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Review

Human Commensal Bacteria: Next-generation Pro- and Post-biotics for Anticancer Therapy

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Abstract

Cancer is a common, deadly disease with an unknown etiology. Meanwhile, current therapeutic options possess significant risks. However, probiotic bacteria and their metabolites have been reported to have antiproliferative and apoptotic effects on cancer cells. Therefore, because of their selective specificity and lack of treatment-associated comorbidities, these bacteria and their metabolites could be potential alternatives to conventional chemical and radiation therapies. Given their superior immunomodulatory and anti-cancer effects and lack of side effects, commensal bacteria derived from healthy humans are currently used as next-generation probiotics. This review summarizes current findings on these probiotic properties and anti-cancer activities of healthy human commensal bacteria. Additionally, the review focuses on small metabolites, proteins, and enzymes secreted by human commensal bacteria for their therapeutic applications against cancer. Further, utilizing a protein engineering strategy to reduce the toxicity of L-asparaginase, an enzyme-based anti-leukemia drug used for the last forty years, is also discussed. A possible workflow outline for isolating, identifying, screening, and characterizing human commensal bacterial strains for their therapeutic applications in cancer treatment is also proposed. This review emphasizes the need to explore various human commensal bacteria, not just mainstream lactic acid bacteria, for novel cancer therapeutics that provide multiple health benefits.

Keywords: anticancer; bacterial metabolites; human commensal bacteria; L-asparaginase; probiotics; postbiotics

1. Introduction

Cancer is a rapidly growing disease and the second leading cause of death worldwide after cardiac diseases [1,2]. Every fourth person is at potential risk of developing cancer during life [3]. Chemotherapy and radiation therapy are the mainstream existing cancer therapeutics, but they possess limited efficacy, ambiguity and adverse side effects [4]. Diagnosis of the cancer at an advanced stage further contribute to the failure of the existing therapeutics [5]. Innovative methods for treating cancer include targeted therapy and cancer vaccinations [6]. Natural resources, such as bacteria, plants, fungi, and marine microorganisms, are observed as abundant therapeutics supply against human diseases, including cancer. In this regard, bacterial metabolites having anti-cancer activity have caught research attention due to their natural origin, ease of production and target-specific action. Moreover, approximately 13,000 naturally occurring chemical compounds with various pharmacological properties have been identified till date from distinct bacterial strains [5,7].

To overcome issues associated with chemo and radiation therapy in cancer treatment, exploration of microbes and their metabolites is crucial because of their advantages to overall health benefits, immunomodulation, cancer prevention and treatment [8,9]. Furthermore, whether used alone or in combination with the traditional anti-cancer therapies, the microbiome treatment ensures improved efficacy and reduced toxicity [10,11]. Human commensal bacteria have co-evolved and naturally adapted to the human host and are involved in maintaining host homeostasis and robustness of the immune system [12,13]. As a result, they can be more beneficial than bacteria from other sources such as food, marine, soil, and plants and could be the most promising candidate for addressing metabolic, immunological and cancer-related health problems [12,14]. Additionally, these commensal bacteria secrete bioactive compounds such as short chain fatty acids, bacteriocins, indoles, indole derivates, and exopolysaccharides that can have anti-cancer activity besides their anti-inflammatory, anti-pathogenic, and immunomodulatory properties [15, 16]. It is intriguing that although two human individuals share 99.9% genetic similarity, their gut microbiome has 80 to 90% genetic diversity [17]. The bacterial secretions such as, toxins, enzymes [*e.g.*, lipases, proteases and L-asparaginases (ASNase)], efficiently target cancer cells [18]. Because of the specificity towards cancer cells, bacterial secretions have the potential to serve as targeted therapeutic agent. However, the Food and Drug Administration (FDA)-approved L-asparaginase for the treatment of blood cancer is associated with therapeutic toxicity. There-

