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Probiotics as a potential tool for gynecological cancers prevention and therapy support

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ABSTRACT

The human body is host to a variety of microbial species that are essential for maintaining general health. About nine percent of these microbial species inhabit the urogenital tract. It is therefore important to establish a symbiotic relationship between the host's immune system and the microbiota in this region. Disruptions of this symbiosis can contribute to the development of pelvic inflammatory disease (PID), bacterial vaginosis (BV), vaginal candidiasis and/or even gynaecological cancers by promoting the proliferation of infectious agents. The chemotherapeutic agents currently used in cancer treatment are associated with undesirable side effects. Therefore, there is an urgent need for antitumor agents that have minimal toxicity compared to conventional treatments. Furthermore, the use of drugs to prevent or alleviate the side effects of cervical cancer treatment could significantly improve the quality of life of patients undergoing such therapy. In this review, we focus our attention on the potential efficacy of probiotics in both the prevention and therapeutic intervention of neoplasms affecting the genital tract.

KEYWORDS: *Lactobacillus*, *Saccharomyces cerevisiae* var. *boulardii*, cervical cancer, microbiome

Introduction

Probiotics are living microorganisms, usually bacteria, which provide health benefits when consumed in sufficient quantities. Similar beneficial microorganisms occur naturally in certain foods such as yogurt, kefir, sauerkraut, kimchi and kombucha, as well as in dietary supplements. Probiotic microorganisms commonly used in the human diet mainly belong to the genera: *Lactobacillus*, *Bifidobacterium*, *Lactococcus*, *Streptococcus* and *Enterococcus*. Some strains of *Bacillus* and *Saccharomyces* are also used (Markowiak and Śliżewska, 2017). These microorganisms are able to produce anticarcinogenic, antioxidant and

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antimutagenic agents and provide protection against various bacterial diseases, which makes them an interesting tool in cancer research.

In the uterine tract of healthy women, *L. crispatus*, *L. gasseri*, *L. iners* and/or *L. jensenii* have been found to dominate the microbiome and maintain a physiological pH range (3.8–4.5) through the production of lactic acid, which inhibits the proliferation of pathogenic species (Garcia-Grau *et al*., 2018). In addition, lactobacilli are involved in the production of hydrogen peroxide (H_2O_2), which has been shown to have a protective role against bacterial vaginosis (BV) associated bacteria (e.g. *Gardnerella vaginalis*, *Prevotella bivia*, *Atopobium vaginae*) and sexually transmitted infections (Yarbrough *et al*., 2015). In studies by Ravel and colleagues (2011), the 16S rRNA marker gene was used to categorize the vaginal microbiota into four distinct low-diversity CSTs (community state types) dominated by a single *Lactobacillus* spp.: CST-I, in which *L. crispatus* dominates, CST-II with a predominance of *L. gasseri*, CST-III – *L. iners* and CST-V – *L. jensenii*. About 80% of women of reproductive age have a simple vaginal microbiota dominated by *Lactobacillus*, while the remaining 20% have a more diverse community type (CST-IV) with several subgroups (Ravel *et al*., 2011). Studies have shown that women who are not dominated by lactobacilli have an increased susceptibility to various genital infections, an increased risk of unfavourable reproductive outcomes, a poor pregnancy rate, early spontaneous abortion, late miscarriage and premature birth (Pendharkar *et al*., 2023). Current research indicates that the microbiome is integral to the female reproductive endocrine system, and interacts with estrogen, androgen, insulin, and others (Qi *et al*., 2021). Brooks *et al*. (2017) suggested that the oral hormonal contraceptives can positively impact the reproductive system by increasing the abundance of *Lactobacillus* species and reducing bacterial texa associated with bacterial vaginosis. Furthermore, progesterone may enhance the α-diversity of both vaginal and endometrial microbiomes.

Microbiome of gynecological cancer

The precise involvement of the uterine microbiome in pathophysiological processes remains unclear. Recent advances in metagenomic and sequencing technologies offer promising opportunities to solve this mystery. A cross-sectional study of 31 women revealed a significant correlation between the co-occurrence of *Atopobium* and *Porphyromonas* in the lower and upper genital tract, coupled with abnormal vaginal pH (> 4.5), and the incidence of endometrial cancer (Walther-António *et al*., 2016). Subsequently, a separate study examined the microbiome composition in ovarian tissue and normal tissue samples from 25 patients diagnosed with ovarian cancer. The results showed a significant reduction in the abundance of lactobacilli and an increase in anaerobic bacterial populations in ovarian carcinoma tissue, compared to adjacent normal tissue (Zhou *et al*., 2019). Furthermore, a robust case-control study of 360 women, including 176 individuals with ovarian cancer and 184 controls, demonstrated a reduced presence of lactobacilli (<50%) in the cervicovaginal microbiome of women with ovarian cancer (Nené *et al*., 2019). In cervical cancer, dysbiosis has been shown to influence human papillomavirus (HPV) infection by affecting HPV acquisition, clearance and persistence, as well as the host immune response by affecting levels of immune system proteins such as TGF-β1 (transforming growth factor β 1) (Łaniewski *et al*., 2020). Collectively,

