Review

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Effects of *Lactococcus lactis* on colorectal cancer in various terms: a narrative review

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Introduction: Colorectal cancer is one of the most common cancers with an increasing number of cases. Various studies have found an association between the gut microbiota balance and colorectal cancer incidence. *Lactococcus lactis* is a probiotic bacterium found in fermented foods, particularly yogurt and cheese. This probiotic has been shown to reduce various anti-inflammatory and pro-inflammatory agents that trigger cancer, such as interleukin (IL)-6, IL-18, tumour necrosis factor-alpha (TNF-α), and natural killer (NK) cells.

Methods: Full-text articles and original research published in the last ten years were used as references, and "Lactococcus and colorectal cancer" as keywords. The reference search is on several databases, such as PubMed, ScienceDirect, ProQuest, and Nature. Searching results obtained eleven articles.

Discussion: *Lactococcus lactis* does have a perfect role in suppressing cancer cells. *Lactococcus lactis* has anti-proliferative effects associated with decreased cyclin D1 expression in SW480 cell lines, decreased NK cells, reduced cancer cell viability, decreased IL-8 levels, and decreased IL-6.

Conclusion: *Lactococcus lactis* contains nisin, which can suppress various gene, protein, and cytokine expressions that play a role in cancer cell growth. Probiotics can inhibit colorectal cancer without significant side effects.

Keywords: Anti-proliferative, cancer, nisin, probiotic

Introduction

Colorectal cancer (CRC) is a common type of cancer worldwide. It is the third most common cancer and the second leading cause of cancer-related deaths worldwide, with an estimated 1.8 million new cases and ~881 000 deaths worldwide in 2018[1]. CRC is the second most common cancer in women and the third most common cancer in men. However, morbidity and mortality in women are ~25% lower than those in men[2]. The epidemiology of CRC varies widely among different regions of the world and different age groups, sexes, and races. Many factors are involved in this variation, including risk factor exposure, demographic variation, genetic mutations, and their influence on prognosis and treatment response[3].

Various risk factors can lead to CRC development. Patients with a genetic history of cancer are at a risk of ~10–20% of all patients with colorectal cancer[3]. About 5% of colorectal cancers are caused by autosomal dominant or recessively inherited tumour predisposition such as Lynch syndrome and familial adenomatous polyposis[4], including a family history of CRC or hereditary diseases, history of comorbidities (e.g. inflammatory bowel disease and diabetes), diabetes can be a risk factor for CRC due to the activities performed by insulin. Insulin is a growth factor and a key regulator of cellular metabolism. The growth stimulation that occurs is facilitated by the insulin receptor expressed on cancer cells in isoform A, which is known for its dominant mitogenic effect that can stimulate neoplastic proliferation[5]. Next, lifestyle and dietary habits (e.g. smoking, drinking alcohol, consumption of processed meat) as well as bacterial infections such as *Bacteroides fragilis* and *Escherichia coli*[6]. The CRC will result in a low quality of life in the community, so effective therapy that is easy and affordable is needed. The existing therapy modalities for CRC currently encompass chemotherapy, radiotherapy, targeted therapy, immunotherapy, combination therapy, and surgical interventions. However, not all therapies within these modalities are capable of achieving the desired therapeutic outcomes. Therefore, probiotic therapy is being developed to evaluate its efficacy in CRC[7]. Standard cancer adjuvant therapies such as chemotherapy and

HIGHLIGHTS

- Probiotics can be used as adjuvant therapy for colorectal cancer.
- One of the probiotics that has been developed and has anti-cancer potential is *Lactococcus lactis*.
- Based on the articles we obtained, the administration of *Lactococcus lactis* can inhibit cancer cells proliferation through various ways.

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radiotherapy are time-consuming in controlling cancer cells, leading to patient discomfort and non-compliance with these therapies. This supports using other adjuvant therapies in controlling cancer cells, such as probiotics. Currently, the utilization of probiotics in treating inflammation and cancer is widely developed as fermented milk products such as curd, which comes from the fermentation of buffalo milk in bamboo tubes and is a Minangkabau cultural product. Curd contains various probiotics that are very beneficial for health, including *Lactococcus lactis*[8].

Lactococcus lactis is a probiotic bacterium found in fermented foods such as yogurt and cheese[9]. Probiotic bacteria are food supplements in the form of live microorganisms with low pathogenicity or non-pathogenic to normal microbiota. Probiotics can positively affect host health, survive in the gastrointestinal tract, and improve the balance of gut microbes. Probiotics can act as antimicrobials against intestinal pathogens, modulate the immune system, lower blood cholesterol levels, reduce colonic inflammation, regulate energy metabolism, and prevent colorectal cancer[10].