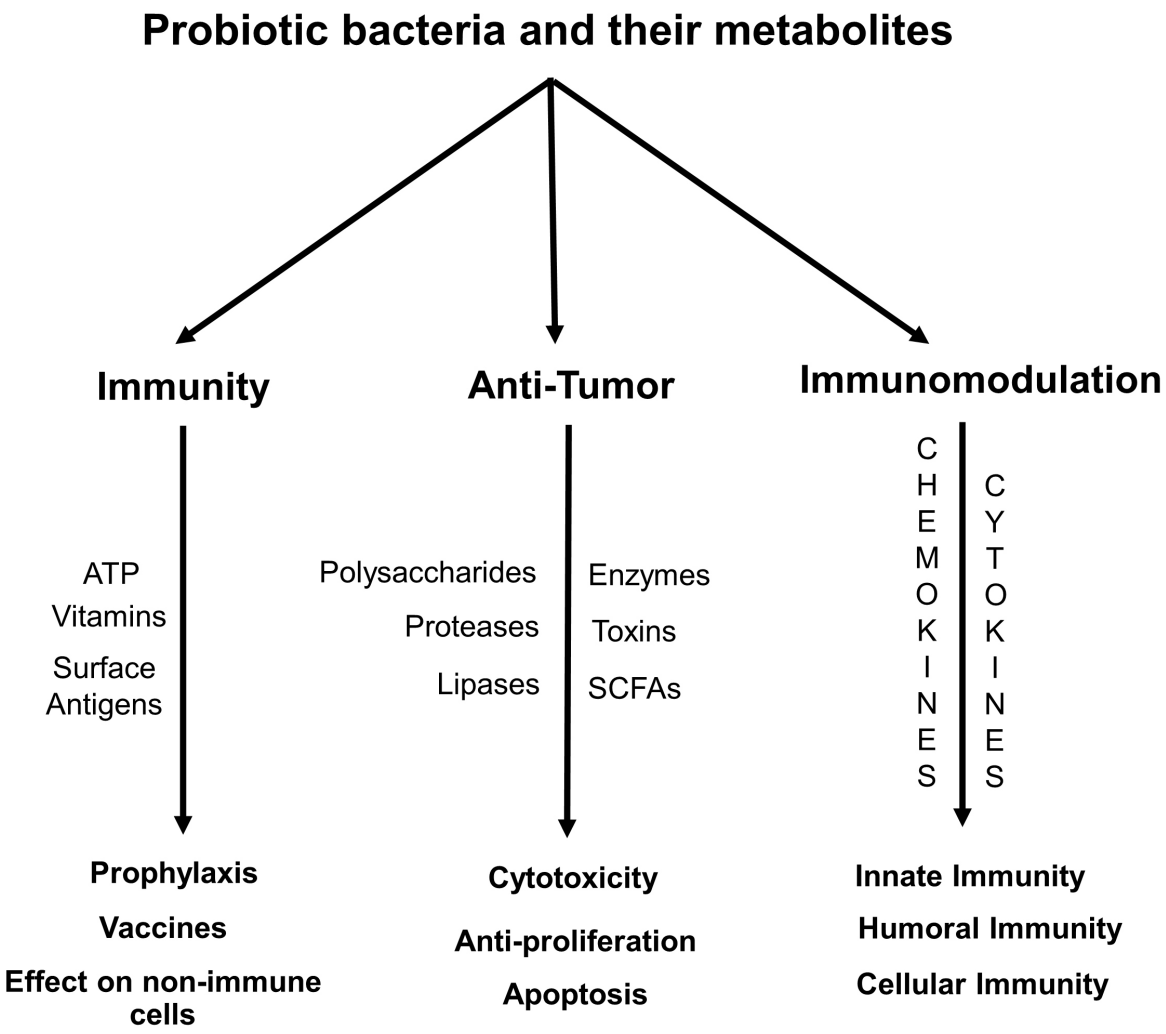

Fig. 1. Flow chart summarizing the role of probiotic bacteria and their metabolites in providing immunity and protection against cancer. SCFAs, short-chain fatty acids.

fore, in the present review, we have proposed the bidirectional approaches—(i) protein engineering of existing L-asparaginase and (ii) exploring alternative L-asparaginase sources-to address the toxicity issues. Further, the current findings of human commensal bacteria and their metabolites in cancer prevention and therapy have also been summarized. Furthermore, the detailed workflow for exploring human commensal bacteria and their metabolites as next-gen probiotics and novel biotherapeutics for ant-cancer therapy is also proposed. The following sections discuss the potential of human commensal bacteria and their metabolites for the treatment and management of different cancer types.

2. Probiotic Bacteria and Anti-cancer Benefits

Probiotics are live and safe microorganisms that are adequately consumed to promote health by rebalancing the intestinal habitat [19,20]. Utilizing undigested food components in the host, these organisms secrete various metabolites that balance the intestinal pH, exhibit antibacterial properties, stimulate and activate immune cells, metabolize cancer-causing substances and maintain the gut microbial equilibrium to rule out dysbiosis [20–22]. Currently, a number of bacteria and their metabolites have been assessed for their anticancer properties and are being further explored as potential alternatives to persisting anticancer therapies [23,24]. Bacterial metabolites are small molecular weight, biologically active natural products that significantly impact health and disease, affecting local and systemic environments [25,26]. Bacteria mainly produce short-chain fatty acids (SCFAs), indole derivates and polyamines by utilizing the host diet, and these metabolites contribute to reducing tissue inflammation and cancer cell proliferation [22,27]. Bile acids and their derivatives are host-derived metabolites modified by bacteria that aid in metabolism and innate immune cell function [16,28]. Bacteria directly synthesize adenosine triphosphate (ATP), Vitamin K, various Vitamin B, and capsular polysaccharides that promote overall antitumor immunity [29,30]. Bacterial

secretions such as enzymes, toxins, lipases and proteases efficiently target cancer cells [24,31]. Gram-negative bacteria (*Salmonella*, *Escherichia*, *Pseudomonas*, and *Proteus*) and gram-positive bacteria (*Clostridium*, *Bifidobacterium*, and *Lactobacillus*) have been reported to have cytotoxic, antiproliferative and apoptotic effects on cancer cells [24,32]. Some bacterial components can be used as adjuvants for vaccine production [33]. Additionally, bacterial metabolites have direct and indirect immunomodulatory effects on both immune and non-immune cells that confer natural and acquired immunity by regulating the proinflammatory cytokines; interleukin (IL) -1 beta (β), IL-6, and tumor necrosis factor (TNF) -alpha (TNF-α) and anti-inflammatory cytokines (IL-1 receptor antagonist, IL-4, IL-10, IL-11, IL-13 and transforming growth factor beta (TGF-β)) as illustrated in Fig. 1 [34–36]. Hence, because of target specificity and ability to prevent multidrug resistance and adverse reactions, bacteria derived metabolites can be alternative to chemical and radiation therapy, and the steroids used for immunosuppression [5,23,26].

