IVYSPRING INTERNATIONAL PUBLISHER

Journal of Cancer

2019; 10(8): 1781-1793. doi: 10.7150/jca.31699

Review

Bacteria in Cancer Therapeutics: A Framework for Effective Therapeutic Bacterial Screening and Identification

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Received: 2018.11.21; Accepted: 2019.02.21; Published: 2019.04.21

Abstract

By 2030, the global incidence of cancer is expected to increase by approximately 50%. However, most conventional therapies still lack cancer selectivity, which can have severe unintended side effects on healthy body tissue. Despite being an unconventional and contentious therapy, the last two decades have seen a significant renaissance of bacterium-mediated cancer therapy (BMCT). Although promising, most present-day therapeutic bacterial candidates have not shown satisfactory efficacy, effectiveness, or safety. Furthermore, therapeutic bacterial candidates are available to only a few of the approximately 200 existing cancer types. Excitingly, the recent surge in BMCT has piqued the interest of non-BMCT microbiologists. To help advance these interests, in this paper we reviewed important aspects of cancer, present-day cancer treatments, and historical aspects of BMCT. Here, we provided a four-step framework that can be used in screening and identifying bacteria with cancer therapeutic potential, including those that are uncultivable. Systematic methodologies such as the ones suggested here could prove valuable to new BMCT researchers, including experienced non-BMCT researchers in possession of extensive knowledge and resources of bacterial genomics. Lastly, our analyses highlight the need to establish and standardize quantitative methods that can be used to identify and compare bacteria with important cancer therapeutic traits.

Key words: cancer, bacterium, therapeutics, bacterium-mediated cancer therapy, screening, microbiology

Introduction

Cancer is a group of diseases caused by disproportionately dividing cells that grow into invasive lumpy masses, commonly referred to as tumors. Approximately 200 human cancers are currently recognised by the National Cancer Institute (http://www.cancer.gov/types/). Some cancers are capable of spreading from their tissue of origin to distant body parts in a process called metastasis. Despite their metastatic ability, cancers are mainly categorized based on their tissue and/or organ of origin. For example, cancers that begin in tissues that line or cover body organs are known as carcinomas. Sarcomas, melanomas, lymphomas, and leukemias are other well-known examples of cancers. It is worth noting that not all cancers are tumorous, for example, blood cancers such as leukemia, lymphoma, and multiple myeloma are known to be non-solid cancers. In addition to pathologically categorizing cancers based on their tissues or organs of origin, molecular taxonomies based on recurrent genetic and epigenetic alterations in human tissue have been suggested.(1) Regardless of the cancer type in question, cancers can indeed have devastating effects on affected and surrounding body organs and are thus deemed a global public health issue.

It is estimated that approximately 18.1 million

new cancer cases occurred globally in 2018, with 92.8% of these cases occurring in Asia, Europe and the Americas. Of these 18.1 million cases, an estimated 6.56 million individuals were newly diagnosed with lung, breast, colorectal, or prostate cancer.[2] An estimated 9.6 million individuals died from cancer in 2018, with approximately 5.5 million of these deaths solely occurring in Asia. It is estimated that lung, liver and stomach cancer were the most fatal cancers, jointly leading to approximately 3.33 million deaths.[2] Other cancers of note estimated to have caused significant global mortality in 2018 are breast and colorectal cancers. They jointly caused a total of about 1.18 million deaths. When compared to their counterparts in developing regions, the cumulative risk of dying from cancer was 7.55% higher in men in developed regions. However, there was no notable difference in the estimated cumulative risk of dying from cancer between women in developing and developed regions.[2] Overall, cancer mortality and incidence trends in 2018 were shown to substantially vary at country and regional levels.[2]

Similar to global cancer mortality and incidence trends, cancer risk factors can often vary by region and country. A wide range of factors including genetic background, age, smoking, alcohol consumption, body weight, diet, exposure to pollutants, and microbial infection have been linked to the risk of developing certain cancer types. Of these risk factors, carcinogenic microbial infection stands out. Together, infectious microbial agents were responsible for causing 2.2 million new cancer cases in 2012.[3] Interestingly, the link between microbial infection and cancer was hypothesized and investigated as early as the nineteenth century.[4] Today, approximately 16% of the global cancer burden is attributed to microbial infections. [3, 5] Curiously, not only can bacteria and other microbes enhance the risk of getting cancer but they can also enhance its treatment.[6] In fact, infection mediated cancer therapy is an age-old therapy that pre-dates the seventeenth century discovery of microorganisms and can be traced to as early as 2600 BC. [7, 8]

Aside from infection mediated cancer therapy, several therapeutic options are currently available to cancer patients. These therapeutic options can vary greatly based on a host of factors, including the locations of the cancer, its size, and the patient's health status. Together, cancer therapies can be broadly classified into two main types: systemic and localised therapies. In cases of metastasized cancers, systemic therapies are often the treatment of choice. Systemic therapies refer to treatments that target the entire body through the bloodstream. Three well-known examples of systemic therapies include chemo-, hormone-, and immuno- therapy. Like everything else, each of these therapies has its advantages and limitations. Generally speaking, one of the main advantages in using systemic therapies is that resection can at times be avoided. On the other hand, systemic therapies can often have unintended consequences on healthy tissues and organs. Furthermore, these treatments rely mostly on blood circulation and could have limited effectiveness in quiescent tumor regions with limited vascularization.[9] Unlike systemic therapies, localised therapies have limited effectiveness against metastasized cancers. However, they can instantly limit cancer or its symptoms by reducing the mass effect of tumors, and in some cases, they can cure cancer. Furthermore, localised therapies, such as surgeries, are able to remove cancers from the body which are inaccessible to certain chemotherapies, the brain for example. Despite recent and impressive advances, there are still many unmet needs in cancer therapeutics.

