Exopolysaccharide APS	*In vitro*	HepG2 hepatocellular carcinoma cells	0, 100, 200, 400, 800 µg/mL	24 h and 48 h	Exopolysaccharide APS from Lactobacillus plantarum WLPL09 exhibited anti-proliferative effect against HepG2 cells. It also could induce apoptosis of HepG2 via increased expression of caspase-3, -8 and -9.	(11)
Capsule of probiotics (Bifidobacterium, Enterococcus, and Lactobacillus acidophilus)	ATCC		*In vivo* (clinical)	Ninety-six patients with type 2 diabetes mellitus combined with gastrointestinal dysfunction and liver cancer		4 weeks and 3 times a day	Probiotics could improve gastrointestinal mucosal function via reduction in D-lactic acid, PCT, and endotoxin. It also improved nutritional status via increasing TP, ALB, Hb, PA and TLC. Moreover, psychological symptoms improved after treatment. Incidence of side effects of treatment including nausea and vomiting decreased.	(12)
Lactobacillus rhamnosus (ATCC 9595)	Cranberry extract biotransformed with Lactobacillus rhamsonus		*In vitro*	HepG2 hepatocellular carcinoma cells	10–500 µg/mL	48 h	Biotransformed Cranberry extract with Lactobacillus rhamsonu could completely supress HepG2 cell proliferation via depletion of ATP in cancer cells and induction of Caspase-3.	(13)
Bifidobacterium longum	ATCC		*In vivo*	Male nude mice and BALB/c mice HepG2 human liver cancer	$1.5x10^{10}$ bacteria/kg	18 days	Bifidobacterium longum used as a delivery system of endostatin gene which could suppress the growth of liver cancer. B. longum with the endostatin gene elevated activity of NK and T cells and provoked the activity of IL-2 and TNF-a.	(14)
Lactobacillus rhamnosus GG and Lactobacillus casei strain Shirota	Fermented milk		*In vivo*	Male Wistar rats	$>10^8$ colony-forming units/g of diet	25 weeks	Probiotic fermented milk and chlorophyllin were used for combating against liver cancer caused by aflatoxin-B1. Probiotic could alone or in combination with chlorophyllin show a hepatoprotective effect against aflatoxin-B1-induced liver carcinogenesis via decreased MDA level and increased antioxidant enzymes levels including GPX, SOD and CAT in the liver.	(15)

Appendix 1 (cont). Effective probiotics on liver cancer

Probiotic bacterium/product	Source	Product/ bacterium	Study model	Participant	Concentration/ dose	Length of treatment	Result	Ref
							Administration of probiotic fermented milk and chlorophyllin caused decreased tumor incidence, reduction in DNA damage and decreased expression of c-myc bax, bcl-2, cyclin D1, p53 and rasp-21 genes.	(16)
Saccharomyces Cerevisiae in combination with herbal medicines	ATCC	CKBM	*In vitro*	Human hepatoma HepG2 cells (ATCC number HB-8065)	2–14%	48 h	This combination showed significant anti-proliferation effect on HepG2. Reduced volume of cancer cells was also observed. Increased expression of p53 and decreased expression of anti-apoptotic protein bcl-2was seen after treatment.	(17)
			In vivo	Male nude mice	0.4, 0.8 ml/day	14 days	Inhibited tumor growth was seen at concentration of 0.4 ml (26.5%) and at concentration of 0.8 ml (54.1%).	
			In vitro	Hep3B and PLC/PRF/5 HCC cell lines		8 h		
Lactobacillus plantarum 2362, L paracasei subsp paracase 19, Pediacoccus pentoseceus 5-33:3 and 32-77:1 L raffinolactis 10 Pediacoccus pentosaceus 5-33:3, 10 Leuconostoc mesenteroides 32-77, 1.10 L paracasei L paracasei subgroup paracasci 19:10 L plantarum alpha 362nd	ATCC		*In vivo* (clinical)	120 patients underwent the hepatic resection due to HCC	100 kcal per day and gradually increased to 400 kcal/dando 3 or 4 days	3 days preoperatively and postoperatively for 7 days	Probiotics caused liver function recovery via decreasing ALT, AST, GGT and ALP and increased total protein and albumin. Probiotics administration also led to provoking immune response, lower tumor markers including CEA and alpha fetoprotein, less surgery complications and morbidity and mortality rates.	(18)