3. Human Commensal Bacteria: A Potential Next-generation Probiotics for Anticancer Therapy

It is known that during tumor development, the microbiota composition changes [37] and, in some instances, the alteration may even start before the formation of tumor [38]. Due to invasiveness and biofilm-forming capabilities, some pathogenic bacteria such as *Escherichia coli* and *Bacteroides fragilis*, can abundantly manifest during the early time point of transformation. Metagenomic sequencing of fecal bacteria from different stages of colorectal cancer patients have revealed increased abundance of *Bacteroides massiliensis*, *Bacteroides ovatus*, *Bacteroides vulgatus*, and *E. coli* [37]. Due to the changes in metabolic, adhesive, and nutrient availability caused by oncogenic transformation, locally, the microbiota also gets redistributed. Interestingly, in some cases resistance to chemotherapy has been linked with enrichment of *Gammaproteobacteria* and *Fusobacterium nucleatum* [37]. It has also been demonstrated that depletion of commensal bacteria can also reduce the effectiveness of chemotherapy or radiotherapy. On the other hand, presence of *Akkermansia muciniphila*, *B. fragilis*, *Enterococcus*, and some *Bifidobacterium* strains have been reported to enhance the effectiveness of immunotherapy in cancer patients.

Known for their beneficial role as probiotics, mainstream lactic acid-fermenting bacteria have been consumed in the diet for decades. However, food-derived lactic acid bacteria have been shown to be delicate and unable to tolerate extended exposure to acidic pH following fermentation, oxygen during refrigeration storage, and human gastric pH [39,40]. Thus, beyond the traditional sources of probiotics such as food and dairy, researchers are searching for human commensal candidates and their metabolites for a wider spectrum of health benefits, either operating alone or in combination with commercial anticancer drugs [41–43]. These findings would also help us develop new therapeutic approaches and modulate the microbiome for prevention and/or cure of cancer.

The application of next-generation sequencing for metagenomics and identification of microbes, has widened the spectrum of traditional probiotics, which include a sizable number of bacteria with potential therapeutic properties [44]. Moreover, natural bacterial strains residing in healthy human individuals can serve as novel sources of probiotics [8,19,45]. Interestingly, gut-derived bacteria have greater adhesion ability and tolerance to high bile salt concentrations and acidic pH, making them plausible candidates for next-generation probiotics [45–47]. Furthermore, probiotic investigations should target the production procedure, stable storage and delivery mode [48,49]. Importantly, different strains of the same bacterial species have varied impacts on metabolic regulation, inflammation and cell proliferation [50,51]. Further, the culture supernatants of *Enterococcus faecalis* strains isolated from the stool of healthy volunteers showed an antiproliferative effect on human colorectal carcinoma cell lines, but *E. faecalis* strains isolated from the stool of colorectal cancer patients did not show any effect [51]. Additionally, the strains isolated from healthy volunteers did not affect the human fetal colon epithelial cell line CRL-1790 [51].

Bazireh *et al*. (2020) [45] reported the potential *in vitro* probiotic properties of five lactic acid bacterial strains isolated from human feces and saliva. The cell-free extract of these strains were found to be effective against colon carcinoma cell line (Caco-2). A microbial biotherapy candidate, *Lactobacillus plantarum 5BL*, a vaginal commensal bacterial strain was found to induce apoptosis and thereby showed significant anticancer activity against the cervical (HeLa), colon (HT-29), gastric (AGS), and breast (MCF-7) cancer cell lines ($p \leq 0.05$) [52]. Interestingly, it did not show any evident cytotoxic effects on normal human umbilical vein endothelial cells (HUVEC) [52].

The examples described in this section imposed that human-derived bacteria, other than mainstream lactic acid-fermenting bacteria, can provide alternative novel probiotic candidates and novel metabolites for anticancer therapy. However, individual human microbiota strains differ in their probiotics and anticancer activities, and therefore more exploratory research is needed.

4. Metabolites and Biomolecules from Human Commensal Bacteria: A Next Generation Postbiotics

Lactic acid bacteria components and their metabolites are widely studied for their *in-vitro* anticancer activity [53]. In a gene expression study by quantitative real-time polymerase chain reaction (qRT-PCR), the bacterial supernatants of the probiotic *L. acidophilus* ATCC 4356 strain

showed dose and time-dependent apoptotic effects on colorectal cancer cell lines through the upregulation and downregulation of the *SURVIVIN* and *SMAC* genes, respectively [54]. Similarly, the proteinous metabolites isolated from vaginal human commensal *Enterococcus* strains had significant anticancer effects on cervical, lung, colon, and breast cancer cell lines [55,56]. Also, culture-free supernatants obtained from vaginal normal-flora, *viz.*, *Enterococcus hirae* 20c, *Enterococcus faecium* 12a and L12b showed pronounced cytotoxic effect on cervical and lung cancer cell lines. Furthermore, these strains showed *in-vitro* probiotic properties such as bile and gastric acid tolerance, potent antimicrobial activity, biofilm formation and adhesion to epithelial cells, etc. [55]. Another vaginal commensal, *E. faecalis* 16H, exhibited potent *in vitro* probiotic properties, and the secreted proteinaceous metabolite showed a selective apoptotic effect on multiple cancer cells (gastric, cervical, breast and colon) and no effect on normal cell lines. Furthermore, the functional characterization of the anticancer metabolite from vaginal *E. faecalis* would be useful for the cancer therapy [56].