Lactococcus lactis is a non-pathogenic lactic acid bacterium that can ferment lactose and suppress tumours[9]. Lactic acid bacteria have anti-inflammatory effects, stimulate the host immune system, compete with pathogenic bacteria to obtain nutrients and ecological sites, inhibit the activity of toxic substances, and reduce lactose intolerance. Lactic acid bacteria provided as food supplements have been shown to protect the balance of gut microbiota, stimulate host immune defense mechanisms, and increase nutrient bioavailability[11]. Lactic acid bacteria can prevent cancer, and numerous ongoing studies are currently evaluating the anti-cancer pathways of these probiotic, especially colon cancer[9]. By looking at the effect of *Lactococcus lactis* on colorectal cancer, this review aims to elaborate on the role of *Lactococcus lactis* against colorectal cancer regarding cytokines and gene expression.

Methods

The writing of this article uses a literature review study method that involves analysis and synthesis of various references. Two keywords, namely "Lactococcus and colorectal cancer", were used. The journals used in this article are full-text articles, original research published within the last ten years, and indexed by Scopus. Reference searches were conducted in several databases: PubMed, ScienceDirect, ProQuest, and Nature. From the search results and selection of articles, 11 main articles were obtained. The systematic review was conducted according to the PRISMA guidelines. The list of references was filtered for relevant studies by using search engines (Fig. 1).

The search resulted in 111 articles that were screened with the following criteria: inclusion criteria consisting of articles published less than 10 years, an experiment, original research, discussing the results of the parameters in CRC given *Lactococcus lactis* while the exclusion criteria consist of articles that are more than 10 years old, articles in the form of abstracts only, posters, do not explain the results of the parameters in CRC given *Lactococcus lactis*.

Results

Various studies *in vitro* and *in vivo* have stated that *Lactococcus lactis* is an effective therapy in reducing various anti-inflammatory and pro-inflammatory that trigger cancer, especially colorectal cancer, summarized in Table 1. Engineered *Lactococcus lactis* (recombinant) reduces a large amount of interleukin-8 (IL-8) from Caco-2 and HT-29 cell supernatants and reduces Interleukin-6 (IL-6) from THP-1 and U-937 cell supernatants as determined by Enzyme-linked immunosorbent assay (ELISA); it can also reduce cell growth processes through the cell cycle carried out by cyclin D1.

Discussion

The role of various parameters on CRC

CRC is one of the leading causes of cancer-related deaths in the world. The incidence of colorectal cancer is influenced by various risk factors, including inflammation and the occurrence of gene mutations that trigger the development of CRC. Inflammasome regulates inflammation, cell death, cytokine release, cascade signalling, and other cellular processes. Inflammasome activation releases bioactive forms of IL-1β and IL-18, inflammatory effector cytokines that trigger CRC[19]. In addition, tumour-infiltrating lymphocytes (TIL) from tumours and colonic mucosa of patients in resected colorectal cancer sections showed that TIL culture supernatants have the potential to promote the growth of human CRC cell lines through activation of signal transducer oncogenic transcription factor and activator of transcription 3 (STAT3) and nuclear factor-kappa B (NF-κB). Several pro-inflammatory cytokines such as IL-17A, IL-21, IL-22, tumour necrosis factor-alpha (TNF-α), and IL-6 are also overproduced in colonic lesions of sporadic CRC mouse models, which is associated with increased STAT3/ NF-κB activation[20]. As with growth regulation in normal cells, cancer cell growth is also inseparable from the role of Cyclin D1[9]. Likewise, natural killer (NK) cells are cytotoxic cells that contain perforin and granzyme and produce various cytokines and chemokines that can be found in inflamed cells and even in tumour cells, namely interferon-gamma (IFN- γ), (TNF-α), interleukin (IL-5), IL-13. IL-13 is found in CRC and colitis[21]. EpCam is also found in CRC, which is a carcinoma marker glycoprotein, but EpCam is also found in normal cells with trim levels compared to cancer cells, especially in the Caco-2 cell line[22].