Coupled to revolutionary cancer therapies such as robot assisted surgery, checkpoint inhibitors and monoclonal antibodies,[10, 11] the scientific community has witnessed the renaissance of bacterium-mediated cancer therapy (BMCT) over the last two decades.[9, 12-26] However, despite notable advances made in improving BMCT in recent years, most bacterial candidates have yet to attain satisfactory efficacy, effectiveness, or safety. The current but rather few therapeutic bacterial candidates are relatively limited in tumor cytotoxicity, immunogenicity, chemotacticcity, or safety. Furthermore, the possibility of an occurrence of septicemia caused by antibiotic resistance and/or the reversion of attenuated pathogenic phenotypes in these candidate bacteria are still causes for concern. Similarly, the impairment of therapeutic effectiveness due to previous bacterial immunization is also possible. As a result, the search for optimal bacterial candidates is still ongoing.

Recent advances in environmental microbiology, in particular, our understanding of microbiomes from diverse ecological niches are contributing to the interest in and the renaissance of BMCT. It is estimated that there may be 1 trillion microbial species on earth, approximately 99% of which cannot be cultured.[27] The candidate pool is therefore huge. Could it be possible that some of these microbes, including the uncultivable ones, are potential therapeutic bacterial candidates? With so many prospective candidates to consider but limited resources to screen every single bacterium, this paper aims to provide a necessary practical guide to screening and identifying bacteria with significant cancer therapeutic potential.

A brief history of bacteria-mediated cancer therapy

Although it was implicitly used prior to the nineteenth century, BMCT was only explicitly used and brought to the forefront of cancer therapeutics in 1891 by Dr. William Coley.[8, 28] Dr. Coley, a bone surgeon, used both attenuated and unattenuated mixtures of *Streptococcus pyogenes* and *Serratia marcescens* to treat sarcoma patients. In spite of his relative success in treating inoperable sarcomas, his treatments, known as Coley's toxins, were met with much skepticism due to their inconsistencies and the extent of their side effects. Also, the emergence of radiotherapy at the time provided a less controversial therapeutic option for cancer treatment.[28, 29] Despite the initial drawbacks and skepticism, research on BMCT persisted. *Clostridium* filtrates and spores were used in cancer treatment for the first time approximately half a century later in 1935 and 1947.[30, 31] In 1988, the very first recombinant *Clostridium* was developed for BCMT,[32] that was followed by the development of an auxotrophic *Salmonella* about a decade later in 1997.[33] The year 2002 marked a monumental milestone in the field of BMCT as the very first clinical trial in recent times was carried out.[34] Despite the limited success of the aforementioned clinical trial, the field of BMCT has generated an unprecedented amount of interest, mainly due to the abundance of potential microbial candidates and the diversity of recombinant DNA techniques being used to further explore relevant bacterial traits.[9-24]

To facilitate BMCT research, we sought to answer the following questions in this paper: (1) what makes a bacterium a good BMCT candidate? (2) How would a good BMCT prospect be identified? (3) How can different BMCT candidates be compared? Building on previously published literature, we discuss key bacterial traits useful in screening for new or better prospects.

Tips necessary for screening promising bacteria prospects

Traditionally, cancer therapeutic bacterial screening was solely a wet lab experimental process. However, recent advances to our understanding of bacterial genomes have added a secondary dimension to the process of cancer therapeutic bacterial screening. Presently, there are over 22,000 complete bacterial genome sequences deposited in the National Center for Biotechnology Information's genome database. *In silico* analyses of these and other data can significantly speed up the screening for cancer therapeutic bacteria. Ideally, a good BMCT prospect should show: (1) cancer selectivity, (2) cancer cytotoxicity and/or immunogenicity, (3) limited toxicity to normal cells, and (4) stability within the human body. Although many bacteria are known to secrete a wide array of cancer cytotoxic substances,[6] little is known about the genes responsible for synthesizing and secreting these substances. Similarly, some bacteria are known to hinder tumor growth through inflammasome and effector T-cell pathways,[6] but little attention has been given to genes necessary for their cancer immunogenicity. Aside from their inherent tumor cytotoxicity and immunogenicity, bacteria can also be used as vectors for the delivery of other anti-cancer drugs as they are able to localise in hypoxic tumor regions.[6, 35] However, to make BMCT more successful, many questions need to be further addressed, including (1) what makes bacteria localise within tumors? (2) Are there niche-specific genes necessary for preferential tumor growth? (3) How can a bacterium prospect with tumor specificity, cytotoxicity, immunogenicity and stability be mined? (4) Does such a bacterium even exist naturally? Or, can it be engineered? And (5) are there better cancer therapeutic bacteria combinations that could improve patient clinical outcomes?