Individual human commensal bacteria and their metabolites have target-specific effects; however, their effectiveness as probiotics and anticancer agents may vary [57]. Several human gut-derived *Lactobacillus* strains exhibit *in vitro* probiotic properties, and their extracellularly secreted metabolites have effective cytotoxic effects on colorectal cancer cell lines, such as Caco-2 and HRT-18, without affecting normal Vero cells, confirming their therapeutic potential in colorectal cancer [58]. Mixed *Bifidobacterium* strains, representative of skin and gut human commensals, showed potent anticancer effects on colon adenocarcinoma cell lines and were ineffective against normal rat-derived epithelial cells. Further, in mouse models, it decreased the expression of colon cancer markers, epidermal growth factor receptors and cyclooxygenase-2 and led to disease stabilization, colon integrity and halting of cancer growth and metastasis. The higher anti-proliferative activity observed was considered to be due to the quorum-sensing ability of mixed *Bifidobacterium* strains [59].

Exploring human commensal bacteria for their additional roles in cancer prevention and therapy, cancer immunity and microbiota maintenance is a need of the hour. Different metabolites with anticancer potential that are produced by human commensal bacteria are summarized in Fig. 2. 6-*N*-Hydroxyaminopurine (6-HAP) from the human skin commensal *Staphylococcus epidermidis* not only selectively reduced melanoma tumor growth but also protected against induced tumors. 6-HAP restricted the growth of murine melanoma cell lines and murine T-cell lymphoma cells by blocking adenosine and thymidine (A = T) base pairing leading to inhibition of DNA synthesis. Compared with vehicle, intravenous inoculation of 6-HAP in melanoma-infused mice reduced tumor growth by more than 60%. Interestingly, when the tumor-induced mice were subjected to ultraviolet (UV) radiation and then tropically injected with 6-HAP, the incidence and frequency of tumor growth were low [60]. Oral administration of fecal samples from healthy humans to germ-free (GF) mice resulted in a notable increase in interferon gamma (IFNγ+) CD8+ T-cell and CD4+ T-cell populations.

The consortia of 11 unique bacteria composed of seven Bacteroidales (with IFNγ-noninducing ability) and four non-Bacteroidales (with IFNγ-inducing activity) showed wide spectrum biotherapeutic potential for treating cancer and also exhibited antipathogenic potential against *Listeria monocytogenes* [61]. Recently, *E. coli* Nissle (EcN), combined with galunisertib (a TGF-β blocker) accelerated tumor growth suppression and tumor migration inhibition in liver and breast cancers. The combination also enhanced the immune response by activating and inducing the recruitment of effector T cells and dendritic cells to the tumor site in hepatic and breast carcinoma-challenged immunodeficient albino mice [62]. Additionally, the probiotic EcN, balanced the gut environment of tumor-infused mice with increased numbers of *Bacteroides*, *Akkermansia* and *Lactobacillus* [62]. *E. coli KUB-36*, derived from healthy adult feces with highest short chain fatty acid production, showed cytotoxicity to colon, breast, and leukemic cell lines. It also showed anti-inflammatory activity by downregulating IL-1β, IL-6, IL-8 and TNF-α and upregulating IL-10 in lipopolysaccharide-induced human monocytic leukemia (THP-1) cell lines [63]. Recently identified next-generation probiotic bacterium, *A. muciniphila* (ATTC BAA-835), combined with IL-2, inhibited colorectal cancer and subcutaneous melanoma growth in mice. Additionally, this combination increased survival by enhancing immunity and maintaining gut microbiota homeostasis. *A. muciniphila* -IL2 combination, strongly lysed patient-derived colon tumor cells *in vitro* compared to *A. muciniphila* alone. Oral administration of *A. muciniphila* in tumor-bearing mice infused with IL-2, inhibited tumor growth and enhanced survival by promoting infiltration of immune cells to the tumor site through activation of the Toll-like receptor signaling pathway [64].

Another metabolite - exopolysaccharides (EPSs), obtained from *Lactobacillus* strains, showed anti-proliferative activity against colon and cervical cancer cell lines by inducing apoptosis and autophagy [65–67]. EPSs with higher mannose/glucose ratio showed better antiproliferative effects via time-dependent apoptosis of HT-29 cells, highlighting the significance of mannose in cancer drug design [65]. The EPSs from the vaginal *L. gasseri* G1 and *L. gasseri* H15 strains inhibited cell proliferation and triggered apoptosis by upregulating *Bax* and *Caspase 3* genes in HeLa cells and also showed an anti-inflammatory effect by increasing IL-10 and decreasing TNF-α production [66]. Choi *et al*. (2006) [68] demonstrated the utility of human gut-derived *Lactobacillus acidophilus* 606, as a probiotic. The heat-killed (HK) strain of *L. acidophilus 606*