The role of Lactococcus lactis in inhibiting the occurrence of CRC

CRC is one of the leading causes of male and female mortality in Western countries but can be prevented by nutritional interventions, including the administration of potential chemoprevention agents. This probiotic has various strains, including strains IBB109 and IBB417. Incubation of Caco-2 cells, a cancer cell line individually with IBB109 or IBB417, can inhibit the proliferation of the Caco-2 cell line[11]. In addition, this probiotic also has a protective effect, as shown in research conducted by Jaskulski and colleagues, where the group that received *L. lactis* subsp. *lactis* (R7) isolate with a standard or hypercaloric diet and with or without 1,2-DMH experienced less aggressive morphological changes in intestinal tissue when compared to the control group (standard diet); this suggests that this microorganism has the

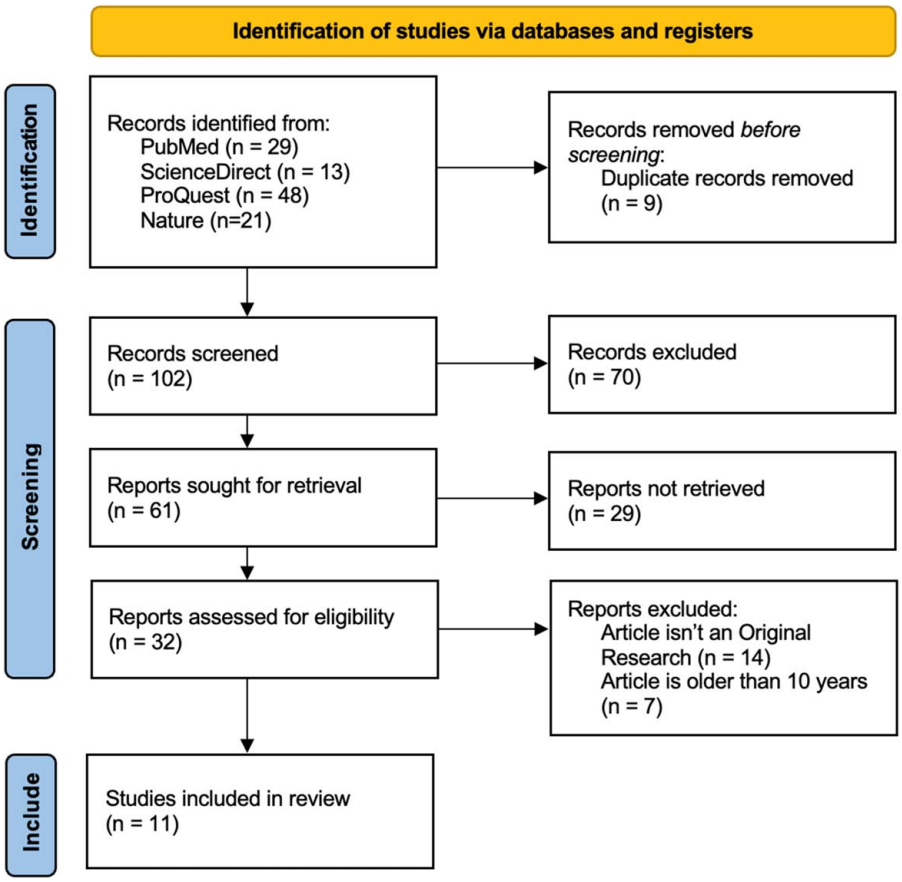

Figure 1. PRISMA flowchart of record selection process.

potential to cause positive effects when given to patients with carcinogenic cell development in the colorectal epithelium and stabilize the development of atypical degrees. Administration of *L. lactis* subsp. *lactis* (R7) isolate also increases the number of white blood cells, which indicates the functioning of the body's immune system. In the study of Li and colleagues, low levels of *L. lactis* in CRC tissue were associated with a decrease in NK cells[10,21].

In addition, *Lactococcus lactis* can produce nisin, which has been shown to inhibit cancer cell development by altering gene expression when added to food, but this requires further investigation[17]. The growth rate of cancer cells treated with

Table 1

The effects of *Lactococcus lactis* administration on colorectal cancer.