The rationale for further bacterial screening is embedded in the above questions and the fact that therapeutic bacterial candidates have been identified for only a few of the approximately 200 existing cancer types. In Figure 1, we suggest a screening framework that entails mining genes important to bacterial cytotoxicity, chemotacticity, immunogenicity, and pathogenicity in currently available therapeutic bacterial candidates, followed by finding analogs or orthologs to those genes in prospect candidates. Fortunately, most present-day cancer therapeutic bacterial candidates have been sequenced and some genes pertaining to cancer therapeutics have been reported. For example, the niche-specific genes necessary for preferential growth of *Salmonella typhimurium* in solid tumors have been identified.[36] In light of the suggested framework, we have provided below a nonexclusive list of cancer cytotoxic bacteria reported in literature, highlighting the cancer's cytotoxic substance they produce and their synthesizing gene(s). We have also provided a list of cancer immunogenic and chemotactic bacteria, highlighting relevant genes whenever possible. It is worth noting that in currently available therapeutic bacterial candidates whose relevant gene clusters are unknown, bioinformatical methods such as gene network, probabilistic algorithms, and metagenomic islands can be used to predict gene function.[37-40] Once predicted, algorithms such as BLAST can be

used to identify analogs or orthologs in bacterial libraries. Our suggested framework could be valuable in advancing BMCT, especially in the current genomic era. More importantly, it could prove to be valuable to new BMCT researchers, including experienced non-BMCT researchers in possession of extensive bacterial genomic resources.

Cancer cytotoxic traits

Cancer cytotoxic traits are attributes that enable bacteria to secrete substances that are toxic to cancerous cells. Given the number of currently identified cancer cytotoxic bacteria, advancing BMCT requires identifying therapeutic bacterial candidates with better selective cytotoxicity and/or other useful cancer therapeutic traits. In view of characterizing cytotoxic gene analogs and orthologs in prospective candidates, we provided a non-exhaustive list of previously identified cancer cytotoxic bacteria, their secreted substances, the chemical nature of these substances, and their synthesizing genes (Table 1). The contents of this table could be useful to researchers seeking to identify the genes responsible for the secretion of certain cancer cytotoxic substances and to those screening necessary genes for bacterial vectors. As shown in Table 1, the main focus of this review was to demonstrate the diversity of bacteria capable of secreting anti-cancer substances. Consequently many compounds produced by *Actinomycetes* and their corresponding biosynthetic genes were omitted. Furthermore, although toxicities to normal cells are not shown in Table 1, they can be referenced accordingly. We note that many bacteria with anti-cancer abilities also produce antimicrobial substances. We hereby encourage researchers working in the field of antimicrobial development to screen their current bacterial libraries for potential anti-cancer activities.

Within the context of identifying bacteria with better or new cancer cytotoxic traits, uncultivable bacteria have been under-explored and represent a huge potential source of anti-cancer substances. This is mostly attributed to our failure to reproduce important aspects of their natural environments under laboratory conditions. It has been previously shown that uncultivable microbes constitute the majority of bacterial genetic diversity in nature and could represent an important BMCT source. [41] The framework suggested in Figure 1 takes into account uncultivable bacteria; however, step 4 for uncultivable bacteria involves processes such as partial genomic digestion and cloning of functional gene fragments into appropriate vectors. Amongst other considerations, isolation and purification of metagenomic DNA from soil, fragment size selection, and choice of vector and host have been previously discussed by Dr. Robin Pettit.[41] Similar and more recent techniques such as primer restriction are also applicable to metagenomic DNA from aquatic environments. More importantly, aside from cloning functional genes belonging to uncultivable bacteria, growing uncultivable bacteria can be achieved through recent advances in bacterial culture, including co-culture with other bacteria, recreating bacterial environment in laboratories, and combining these approaches with micro-cultivation technology.[42]

Cancer immunogenic traits

Cancer immunogenic traits are attributes that enable certain bacteria to induce human immune responses against cancerous cells. Bacteria's inherent ability to elicit the immune system makes them crucial to BMCT. Immune system stimulation within the context of cancer treatment can be achieved in several ways including: (1) inflammasome activation, (2) tumor associated macrophage repolarization, (3) tumor-associated myeloid derived suppressor cell alteration and, (4) effector T-cell responses. [6, 170-172] Of major interest to the process of cancer therapeutic bacterial screening is identifying previously studied immunogenic bacteria and genes necessary for their immunogenicity. In Table 2, we provided a non-exclusive list of cancer immunogenic bacteria, their respective immunogen(s) and synthesizing gene(s). Furthermore, within the context of exploring uncultivable bacteria, analogous or orthologous immunogenic genes from uncultivable bacteria can be cloned into suitable vectors and tested *in vivo*, or grown by means of recent advances in bacterial culture.[42]

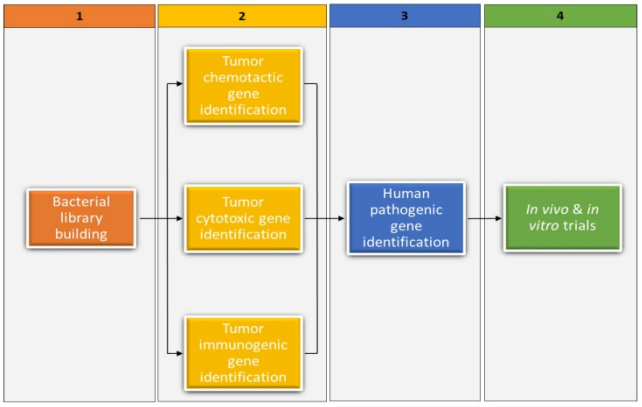

Figure 1. A four-step framework for screening and identifying bacteria with cancer therapeutic potential. Bacterial library building within the context of this review entails all procedures involved in obtaining various bacterial DNAs (prospective candidates) from diverse sources (step 1). The procedure depicted in step 2 entails identifying genes important to bacterial cytotoxicity, chemotacticity, and immunogenicity in currently available therapeutic bacterial candidates, and then identifying the analogs or orthologs of those genes in prospective candidates. Step 3 identifies whether the prospects from step 2 are pathogenic to humans that need attenuation. Lastly, step 4 tests the prospects from step 3 *in vivo* and *in vitro* trials, including in cell lines that have not been previously investigated.