these studies provide compelling evidence linking disruption of the genital microbiome to the pathogenesis of gynaecological malignancies. Therefore, probiotics may serve as adjunctive agents to enhance or modulate other diagnostic and therapeutic approaches.

Kailasapathy *et al*. (2000) have proposed mechanisms by which probiotics may exert their role as antitumour agents. These mechanisms include activation of the host immune system, alteration of colonic transit time and motility, suppression of pro-carcinogens and carcinogens, inhibition of bacteria involved in the conversion of pro-carcinogens to carcinogens, and lowering of intestinal pH (Kailasapathy *et al*., 2000). Research suggests that probiotics have a significant impact on several biological processes involved in cancer, including apoptosis, oxidative stress, proliferation, inflammation and metastasis (Johanshahi *et al*., 2020).

Probiotics and their use in gynecological cancer treatment

Cervical cancer is one of the most common malignancies affecting women's health worldwide, although its incidence is significantly lower in developed countries. Approximately 80% of cases are reported in developing or less developed countries. It is typically asymptomatic in its early stages, but as the disease progresses, symptoms may manifest, such as dyspareunia, pelvic pain, irregular vaginal bleeding, fatigue and leg swelling (Simms *et al*., 2020). Findings have shown that *Lactobacillus plantarum*, sourced from vaginal secretions of young adult and adolescent women, has been demonstrated to possess probiotic attributes and exhibit anticancer properties against the HeLa line of cervical cancer (Nami *et al*., 2014). In another study involving the HeLa cell line, research on human milk-derived strains of *Lactobacillus* (*L. casei* SR1, *L. casei* SR2, and *L. paracasei* SR4) demonstrated notable probiotic effects. Results indicated that supernatants from cell-free cultures exhibited anticancer activities, including the BCL-2 downregulation and upregulation of genes of such proteins as caspase 3, caspase 8, caspase 9, BAX (BCL2-associated X protein), and BAD (BCL2-associated agonist of cell death) (Rajoka *et al*., 2018). Treating HeLa cells with supernatants of *L. rhamnosus* and *L. crispatus* has been observed to decrease the expression of the *CASP3* (caspase-3) gene, as well as *MMP2* (matrix metalloproteinase 2) and *MMP9* (matrix metalloproteinase 9), resulting in an inhibitory effect on metastasis (Nouri *et al*., 2016). Research findings of Cha *et al*. (2012) indicate that *Bifidobacterium adolescentis* SPM1005-A exhibits antiviral activity in the SiHa cervical cell line, which expresses HPV type 16 (oncogenic), potentially contributing to the prevention of cervical cancer. Treatment with this bacterium has been shown to decrease the expression of the *E6* and *E7* oncogenes at both mRNA and protein levels. *L. gasseri* 3396 and *L. crispatus* 2743 had an inhibitory effect on the expression of *E6* and *E7* at the mRNA level (Li *et al*., 2019). The same effect was noticed in the case of cell-free supernatants (CFS) of *L. crispatus*, *L. jensenii* and *L. gasseri* treatment of Ca Ski cells (Wang *et al*., 2018).

It has been observed that cisplatin pro-apoptotic and cytotoxic effects against cervical cancer cells are enhanced by co-treatment with *Lactobacillus* bacteria. In a recent study, Negi *et al*. (2020) proposed a drug delivery strategy involving the application of probiotic strains. They concluded that pessaries containing cisplatin and probiotic biomass could offer an improved therapeutic approach for cervical cancer, as probiotic

strains exhibited favourable effects such as free radicals' elimination. Conversely, Kim *et al*. (2015) reported that the extract of *L*. *casei* did not demonstrate synergistic effects with anticancer drugs in suppressing the growth of HeLa and Ca Ski cancer cells. Despite numerous studies investigating the role of probiotics in cervical cancer, little is understood about their synergistic effects with drugs used to treat this cancer. Nonetheless, probiotics have the potential to enhance the antitumor effects of other drugs (Villeger *et al*., 2019). The knowledge of exact mechanisms associated with antitumour actions of probiotic bacteria is still limited and requires further research. Little is known about the effects of probiotics in other types of gynaecological cancer.