No	Type of study	Intervention	Result	Reference
1	*In vitro* (HCT116 cell line) and in vivo (NOD-SCID mice injected with HCT116 cells)	*Lactococcus lactis* NZ9000 (hsTRAIL+)	HCT116 cell viability ↓, HCT116-derived tumorspheres ↓	[1]
2	*In vitro* (SW480 cell line)	*Lactococcus lactis* ssp. *Lactis*	Cyclin D1 ↓	[9]
3	*In vivo* (male Wistar rats induced by 1,2-DMH)	*Lactococcus lactis* subsp. *lactis* (R7)	Degrees of atypia ↓, T lymphocytes ↑, monocytes ↑	[10]
4	*In vitro* (Caco2 cell line)	*Lactococcus lactis* IBB109 and IBB417	IL-18 ↑	[11]
5	*In vitro* (HT-29 cell line)	*Lactococcus lactis* subsp. *Lactis* 44Lac	HT-29 cell viability ↓	[12]
6	*In vitro* (DLD-1, HT-29, and LoVo cell line)	*Lactococcus lactis* NK34	DLD-1, HT-29, and LoVo cell proliferation ↓	[13]
7	*In vitro* (HT-29 cell line)	*Lactococcus lactis* NZ9000-401-kiss1	HT-29 cell proliferation ↓, MMP-9 ↓	[14]
8	*In vitro* (HCT116 cell line)	*Lactococcus lactis* NZ9000 (hsTRAIL+)	HCT116 cell apoptosis ↑	[15]
9	*In vitro* (SW480 and HCT116 cell line)	*Lactococcus lactis*/TRAILcwa	Bax ↑, Bcl-2 ↓	[16,22]
10	*In vitro* (SW480 and HCT116 cell line)	Nisin from *Lactococcus lactis*	HERV-K ↑, HERV-W ↑, miRNA-9-5p ↑, miRNA-9-192-5p ↑, miRNA-9-205-5p ↑	[17,20]
11	*In vitro* (Caco2 and HT-29 cell line)	*Lactococcus lactis*-AFFI-EVA and ZHER-EVA	IL-8 ↓	[18,21]

IL, interleukin.

nisin decreased significantly compared to those treated with the other two substances, namely cytoplasmic extract and cell wall ($P < 0.05$). Probiotic products such as nisin, bacterial cytoplasmic extract, and bacterial cell walls have anti-proliferative effects associated with decreased Cyclin D1 expression in the SW480 cell line[9].

Administration of *L. lactis* can reduce IL-6 cytokine levels in supernatants of immunostimulation THP-1 colorectal cancer cell lines and U-937 monocyte cell-like and reduce IL-8 levels in Caco-29 and HT-29 cancer cell lines[18]. Some studies support the use of *L. lactis* strains as therapeutic agents. For example, *Lactococcus lactis* strain NZ9000 is used as a vector for the production of human TRAIL, which plays a role in killing tumour cells. This is done by transforming the human soluble TRAIL-cDNA (hsTRAIL-cDNA) into *L. lactis* NZ9000 through the electropolishing process. This cloning process will produce hsTRAIL, cytotoxic to colon cancer cell line HCT116[15]. *L. lactis* subsp. *Lactis* 44Lac is known to have moderate anti-bacterial activity against *S. marcesens*, *K. pneumoniae*, and *E. coli*. This anti-bacterial activity is derived from bacteriocin (nisin), so this strain has anti-pathogenic effects and is used in food preservation[12]. *L. lactis* NK34 is also known to have the ability to inhibit cancer cell proliferation and pro-inflammatory cytokine production in several cancer cell lines SK-MES-1, DLD-1, HT-29, LoVo, AGS and MCF-7[13]. Through the Nisin Controlled Gene Expression System (NICE), *L. lactis* bacterial modification (hsTRAIL +) can induce the death of HCT116 and SW480 cancer cell lines and reduce the growth of HCT116-tumour sphere *in vitro*[1].

Lactic acid bacteria can cause cancer cells' apoptosis through intrinsic and extrinsic pathways. Recombinant *Lactococcus lactis* that produce TRAIL protein has been proven to induce apoptosis of colon adenocarcinoma cell lines SW480 and HCT116. This is evidenced by a significant decrease in Bcl-2 gene expression in the HCT116 cancer cell line. Bax gene expression as a proapoptotic gene increased significantly in both groups of cancer cell lines studied[16]. Other studies have shown a particular binding of *Lactococcus lactis* with surface proteins displayed by EpCAM-targeting and HER2-targeting with tumour antigens on human cells under static and constant flow conditions. This system is a step forward in utilizing bacteria-based colorectal cancer theranostics[22]. Exopolysaccharide (EPS) from *Lactococcus hircilactis* strain CH4 and *Lactobacillus delbrueckii* strain GRIPUMSK is an antimicrobial that can inhibit *H. pylori*, *S. flexneri*, *E. faecalis*, and *C. albicans* and an excellent antioxidant in preventing free radical-induced tissue damage. It can potentially kill cancer cells[23].

Conclusion

Lactococcus lactis contains nisin, which can suppress various gene, protein, and cytokine expressions that play a role in cancer cell growth. Probiotics can inhibit colorectal cancer without significant side effects.

Ethical approval

Ethics approval was not required for this review.

Consent

Informed consent was not required for this review.

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Author contribution

M.I.R.: writing the paper, study concept and design, data collection and interpretation. R.E.L.: study concept and design. A.E.P.: study concept and design. A.E.: editing the paper, study concept and design, data analysis and interpretation.

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The authors declare no conflicts of interest.

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