Table 1. Representative list of bacteria with cancer cytotoxic traits

Bacteria	Cytotoxic substance	Chemical nature	Active against	Synthesizing gene(s)	Growth Inhibition	Reference(s)
Actinoalloteichus cyanogriseus	Caerulomycin F–K	Bipyridines	K562, HL-60 (leukemia), KB(epidermoid carcinoma), and A549 (alveolar adenocarcinoma)	The caerulomycin A gene cluster is known	IC_{50} = 0.37 and 25.7 μM	(43, 44)
Actinomadura sp.	Chandrananimycin A-C	Phenoxazines	CCL HT29 (colon carcinoma), MEXF 514L (melanoma), LXFA 526L (lung carcinoma), LXFL 529L (lung carcinoma), CNCL SF268, LCL H460, MACL MCF-7 (breast carcinoma), PRCL PC3M, and RXF 631L (kidney tumor)	The chandrananimycin gene cluster	IC_{70} values down to 1.4μg/mL	(45, 46)
Actinomadura verrucosospora	Esperamicin A	Enediyne	B16-F1O (melanoma), HCT116, MOSER (colorectal carcinoma), H298, and SW900 (lung adenocarcinoma)	Although unknown in *A. verrucosospora*, esperamicin A gene analogs have been localised in *Actinomadura madurae* and *Streptomyces carzinostaticus*	IC_{50} =0.3–8.3ng/mL	(47, 48)
Actinomyces sp.	Chlorinated dihydroquinones	Terpene	Human colon adenocarcinona (HCT-116)	Unknown	IC_{50} = 0.97–2.40μg/mL	(49)
Bacillus amyloliquefaciens	Exopolysaccharide	Polysaccharide	MC-4 and SGC-7901(gastric carcinoma)	Unknown	IC_{50} = 19.7 and 26.8μg/μL	(50)
Bacillus licheniformis, *Nocardiopsis alba*, *Enterobacter cloacae* and several other bacteria including *Escherichia coli, Pseudomonas otitidis, Streptomyces* sp., *Erwinia carotovora etc.*	L-asparaginases	Peptide	Jurkat clone E6-1, K-562, HL-60, MOLT-4 (leukemia), MCF-7, MDA-MB-231 (breast carcinoma), and Caco2(colorectal adenocarcinoma)	The *ansA1* and *ansA3* genes in *Bacillus licheniformis* have been identified. The *ansA* in *Nocardiopsis alba* NIOT-VKMA08 is partially analogous to that of several unreported actinobacteria	IC_{50} = 0.15–11.2 IU/mL	(51-55)
Bacillus mojavensis	Iso-C16 fengycin B, and anteiso-C17 fengycin B	Lipopeptides	HL-60 (leukaemia)	Although unknown in *B.mojavensis* , the fengycin gene cluster in sister species *Bacillus subtilis* have been localised	IC_{50} = 1.6 and100μM	(56, 57)
Bacillus silvestris	Bacillistatin 1 and 2	Peptide	P388 (leukemia), BXPC-3 (pancreatic carcinoma), MCF-7 (breast carcinoma), SF-268 (glioblastoma), NCI-H460(lung cancer), KM20L2 (colon cancer), and DU-145 (prostate cancer)	Unknown	IC_{50} = 0.26–13 ng/mL	(58)
Bacillus sp.	Mixirin A-C	Acylpeptide	HCT116 (colorectal carcinoma)	Unknown	IC_{50} = 0.65–1.6 μg/ml	(59)
Bacillus sp.	Halobacillin	Peptide	HCT116 (colorectal carcinoma)	Unknown	IC_{50} = 0.98 μg/mL	(60)
Bacillus subtilis and *Streptomyces albulus*	Epsilon-poly-L-lysin (ε-PL)	L-lysine homopolymer	HeLa (cervical adenocarcinoma), and HepG2 (hepatocellular carcinoma)	The *Pls* gene	The culture supernatant of *Bacillus subtilis* SDNS inhibited 56.2 and 77.2 % of HepG2 and HeLaS3 after 72 hrs. The IC_{50} for *S. albulus* derived ε-PL against HepG2 is 13.49 and 8.664 μg/mL at 24 and 48h, respectively	(61-63)
Brevibacillus sp.	Laterosporulin 10	Peptide	MCF-7(breast cancer), HEK293T (embryonic kidney cancer), HT1080 (fibrosarcoma), HeLa (cervical carcinoma), and H1299(lung carcinoma)	The laterosporulin10 gene cluster	A minimum of 70% cytotoxicity was observed at 10 μM in all tested cells	(64, 65)
Chromobacterium violaceum	Romidepsin (FK228)	Peptide	In over 20 cell lines including nueroblastoma, T-cell lymphomas, lung, mammary, stomach, and colon adenocarcinoma.	The romidepsin gene cluster	IC_{50} = 0.3–6.3 ng/mL	(66-69)
Clostridium botulinum	Botulinum neurotoxin type A	Peptide	T47D (breast cancer), PC-3, LNCaP, (prostate cancer), and SH-SY5Y(neuroblastoma)	The *boNT/A* gene	IC_{50} = 0.54–300 nM	(70, 71)
Corynebacterium diphtheriae	Diphtheria toxin	Peptide	MCF 7(breast carcinoma), H295R (adrenocortical carcinoma), HeLa (cervical adenocarcinoma), CTCL (cutaneous T cell lymphomas), U118MG, U373MG, and U87MG(glioblastomas)	The *tox* gene	IC_{50} =0.55–2.08 μg/mL	(70, 72)
Dermacoccus abyssi	Dermacozine F and G	Phenazine	K562 (leukemia)	Unknown	IC_{50} = 7 and 9 μM	(73)
Enterococcus sp.	Enterococcal anti-proliferative peptide	Peptide	MDA-MB-231(breast adenocarcinoma), HeLa(cervical adenocarcinoma), and AGS (gastric adenocarcinoma)	Unknown	29.1-38.4% reduction in proliferative activity	(74, 75)
Escherichia coli	Colicin A and E1	Peptide	MCF7, MDA-MB-231, ZR75, BT474BT549, SKBR3, T47D (breast carcinoma), SKUT-1 (leiomyosarcoma), HOS (osteosarcoma), and HS913T (fibrosarcoma)	The *caa* and *cea* genes	Inhibition of cellular growth was 17–40% for Colicin E1 and 16-56% for Colicin A	(76, 77)
Escherichia coli	Cytosine deaminase (often used with 5-fluorocytosine)	Peptide	A549 (alveolar adenocarcinoma), C6, U251 (glioma), HCT116 (colorectal carcinoma), and DU145 (prostrate carcinoma)	The *codA* gene	IC_{50} =0.3mM – 30 mM	(78-81)
Geitlerinema sp.	Ankaraholide A	Macrolide	NCI−H460 (lung cancer), Neuro-2a (neuroblastoma), and MDA-MB-435(breast cancer)	Unknown	IC_{50} = 8.9-262 nM	(82)
Klebsiella pneumoniae	Microcin E492	Peptide	RJ2.25 (B-lymphoblastoid cells), HeLa (cervical adenocarcinoma), and Jurkat (acute T cell Leukaemia)	The microcin E492 gene cluster	4 ±3 to 57 ±11% cell survival	(83, 84)
Lactococcus lactis	Nisin A	Peptide	UM-SCC-17B, UM-SCC-14A, HSC-3 (head and neck carcinoma), MCF 7(breast carcinoma), HepG2 (hepatocellular carcinoma), and Jurkat (T cell leukaemia)	The nisin A gene cluster	IC_{50} =105.5–225 μM	(70, 85)
Listeria monocytogenes	Listeriolysin O	Peptide	SKBR-3, MCF 7(breast carcinoma), and Jurkat (T cell leukaemia)	The *hly* gene	IC_{50} = 50 pM to 0.1 nM	(70, 86)