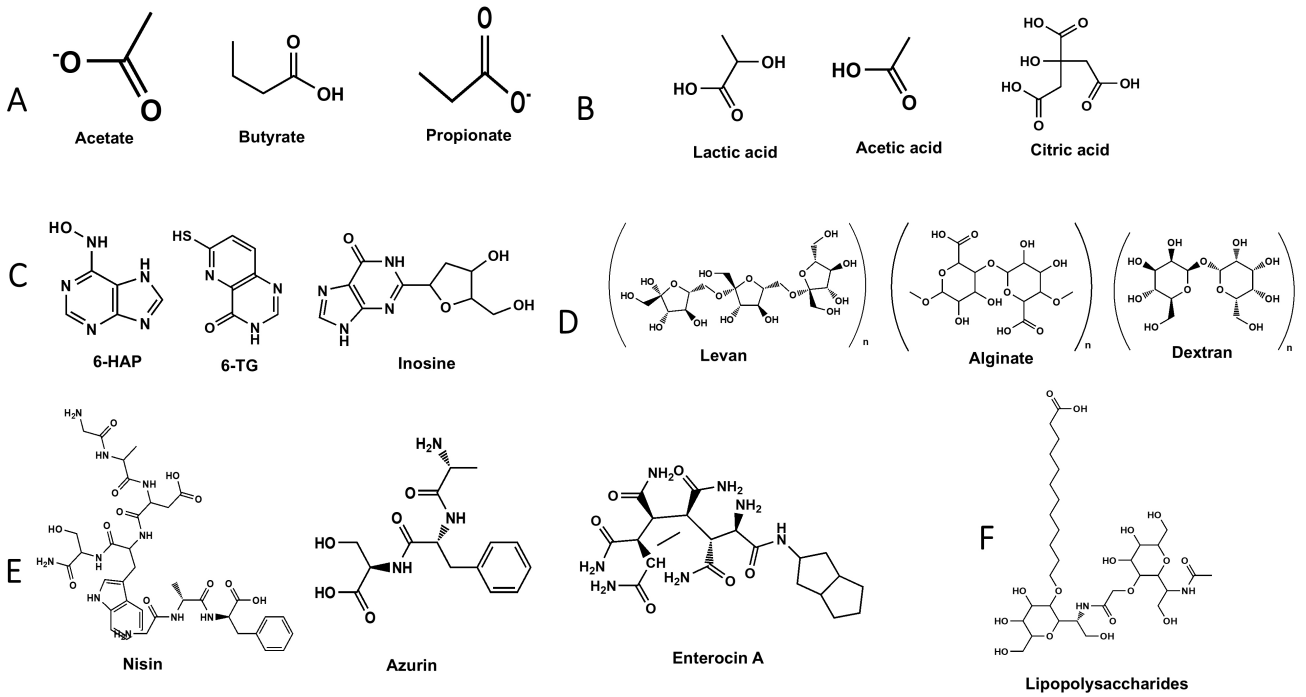

Fig. 2. Metabolites and molecules with anticancer potential obtained from human commensal bacteria. These metabolites include short chain fatty acids (A), organic acids (B), nucleotide derivatives (C), polysaccharides (D), small peptides (E) and lipopolysaccharides (F). 6-HAP, 6-N-hydroxyaminopurine; 6-TG, 6-thioguanine.

and its soluble polysaccharide component significantly inhibited colon, cervical and pancreatic cancer cell lines. Importantly, the polysaccharide component showed less toxicity to healthy human embryo fibroblasts (hEFs) than to HK cells. However, the protein and lipid components did not show anticancer activity against tested cancer cell lines [68]. Further, Kim *et al*. (2010) [67] demonstrated the potential of cell-bound exopolysaccharides (cb-EPS) obtained from *L. acidophilus* 606 to arrest the proliferation of HT-29 colon cancer cells *in vitro*. The cb-EPS induced the autophagy-associated proteins Beclin-1 and GRP78 as well as indirectly triggered the apoptotic signalling factors Bak and Bcl-2, that in-turn led to alteration in the cell morphology [67].

On the contrary, decreased production of short chain fatty acids – butyrate and propionate have been linked with the complication during chemo and radio therapies in cancer patients. Interestingly, a study by Okubo and colleagues [69] showed that higher abundance of Bacteroides was associated with increased fear of breast cancer recurrence. On the other hand, higher abundance of *Lachnospiraceae*, and *Ruminococcus* or increased alpha diversity was associated with reduced fear of cancer recurrence [69]. Therefore, studying the bacterial strains and the associated metabolites found amongst the patients showing reduced morbidity and cancer recurrence during treatment might lead to better therapy regimens.

In the light of above evidence, it can be suggested that human commensal bacteria-derived proteinaceous compounds, exopolysaccharides, and short-chain fatty acids (SCFAs) have selective cytotoxicity against cancer cells while sparing healthy ones. These metabolites regulate important cancer markers like *SURVIVIN* and *SMAC*, induces apoptosis, suppress the inflammatory pathways, and thereby display their potential as anticancer agents. Interestingly, these metabolites in combination with immune-modulating agents like IL-2 or TGF-β inhibitors, showed enhanced tumor suppression in colon, breast, and melanoma cancer models. Moreover, as heat-killed bacteria and extracellular polysaccharides are non-proliferative, they can be safer and more stable therapeutic alternatives in modern day anticancer therapy. However, to understand their mode of action, delivery to the target site, and to access efficacy and safety, human clinical trials are essential. Moreover, integrating the human commensal bacteria and/or their metabolites with existing therapies, could pave a new way for development of affordable, less toxic and more effective anticancer treatments.

The human commensal bacteria proven for their beneficial role against different types of cancer are listed in Table 1 (Ref. [45,52,54–56,58–60,62,63,65–68]).

5. L-asparaginase from Human Commensal Bacteria: For Improved Efficacy and Reduced Toxicity

In addition to small metabolites, proteins and enzymes secreted by human commensal bacteria have also been

Table 1. *In vitro* and *in vivo* anticancer properties of human commensal probiotic bacteria.