Appendix 1 (cont). Effective probiotics on liver cancer

Probiotic bacterium/product	Source	Product/ bacterium	Study model	Participant	Concentration/ dose	Length of treatment	Result	Ref
Lactobacillus Plantarum EMCC-1039	ATCC		*In vivo*	Forty eight male Wister rats	$1.2x10^9$ cfu/ml	6 weeks	L. Plantarum significantly improved liver function via decreased the levels of ALT, AST, ALP and AFP and the hepatic expression of CXCL9, PREX-2 and TLR4 expression. Improvement in liver pathological changes such as reduction in collagen fibers and cytokeratin AE1/AE3nwas observed.	(19)
Lactobacillus paracasei strain TD3	ATCC		*In vitro*	HepG2 cell line	109 CFU/ ml with concentration of 5, 10, 20, 30 and 40% (v/v)	24 h	L. paracasei showed a significant inhibitory effect on HepG2 cell growth.	(20)
Streptococcus salivarius BP8 S. salivarius BP156, and S. salivarius BP160	Human breast milk		*In vitro*	Human liver cancer cells (HepG2)	2 ml of each probiotics was added to 8 ml of MRS broth	24 h	Probiotics could inhibit 44.83–59.65% of HepG2 cells proliferation. Probiotics provoked HepG2 cells death through DNA fragmentation.	(21)
Bifidobacterium bifidum 86321	ATCC	Lipoteichoic acid	*In vivo*	Six-to-eight-week-old female Balb/c mice	150 mg Bifidobacteria lipoteichoic acid	10 days	Bifidobacteria lipoteichoic acid alone and combined with 5-fluorouracil could significantly prevent the tumor proliferation and induced apoptosis through up-regulation of Bax and downregulation of Bcl-2 and Caspase-3.They also stimulated necrosis and elevate infiltration of immune cells and increasing the proliferation of immune organs and CD4+ T cell. The significant reduction in ratio of CD4+CD25+ Treg and proteins expression of Foxp3 and TIM-3 was also seen.	(22)
Lactobacillus acidophilus ATCC 4356	ATCC	Exopolysaccharides	*In vivo*	50 adult male Swiss albino rats	100 mg EPSs/kg b.w.	Eight weeks	Exopolysaccharides of Lactobacillus acidophilus caused reduction in ALT and GGT, MDA, IL-17, TGF-β1 levels and elevation in glutathione, IL-10. The gene expression of TLR-2, STAT-3 and p38MAPK signaling pathways were also decreased after treatment. Moreover, treatment caused normal hepatic architecture of parenchyma, vascular and stroma without inflammatory cells infiltration.	(23)