Lyngbya majuscula	Aurilides B & C	Depsipeptide	NCI-H460 (lung cancer) and neuro-2a (neuroblastoma)	Unknown	IC_{50} = 0.01-0.13 µM	(87)
Lyngbya majuscula	Hermitamide A and B	Alkaloid	CCL-131(neuroblastoma)	Unknown	2.2–5.5 µM	(88)
Lyngbya majuscula	Lyngbyabellin E-I	Peptide	NCI-H460 (lung tumor) and neuro-2a (neuroblastoma)	Unknown	IC_{50} = 0-2–4.8 µM	(89)
Lyngbya semiplena	Wewakpeptin A and B	Peptide	H460 (lung tumor) and neuro-2a (neuroblastoma)	Unknown	IC_{50} =0.2–0.65 µM	(90)
Marinispora sp.	Marinomycin A-C	Polyketide	NCI's panel of 60 cancer cell lines	Unknown	Mean IC_{50} = 0.2–2.7µM	(91)
Mechercharimyces asporophorigenens	Urukthapelstatin A	Peptide	A549, DMS114, NCIH460(lung cancer), MCF-7 (breast cancer), and HCT-116 (colorectal carcinoma)	Unknown	log GI_{50} = 3.5-5.2 nM	(92)
Microbispora rosea	Hibarimicin A-D	polyketide	16-F10(skin melanoma) and HCT-116(colorectal carcinoma)	Unknown	IC_{50} = 0.7–3.6 µg/mL	(93)
Microcystis aeruginosa	Microcyclamide	Peptide	P-388 (leukemia)	The microcyclamide gene cluster	IC_{50} = 1.2 µg/mL	(94, 95)
Micromonospora sp.	IB-96212	Polyketide	P-388 (leukemia), A-549(alveolar adenocarcinoma), HT-29 (colorectal adenocarcinoma), and MEL-28(melanoma)	Unknown	IC_{50} = 0.0001–1 µg/mL	(96)
Micromonospora sp.	Arisostatin A and B	Tetrocarcin group spirotetronate	Myeloid leukemia U937	The tetrocarcin A gene cluster in *M. chalcea* NRRL 11289 has been localised	IC_{50} = 0.4 and 4 µg/mL	(97, 98)
Nocardia dassonvillei	N-(2-hydroxyphenyl)-2-phenazinamine	Phenazine	HepG2(hepatocellular carcinoma), A549(alveolar adenocarcinoma), HCT-116 (colorectal carcinoma), and COC1(ovarian cancer)	Unknown	IC_{50} = 28.11 and 40.33 µg/mL	(99)
Nocardiopsis lucentensis	Lucentamycin A and B	Peptide	HCT116 (colorectal carcinoma)	Unknown	IC_{50} = 0.2 and 11µM	(100)
Nostoc sp.	Cryptophycins-1	Depsipeptide	KB(cervical carcinoma), B16V(murine melanoma), and LoVo (colon carcinoma)	The cryptophycin gene cluster	IC_{50} =3 pM –I.3nM	(101-103)
Paenibacillus profundus	Heptapeptide	Peptide	SK-MEL-28 (human melanoma)	Unknown	IC_{50} = 3.07µM	(104)
Pediococcus acidilactici	Rec-pediocin	Peptide	HepG2 (hepatocarcinoma), HeLa (cervical adenocarcinoma), MCF7 (mammary gland adenocarcinoma), and Sp2/0-Ag14 (spleen lymphoblast)	The *CP2* gene	25µg/ml of rec-pediocin reduced cell viability by at least 89% in all cell line tested	(105, 106)
Pelagiobacter variabilis	Pelagiomicin A	Phenazine	HeLa (cervical carcinoma)	The pelagiomicin gene cluster	IC_{50} = 0.04–0.2 µg/mL	(107, 108)
Pseudomonas aeruginosa	Exotoxin A	Peptide	PaCa-2 (pancreatic cancer, FEMX, Melmet-1, Melmet-5, Melmet-44, MelRM, MM200 (melanomas), Daudi, CA46 (Burkitt's lymphoma), EHEB, MEC1(leukemias), head and neck squamous carcinomas	The *PE* gene	0.3–8.6 ng/mL	(70, 109)