S. No.	Beneficial bacterial strains	Isolation source	Effective in cancer type	Findings	Reference
1.	*Lactobacillus fermentum* strains	Gut and mouth	Colon	Showed probiotics properties and cytotoxicity on Caco-2 cell lines.	[45]
2.	*Lactobacillus plantarum* 5BL	Vagina	Breast, Cervical and Gastric	Induced apoptosis in breast cancer cell line (MCF-7). No cytotoxic effects on normal cells. Also effective in gastric cancer.	[52]
3.	*L. plantarum* GD2, *L. rhamnosus* E9, *L. brevis* LB63	Gut	Colon	Secreted exopolysaccharides (EPS) with high mannose to glucose ratio induced apoptosis in HT-29 cell lines.	[65]
4.	*L. gasseri* strains (G10 and H15)	Vagina	Cervical	Bacterial strains and secreted exopolysaccharides showed anti-proliferative, apoptotic and anti-inflammatory effect on HeLa cells.	[66]
5.	*L. acidophilus*	Gut	Colon	Cell-bound exopolysaccharide (cb-EPS) induced autophagy and arrested the proliferation of HT-29 colon cancer cells.	[67]
6.	*L. acidophilus* 606	GI Tract	Cervical, Colon and Leukemia	Heat killed (HK) bacteria and its soluble polysaccharide showed dose and time dependent cytotoxicity against cervical cancer cell lines. Also effective against leukemia and colon cancer.	[68]
7.	*Lactobacillus acidophilus* (ATCC 4356)	Human microbiota	Colon	Secreted metabolite regulates expression and induced apoptosis in colon cancer cell.	[54]
8.	*Enterococcus* sp.	Vagina	Cervical, Lung and Hepatic	Secreted metabolites showed cytotoxicity against cervical cancer cell lines. Also effective in hepatic and lung cancer.	[55]
9.	*Enterococcus faecalis* 16H	Vagina	Breast, cervical, Colon, and Gastric	Secreted metabolites induced apoptosis in breast cancer cells. Also effective against cervical, colon, and gastric cancer cells.	[56]
10.	*Lactobacillus acidophilus* strains	Gut	Colon	Cell-free supernatants showed cytotoxicity on Caco-2 cells. No effect on Vero cells.	[58]
11.	*Bifidobacterium* species*	Skin and Gut	Colon	Mixed *Bifidobacterium* strains showed potent anti-cancer activity against colon adenocarcinoma cell lines.	[59]
12.	*Staphylococcus epidermidis**	Skin	Skin	Purified 6-HAP molecule restricted growth of murine melanoma cell and mice T-cell lymphoma cells by inhibiting DNA synthesis.	[60]
13.	*Escherichia coli* strain Nissle 1917 (EcN)*	Intestine	Breast and Gastric	Enhanced immunity and Suppressed breast tumor growth and malignant transformation in combination with galunisertib (transforming growth factor beta (TGF-β) blockade). Also effective in gastric cancer.	[62]
14.	*Escherichia coli* KUB-36	Gut	Breast and Gastric	Produces SCFA and showed anti-inflammatory activity and cytotoxicity against breast cancer cell lines. Effective against colon and gastric cancer cells too.	[63]

'*' denotes *in-vivo* study.