Appendix 1 (cont). Effective probiotics on liver cancer

Probiotic bacterium/product	Source	Product/ bacterium	Study model	Participant	Concentration/ dose	Length of treatment	Result	Ref
Bifidobacterium longum and Enterococcus hirae	ATCC		*In vivo* (clinical)	18 HBV-related primary HCC patients			Probiotics showed their antitumor effect via their immunomodulatory effect and stimulation of CD8+ T cell response and better prognosis.	(24)
L. acidophilus HM1, L. fermentum HM3, and L. buchneri FD2, Lactobacillus casei Shirota	ATCC		*In vitro*	Liver cancer (HepG2)	107 CFU/mL	24 h	Probiotics could inhibit cancer cell growth with IC50=14.81 to 24.81.	(25)
Lactobacillus casei ATCC393	ATCC	Extracted protein	*In vitro*	Huh7, human hepatoma cells	0.1 mg	72 h	L. casei extract howed its anti-tumor effects via reduction in cell viability of Huh7 cells by 77%. L. casei extract also elevate arrested cells in G2/M phase and apoptosis. Enhancement in expression of TNFR1, DR3 mRNA, and P21 and P27 cell cycle proteins was observed. Reduction in caspase-8, -9, phospho-Bad and Bcl-2 proteins was seen.	(26)
Lactobacillus reuteri (L. reuteri)	ATCC		*In vitro*	HEPG2 cell			L. reuteri in combination with selected antioxidants enhanced cell death and cancerous morphology in hepatocellular carcinoma.	(27)
Lactobacillus sps (MTCC 4184, 9496, and 10093), Bacillus cereus (MTCC 7166, 9017 and 10202)	ATCC	Protein extracts	*In vitro*	Hep-G2 (Liver Cancer)			Protein extracts of Lactobacillus sps and Bacillus cereus showed their anti-cancer effects by inhibiting Hep-G2. The cancer cell death was seen from 52% to 71% through increased Caspase-3 activity, DNA fragmentation and cell cycle arrest.	(28)
Four Lactobacilli, three Bifidobacteria, and one Streptococcus thermophilus subsp Salivarius	ATCC		*In vivo*	Pathogen-free male Sprague–Dawley rats	Low dose, $6x10^9$ CFU or high dose, $6x10^{10}$ CFU	One week before DEN injection	Probiotics decreased liver tumor growth and multiplicity via affecting p53 signaling. Probiotics could also decrease dysbacteriosis and hepatic and intestinal inflammation through reduction in LPS and IL-6 levels and elevation in IL-10 levels.	(29)
Lactobacillus casei strain Shirota, Bifidobacterium breve strain Yakult	Yakult Bifiel galactooligosaccharides		*In vivo* (clinical)	101 patients	$1–4x10^{10}$	2 weeks before after operation	Probiotics could decrease infections after biliary cancer. Preoperatively administration of probiotics caused increased NK activity and lymphocyte counts and significant reduction in IL-6. They also and improve gut microbia. Thus,	(30)

Appendix 1. Effective probiotics on liver cancer

Probiotic bacterium/product	Source	Product/ bacterium	Study model	Participant	Concentration/ dose	Length of treatment	Result	Ref
Lactobacilli bacteria (L. plantarum MYL26, L. bulgaricus MYL101, L. plantarum MYL31, L. bulgaricus MYL102, L.acidophilus MYL 201, L.acidophilus MYL 202 and L. casei MYL01)	New born infant faeces and breast milk	Crude cell wall extracts	*In vitro*	Human he patocellular carcinoma cells (HepG2)	10±0.2 mg/ 1 · 108 cfu	20 h	Lactobacilli bacteria participated in activation of NOD2 expression, induction of IL-10, suppressor of cytokine signaling and PPAR-α via NOD2-NF-kB pathway, and cross-regulated Toll-like receptor 4 (TLR4) downstream signal transduction.	(31)
Lactobacillus plantarum C88	ATCC		*In vivo*	Ninety male ICR mice	$4.0x10^{10}$ CFU/ kg bw viable or heat-killed	21 days	L. plantarum C88 administration could ameliorate ALT, AST, ALP, total cholesterol, triglyceride, total protein and albumin. Moreover, L. plantarum C88 decreased inflammatory response through reduction in IL-1β, IL-6, IL-8, IFN-γ, and TNF-α levels. In addition, down-regulation of NF-κB, TLR2, and TLR4 was seen. Decreased levels of Bax and Caspase-3, Fas, FADD, TRADD Caspase-8 and increased level of Bcl-2.	(32)
Bifidobacterium infantis strain	ATCC	Thymidine kinase	*In vivo*	Twenty-four mice (Balb/c-nu) and Balb/c mice liver cancer (SSMC-7721) cell line	$1.0x10^{6}$ cell/ tumor	48 h post injection	Bifidobacterial recombinant thymidine kinase prevented more than 83% death of liver cancer through inhibiting expression of TNF-α, induction of apoptosis through TNFR2, FasL and Bid and Bim, and increased P53 expression.	(33)
Prohep, a probiotic mixture (Lactobacillus rhamnosus GG (LGG), viable Escherichia coli Nissle 1917 (EcN) and heat-inactivated VSL#3 (1:1:1))	ATCC		*In vivo*	Male C57BL6/N mice	108 CFU/day and 1010 CFU/day	38 days	Probiotics decreased tumor growth, tumor size and weight by 40%. Reduction in tumor growth through the inhibiting angiogenesis, down-regulation of IL-17 cytokine and Th17 cells, upregulation of anti-inflammatory cytokines and downregulation of IL-17, angiogenic factors, and receptors.	(34)