Pseudomonas aeruginosa	Azurin	Peptide	MCF7, MDA-MB-157, T- 47D and ZR-75-1 (breast carcinoma) HCT-116 (colon adenocarcinona) and UISO-Mel-2 (melanoma)	The azurin gene	IC_{50} = 32 and 53 µM for MCF-7 and MDA-MB-157, respectively. In T- 47D and ZR-75-1, IC_{50} = 72± 3µg/mL. Approximately 40% of HCT-116 were inhibited by 10ug/uL	(110-113)
Pseudomonas aeruginosa	Pyocin S2	Peptide	HepG2 (hepatocellular carcinoma) and Im9 (lymphoblast myeloma),	The *pys2* gene	IC_{50} = 3.5- 50 U/mL for both crude and purified pyocin S2 in HepG2 and Im9	(114, 115)
Pseudomonas aeruginosa, *Sphingobacterium* sp., *Streptococcus pyogenes*	Arginine deiminase	Peptide	HCT-116(colon adenocarcinona), K-562(leukemic cell lines),PC-3(prostrate carcinoma), T47D(breast carcinoma) HROG02, HROG05, HROG10, and HROG17 (Glioblastoma)	The *arcA* gene in *Pseudomonas aeruginosa* and *Streptococcus pyogenes* have been identified.	IC_{50} in *Streptococcus pyogenes* is ~ 35mU/mL in HROG05 cell lines; in *Pseudomonas aeruginosa* PS2 it ranges from 0.8-1.4 IU/mL in various cell lines, while in *Sphingobacterium sp.* the same ranges from 0.8-1.8 IU/mL	(116-119)
Pseudomonas chlororaphis subsp. *aurantiaca*	Phenazine-1-carboxylic acid	Phenazine	HCT-116 (colon adenocarcinona)	The phenazine gene cluster (*phzXYFABCD* genes)	IC_{50} =15.6 µM	(120,121)
Pseudomonas sp.	DOB-41	Phenazine	Leukemia P388 in mice.	Unknown	T/C: 113-153%	(120)
Saccharothrix aerocolonigenes	Bromo analog of rebeccamycin	Indolocarbazole	P388(murine lymphocytic leukemia)	The rebeccamyacin gene cluster	T/C: 115-165%	(121, 122)
Salinispora arenicola	Arenamides A-C	Peptide	HCT-116 (colorectal carcinoma)	Unknown	IC_{50} = 13.2 and 19.2 µg/mL	(123)
Salinispora tropica	Salinosporamide A	Polyketide	NCI's 60-cell-line panel and HCT-116 (colorectal carcinoma)	The *sal* gene cluster	Mean GI_{50} (NCI's cell-line) = < 10 nM and IC_{50} = 11 ng/mL in HCT-116	(124, 125)
Salmonella enterica and Escherichia coli	Cytolysin A	Peptide	CT26 (colon cancer)	*ClyA* gene	4.5 × 10^7 colony-forming units of *Salmonella typhimurium* vector suspended in 100 µL of PBS mediated complete tumor eradication.	(126, 127)
Serratia marcescens	Prodigiosin	Pyrrolylpyrromethane	A2780, A2780RCIS (ovarian cancer), EPG85-257, EPG85-257RDB, EPG85-257RNOV(gastric carcinoma),SW-620 (colorectal cancer), HeLa (cervical adenocarcinoma), Hep2 (laryngeal carcinoma), B-CLL (leukemia), LNCaP, DU145(prostate cancer) and MCF-7 (breast carcinoma)	The *pig* cluster	IC_{50} = ~116 nM –127 µM	(128-133)
Serratia surfactantfaciens	Serrawettin W2	Cyclic lipopeptide	Hela (cervical carcinoma) and Caco2 (colorectal adenocarcinoma)	A hybrid polyketide synthases-non-ribosomal peptide synthetases gene cluster	IC50= 20.9 and 54.1 µM in the Hela and Caco2 cell line, respectively	(134)
Sorangium cellulosum	Epothilone A &B	Lactones	CCRF-CEM/VBL_{100} (multidrug-resistant lymphoblastic leukemia)	The epothilone gene cluster	IC_{50} = 0.02 and 0.002 µM for epothilon A and B in	(135-137)