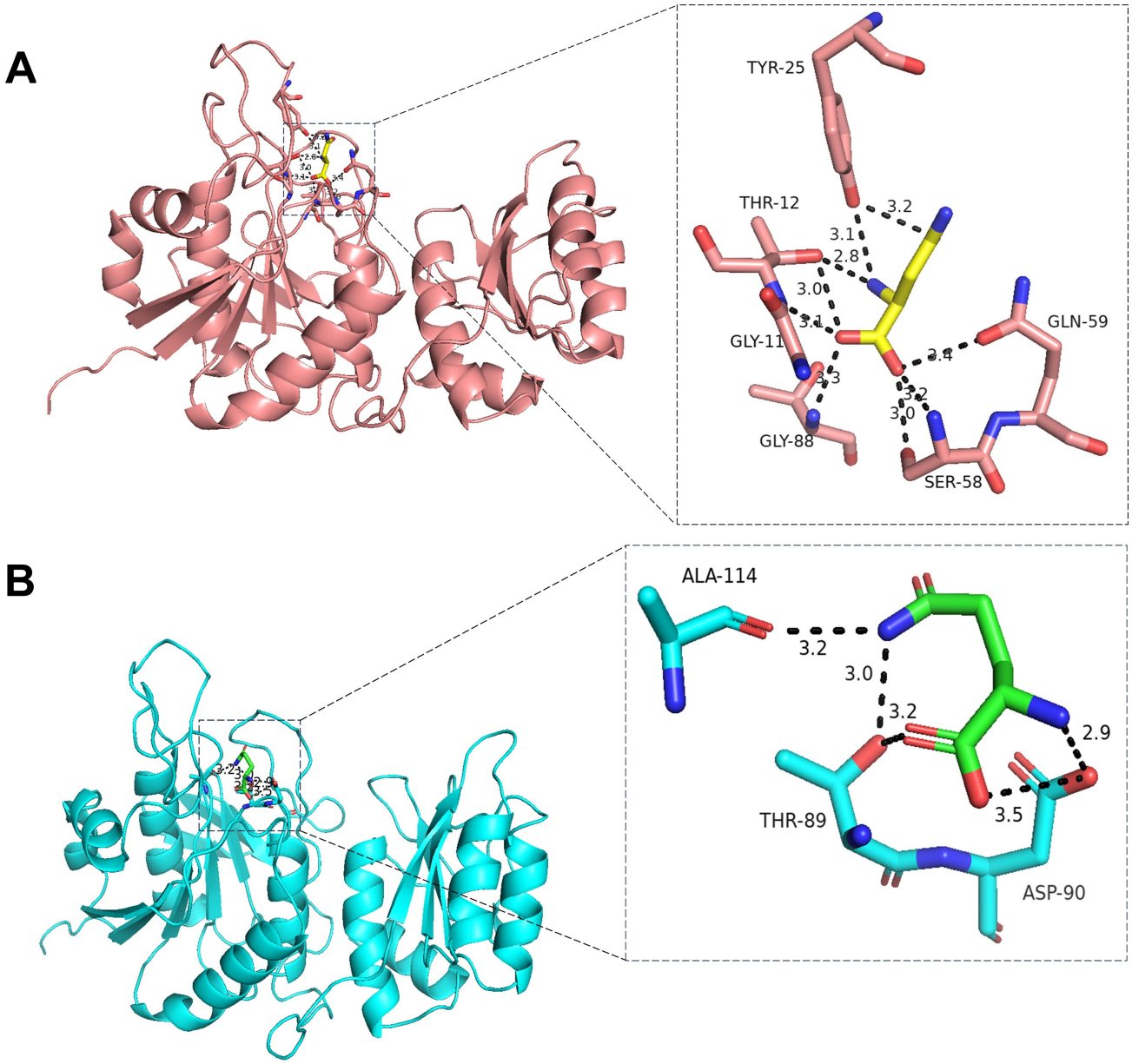

Fig. 3. Molecular docking of L-asparaginase from *E. coli* K12 (PDB Id 6PAC) with L-asparagine (A) and L-glutamine (B) was performed using AutoDock Vina v 1.1.2. The protein data bank (PDB) with partial charges (Q) and atom types (T) (PDBQT) files for protein and ligand molecules were generated with the help of AutoDock Tools v.1.5.6 (https://ccsb.scripps.edu/mgltools/1-5-6/). The ligand binding site was already known so grid box was set at this point, all other parameters were assigned to their default values, the best conformations were selected based on docking energy scores and visual inspection of the docked molecules within catalytic pocket.

shown to have therapeutic applications in cancer treatment. For instance, L-asparaginase obtained from *E. coli* K12 strain is FDA-approved drug for the treatment of leukaemia for last 45 years [70]. Though L-asparaginase selectively targets cancer cells, it also shows some side effects on normal human cells [71]. In contrast to normal cells, cancer cells lack the L-asparagine synthetase enzyme and thus cannot synthesize L-asparagine. Hence, the malignant cells rely on circulating plasma L-asparagine to grow and proliferate [31,71,72]. Externally supplied L-asparaginase (~3 μM) utilizes circulating plasma L-asparagine and deprive the tumor cells of this amino acid, leading to starvation and, ultimately death by p53-dependent apoptosis [73,74]. However, the treatment is associated with adverse reactions mainly exhibiting hypersensitivity reactions and organ related toxicities [72]. This is primarily due to the antigenicity and intrinsic L-glutaminase activity of the currently used L-asparaginases [75]. The intrinsic L-glutaminase activity deprives L-glutamine in the blood, which is crucial for normal cell division, tricarboxylic acid (TCA) cycle, asparagine synthesis and cell survival [76,77]. This can result in organ toxicity (liver, pancreas and brain), diabetes melli-

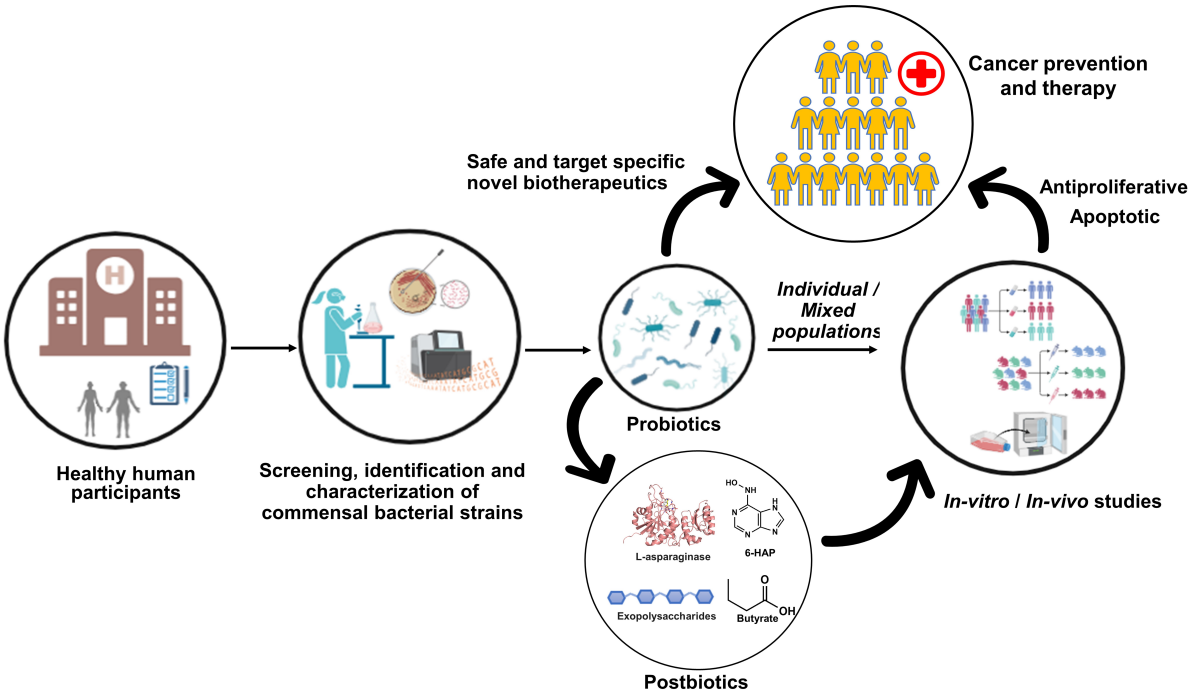

Fig. 4. The overall workflow to obtain human commensal bacterial strains from healthy individuals and exploring them as pro- and post-biotics in prevention and treatment of cancer.