Appendix 2. Effective probiotic's metabolites on liver cancer

Probiotic bacterium/product	Source	Study model	Participant	Concentration/dose	Length of treatment	Result	Ref
OK-432	Streptococcal preparation	*In vivo* (clinical) *In vitro*	Thirteen patients with HCC Hep3B and PLC/PRF/5 HCC cell lines	0·1 KE/ml OK432 for stimulation of $5x10^6$ of DCs for 2 days	7 days 8 h	OK-432 was used for stimulation of dendritic cell to combat HCC. Administration of OK432-stimulated dendritic cells could increase the life expectancy of patients without recurrence. OK432-stimulated dendritic cells showed its anti-cancer effects via increased levels of cytokines IL-9, IL-15 and tumor necrosis factor-α and the chemokines CCL4 and CCL11.	(35)
OK-432	Streptococcal preparation	*In vivo*	Male C3H/HeN and C3H/HeJ mice HCC cell line (MIH-2) MC38 cells	0.1 mg of OK-432 dissolved in 0.1 ml of phosphate buVered saline (PBS)	Every 2 days, six times totally	OK-432 showed suppressive effect on tumor. It also caused IFN-gamma production by activating CD4+ or CD8+T cells. Moreover, IFN-gamma induces apoptosis.	(36)
OK-432	Streptococcus pyogenes	*In vivo* (clinical)	A 51-year-old Japanese man	10KE	Four times into the peritoneal cavity, within 1 week	OK-432 decreased AFP level and massive ascites caused by HCC.	(37)
Nisin	ATCC	*In vitro*	Hepatocellular carcinoma cell line (HepG-2)	100 µg/mL	24 h	Nisin showed anti-proliferative effect and decreased PI3K/AKT mRNA and protein expression and SIRT1/NRF2 mRNA expression and VEGF protein level.	(38)
Bovicin HC5	Streptococcus bovis HC5	*In vitro*	HepG2 (human liver hepatocellular carcinoma cell line)	0–333 µM	24 h	Bovicin HC5 exhibited cytotoxic effects against HepG2 (IC50 = 289.30 µM).	(39)
Nisin	Lactococcus lactis	*In vitro*	HepG2 (human liver hepatocellular carcinoma cell line)	0–135 µM	24 h	Nisin exhibited cytotoxic effects against HepG2 (IC50 = 112.25 µM).	(39)
Nattokinase	Bacillus subtilis	*In vivo*	C57BL/6 wild-type mice	7.5 µl/g according to body weight	20 days	Nattokinase enhanced the survival of HCC mouse to 31% and depressed ascites. It also decreased tumor size and growth. Moreover, the expression of FOXM1 was inhibited and the expression of CD31, CD44 and vimentin was decreased.	(40)
	Streptococcus and Lactococcus	*In vitro*	Human liver cancer cell line (HepG2)	1–200µg/ml	24 and 48 h	Nisin treatment could decrease viability of HepG2 cells (IC50 ~ 40 µg/ml) and play role in induction of apoptosis via increase in gene expression of caspase-9 and BID and BCL2.	(41)
Nisin *Nisin and Nisin-Loaded PLA-PEG-PLA Nanoparticles*	Lactococcus lactis	*In vitro*	HepG2	0, 15, 30, 40, 60, 75, 150, 250, 350, and 450 µM	24, 48, and 72 h	Nisin and especially Nisin nanoparticles showed cytotoxic effects against HepG2. Moreover, elevation in the percentage of apoptotic cells was seen.	(42)