					CCRF-CEM/VBL_{100}	
Staphylococcus aureus	α-hemolysin	Protein	MCF-7 (mammary carcinoma)	The α-hemolysin gene	A reduction in cell viability of 7.1% per min and a saturation constant of 0.14	(138, 139)
Streptococcus pneumoniae	Pep27anal2 (pep27 analog)	Peptide	AML-2, HL-60, Jurkat (leukemia), SNU-601 (gastric cancer), and (MCF-7) breast cancer	The *Pep27* gene	IC_{50} = <10–29 μM	(140, 141)
Streptococcus bovis	Bovicin	Peptide	HepG2 (hepatocellular carcinoma) and MCF 7(breast carcinoma)	The bovicin gene cluster	IC_{50} = 279.39 and 289.3 μM in MCF-7 and HepG2	(142, 143)
Streptomyces verticillus	Bleomycin	Polyketide	Has been tested against over 30 different cancer type including leukemias, lymphomas, myelomas, and carcinomas	The bleomycin gene cluster	IC_{50} =25.2 nM - 2.93 mM	(70, 144)
Streptomyces peucetius var. *caesius*	Doxorubicin (Adriamycin)	Anthracycline	Tested in over 900 cell types including various carcinomas, sarcomas, melanomas, lymphomas, and leukemias	The doxorubicin gene cluster	IC_{50} = 0.0044 – 44.7μM	(70, 145, 146)
Streptomyces caespitosus or *Streptomyces lavendulae*	Mitomycin C	Aziridine	Tested in over 900 different cell lines	The mitomycin C gene cluster	IC_{50} = 0.00948–249 μM	(147, 148)
Streptomyces griseofuscus	Azinomycin A & B	Aziridine	L5178Y (leukemia)	The azinomycin gene cluster	IC_{50} = 0.07 and 0.11μg/mL for azinomycins A and B, respectively	(149)
Streptomyces iakyrus	Actinomycin G_2	Chromopeptide	HM02 (gastric adenocarcinoma), HepG2 (hepatocellular carcinoma), and MCF7 (breast adenocarcinoma)	The actinomycin G gene cluster	IC_{50}= 0.0013 - 0.0039 μM	(150, 151)
Streptomyces macromomyceticus	Auromomycin	Polypeptide	Ehrlich ascites (carcinoma), ascites sarcoma 180, L1210 (leukemia), and LEWIS lung carcinoma	The macromomycin gene (auromomycin apoprotein) has been sequenced	IC_{50} =3mg/kg	(152, 153)
Streptomyces peucetius	Daunorubicin	Anthracycline	L3.6 (pancreatic carcinoma) and HeLa (cervical adenocarcinoma)	The daunorubicin gene cluster	IC_{50} =0.02 – 0.4μM	(146, 154, 155)
Streptomyces sp.	Chromomycin	Polyketide	Wide range of cancer cells including ovary, breast, prostrate, pancreas, skin, lung,	The chromomycin gene cluster	IC_{50} =0.26 nM - >50μM	(156-159)
Streptomyces sp.	IT-62-B	Baumycin group anthracycline	LI210 (murine leukemia cells), P388(murine lymphocytic leukemia), P388/ ADR (doxorubicin resistant), and KB(human nasopharyngeal carcinoma)	The baumycin gene cluster (*dox*) within the *Streptomyces* genus is known	IC_{50} = 0.006-0.04 μg/mL	(160, 161)
Streptomyces sp.	Diketopiperazine derivative	Piperazine	HCT-116 (colon adenocarcinona) and HepG-2(hepatocellular carcinoma)	Type II polyketide gene cluster	IC_{50}=3.3 and 1.1 μg/mL against HCT-116 and HepG-2 cell lines	(162)
Streptomyces sp.	SF2575	Tetracycline	P388(murine lymphocytic leukemia)	Unknown	IC_{50} = 7.7 ng/mL	(163)
Streptomyces sp.	1, 2- benzene dicarboxylic acid, mono 2- ethylhexyl ester	Dicarboxylic acid	HepG 2 (hepatocellular carcinoma) and MCF-7 (breast adenocarcinoma)	Unknown	IC_{50} = 42 and 100 μg/mL for HepG 2 and MCF-7, respectively	(164)
Streptomyces sp.	2-bromo-1-hydroxy phenazine	Phenazine	Human colon adenocarcinona (HCT-116)	Unknown	IC_{50} = 0.1 μM	(165)
Streptomyces violaceochromogenes	Arugomycin	Anthracycline	Sarcoma S-180	Unknown	I.7mg/kg of interperi toneal injection	(166)
Symploca sp.	Belamide A	Tetrapeptide	HCT-116 colon cancer	Unknown	IC_{50}=0.74-1.6 μM	(167)
Thermoactinomyces sp.	Mechercharmycin A (Mechercharstatin)	Peptide	A549 (lung cancer), and Jurkat (T cell leukaemia)	Unknown	IC_{50} = 0.04–0.046μM	(168)
Verrucosispora spp.	Proximicin A–C	Peptide	MCF-7(breast carcinoma) AGS (gastric adenocarcinoma), and HepG2 (hepatocellular carcinoma)	Unknown	GI_{50} = 0.25-9.5 μg/mL	(169)