tus, and blood abnormalities (hyperammonaemia, leukopenia and thrombosis) due to reduced protein synthesis such as albumin, fibrinogen, insulin and coagulation factors [72,75,78]. To circumvent the treatment challenges associated with currently available L-asparaginase, numerous bacterial sources *viz*, soil, plants, water, and extreme environments have been explored so far [79–83] and human commensal bacteria were still unexplored for screening and analysis of glutaminase-free L-asparaginase. Recently, we reported three strains (two of *E. coli* 3F1, 3F2 and one *Klebsiella pneumoniae* 3S3) that produced glutamine-free-asparaginase and also showed probiotic properties [84].

Alternatively, engineering of commercially available L-asparaginase can also be attempted to reduce its affinity towards L-glutamine. To attempt the same, we have inspected the structure of L-asparaginase (PDB Id 6PAC), there are four molecules in the asymmetric unit (Fig. 3). As calculated using computed atlas of surface topography of proteins (CASTp) program, the volume of the catalytic site is ~35.27 Å^3. The L-asparagine and L-glutamine binds to the catalytic pocket with energy values ~–4.29 kcal/mol and ~–4.20 kcal/mol, respectively. The L-asparagine forms hydrogen bond with Gly11NB, Thr12OG1, Tyr25OH, Gln59OE1, Ser58NB/OG and Gly88NB (Fig. 3), whereas L-glutamine forms hydrogen bond interaction with Thr89OG1, Asp90OD2, Ala114OB residues (Fig. 3). The later three amino acids at active site, can be explored for engineering commercial L-asparaginase to reduce its affinity towards L-glutamine and thereby reduce the side effects.

6. Prospects for the Use of Human Commensal Bacteria in Anticancer Therapy

As discussed in previous sections, human-derived, non-lactic acid fermenting bacteria can provide novel probiotic candidates and novel metabolites for anticancer therapy. In this context, we proposed the possible overall working protocol to obtain such strains and exploring them for their anticancer potential (Fig. 4). For instance, based on study's objectives, first, to get the required number of samples, identification of the volunteers within the framework of inclusion and exclusion criteria should be done [84]. Prior Institutional ethical clearance (IEC) is a must for a study involving human participants. Once the specimens are obtained one would follow a standard protocol to isolate, screen, identify and characterize the desired bacteria at the strain level. Further, the selected bacterial strains and their metabolites can be checked *in vitro* for effectiveness and preliminary safety assessment, followed by validation through *in vivo* experiments (using experimental animal models) and human trials. Finally, upon approval from regulatory authorities, lead human commensal bacterial strains and their metabolites can be recommended for use in humans as cancer therapeutics.

7. Conclusions

A number of selected bacterial strains obtained from various sources and their metabolites have been recognized for the health benefitting properties. However, the repository of desired bacterial isolates obtained from human com-

mensal pool is still relatively small and even more so with reference to cancer prevention and therapy perspectives. Due to pre-established association with human tissues, the commensals and their metabolites would have a better biocompatibility than the ones from other sources. Identification of bacterial strains present in healthy individuals but particularly absent in cancer patients would help in enriching the pool of therapeutic probiotics with potential application for the cancer treatment. Additionally, the human commensal sources of metabolites proven to have anti-cancer activity should be actively explored. For instance, new commensal source of L-asparaginase—a FDA approved anticancer drug would help in addressing the toxicity issues associated with the currently available L-asparaginase. In this regard, L-asparaginase producing human commensals recently isolated by us is a significant step forward, however, further studies are essential to prove its utility in treatment against cancer. Additionally, the modifications at molecular level using available protein engineering tools, as exemplified for L-asparaginase in this review, would also significantly help in eliminating the unwanted side effects associated with therapeutic biomolecules already in use. Importantly, the effective workflow as suggested in this review would help the exploration aimed to identify, characterize and employ the next-generation pro- and postbiotics for anticancer therapy.

Author Contributions

HS - Data curation (Lead), Formal analysis (Lead), Investigation (Lead), Methodology (Lead), Software (Lead), Validation (Supporting), Visualization (Supporting), Writing - original draft (Lead), Writing - review & editing (Supporting). SD - Data curation (Supporting), Formal analysis (Supporting), Methodology (Supporting), Software (Equal), Validation (Supporting), Visualization (Supporting), Writing - review & editing (Supporting). BR - Formal analysis (Supporting), Validation (Supporting), Visualization (Supporting), Writing - review & editing (Supporting). EKP - Conceptualization (Lead), Data curation (Supporting), Formal analysis (Equal), Funding acquisition (Lead), Investigation (Supporting), Methodology (Supporting), Project administration (Lead), Resources (Lead), Supervision (Lead), Validation (Equal), Writing - review & editing (Equal). All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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