Yeast probiotics and their potential use in cancer therapy support

A wide array of yeasts, such as *Saccharomyces cerevisiae* var. *boulardii*, *Kluyveromyces*, *Debaryomyces*, *Candida*, *Pichia*, *Hanseniaspora*, and *Metschnikowia*, have been demonstrated to exhibit probiotic properties. Similarly, the anticancer properties of yeast probiotics have been extensively examined through various methods in numerous studies, including cell-based investigations, animal models, and clinical trials (Sambrani *et al*., 2021). The promotion of traditional fermented foods enriched with a spectrum of yeast probiotic strains holds promise in mitigating the risk of colorectal cancer (CRC) development significantly. Multiple investigations have underscored the favourable impact of yeast probiotics in cancer therapy support, highlighting their capacity to engage apoptotic pathways and modulate immune responses (Shamekhi *et al*., 2020). *In vivo* studies, coupled with high-throughput metagenomic analysis of 281 stool samples, have substantiated efficacy of *S. cerevisiae* var. *boulardii* use in significantly impeding colorectal cancer metastasis. The inhibition of cancer cells development was achieved through the stimulation of cancer cell apoptosis and the promotion of gastrointestinal health via immunomodulation. Notably, *S. cerevisiae* var. *boulardii* demonstrates a capacity to downregulate the expression of various genes encoding proteins implicated in tumour genesis, including TNF-α (tumour necrosis factor alpha), interleukin-1β, and interleukin-17. *S. cerevisiae* var. *boulardii*-based probiotics has been also used in vaginal candidiasis treatment, which is caused by *Candida albicans*. The yeast-based probiotics and fungus interaction results in prohibiting the cohesion of *Candida albicans* to the vaginal epithelial cells (Pericolini *et al*., 2017). Despite reports suggesting the advantageous effects of yeast probiotics in support of cancer treatment, the range of their application in the treatment and prevention of gynecological malignancies remains incompletely investigated. A summary of the presented probiotics and their effect on cancer is shown in Table 1.

Conclusion

Because of their ability to restore and maintain a healthy microbiome in the gynaecological tract and modulate the immune system, probiotics hold promise for the treatment of several gynaecological conditions, including bacterial vaginosis, vaginal candidiasis, sexually transmitted infections, and human papillomavirus (HPV) infection. While numerous studies have highlighted the potential benefits of probiotics in cancer prevention and treatment support, research into their impact on endometrial and ovarian cancer prevention remains limited. Furthermore, the therapeutic potential of yeast in the treatment of

Table 1. Studies investigating probiotics on chosen cancer types.

Probiotics	Cancer type	Cell line/ Research material	Findings	Publication
Lactobacillus plantarum	Cervical cancer	HeLa	Inhibition of cancer cells growth	Nami *et al*. 2014
Lactobacillus casei SR1, *Lactobacillus casei* SR2, *Lactobacillus paracasei* SR4		HeLa	Apoptosis-related genes upregulation	Rajoka *et al*. 2018
Lactobacillus rhamnosus, *Lactobacillus crispatus*		HeLa	Inhibitory effect on metastasis	Nouri *et al*. 2016
Bifidobacterium adolescentis SPM1005-A		SiHa	Antiviral properties against HPV infection	Cha *et al*. 2012
Lactobacillus gasseri 3396, *Lactobacillus crispatus* 2743		SiHa	HPV oncogene downregulation	Li *et al*. 2019
Lactobacillus crispatus, *Lactobacillus jensenii, Lactobacillus gasseri* CFS		Ca Ski	HPV oncogene downregulation	Wang *et al*. 2018
Saccharomyces cerevisiae var. *boulardii*	Colorectal cancer	Metagenomic analysis of stool samples	Downregulation of expression of the genes implicated in tumor genesis	Shamekhi *et al*. 2020

gynaecological cancers has been largely overlooked.

Further investigation of the anticancer properties of specific probiotic strains and their underlying mechanisms is essential. Randomized, double-blind, placebo-controlled clinical trials are needed to gain approval from the medical community and validate probiotics as a viable assistance in cancer therapy (Śliżewska *et al*., 2020). In conclusion, further research is needed to clarify the precise mechanisms of action of probiotics, although the existing evidence strongly supports their use to improve women's health.

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