IU: the amount of enzyme that liberates 1 μM; T/C: the ratio of mean survival days of the treated group divided by that of the control group; IC50: the drug concentration that inhibits biological activity by 50%; GI50: the drug concentration that inhibits the growth of cancer cells by 50%.

Traits necessary for preferential tumor growth

The most important and well explored bacterial traits for efficient BMCT are the abilities to differentiate cancerous cells from healthy cells or recognize the peculiar bacterial growth environment provided by the cancer cells. This is typically achieved by bacteria recognizing specific chemical signals emitted by cancer cells. Due to recent advances in recombinant DNA techniques, these traits have been leveraged by engineering bacterial vectors for the precise delivery of diverse anti-cancerous proteins to tumors. This has been mostly achieved by cloning genes coding for diverse anti-cancerous proteins into bacterial vectors, including those coding for immunogenic antigens, cytokines, cell cycle check-point inhibitors, antibodies, and cytotoxic agents. [186] Although bacterial vector systems are typically designed to enable the direct expression of these anti-cancerous proteins, they have also been used for bactofection or gene transfer to mammalian cancer cells. [186] Within the context of cancer therapeutic bacterial screening, we provided examples of bacteria that are currently used as vectors for the delivery of diverse anti-cancerous molecules (Table 3). We however note that, for most of these bacterial vectors, genes responsible for preferential growth around tumor environment are either unreviewed or unknown. Advancing BMCT within the context of bacterial screening would not only entail identifying these genes in currently used vectors for the delivery of diverse anti-cancerous molecules, but also randomly screening both facultative and obligate anaerobes in order to identify new prospects with better tumor discriminatory abilities or other useful cancer therapeutic traits in addition to their tumor discriminatory abilities. This is particularly important as the future of BMCT hinges on bacteria with improved tumor discriminatory abilities.

Table 2. Representative list of bacteria with cancer immunogenic traits

Bacteria	Immunogen(s)/ Immunogenic properties	Active against	Synthesizing gene(s)	Reference(s)
Clostridium novyi	Phospholipases	Sarcomas, gliomas, squamous and colon carcinomas	The phospholipase C gene (*NT01CX0979*)	(170, 173, 174)
Escherichia coli	Lipopolysaccharide	Colon and breast carcinomas	Lipid A, core polysaccharide, and O-antigen genes	(175, 176)
Mycobacterium bovis	α antigen	Cervical adenocarcinoma and bladder cancer	The *a antigen* gene	(177-179)
Streptococcus pyogenes	Emm55, speA, speB and speC	Pancreatic carcinoma and lymphosarcoma	*speA*, *speB*, *speC*, and *Emm55* genes	(180-182)
Listeria monocytogenes	Listeriolysin O	Ovarian and breast carcinomas.	The *hly* gene	(171, 172, 183, 184)
Salmonella typhimurium	Lipopolysaccharide/ survival within macrophages	Colon carcinoma	Lipid A, core polysaccharide, and O-antigen genes; *slyA, STM3120* and *htrA* genes	(16, 185)

Table 3. Representative list of bacteria that are known to preferential accumulate in tumors

Bacteria	Comments	Effective against	Important gene(s)	Reference(s)
Bifidobacterium longum	At 168 hours, tumors had 60,000 bacilli per gram of tumor tissue in contrast to no germination in livers, spleens, kidneys, or lungs.	Diverse solid tumors including B16-F10 melanoma and Lewis lung carcinoma	Unknown	(187)
Clostridium novyi	Within 16 hours the bacterium had floridly germinated within the tumors in contrast to no germination in livers, spleens, kidneys, lungs, or brains	Diverse solid tumors including HCT116 colon and B16 skin carcinomas	Unknown	(188)
Escherichia coli	Preferential accumulated in tumor at a ratio of >10^8:1	Diverse solid tumors including gliomas, breast, skin and colon and carcinoma	Unknown	(189, 190)
Listeria monocytogenes	Selectively infected, survived and multiplied in tumors; 27 hours after injection, the bacterium was only detected in tumors	Diverse solid tumors including 4T1 mammary and PC-3 prostate tumors	Unknown	(189, 191)
Magnetococcus marinus	Aerotactic bacteria with the ability to swim along magnetic field lines. Approximately 55% of the bacterium penetrated into hypoxic regions	HCT116 colorectal carcinoma	Unknown	(192, 193)
Salmonella typhimurium	Preferential accumulated in tumor at a ratio of >1,000:1	Multiple solid tumors	*cheY*, *motAB*,and *eutC* genes	(36, 189)
Vibrio cholerae	Selectively infected and multiplied in tumors and metastases, with titres reaching approximately 5.97 × 10^8 after 32 hours	Bladder carcinomas, gliomas and fibrosarcoma	Unknown	(189)

Pathogenic traits

The rationale for identifying bacterial pathogenic traits or genes as proposed in our four-step framework (Figure 1), lies in the fact that pathogenicity of the candidate bacteria has been one of the major drawbacks to BMCT. Nonetheless, a significant number of bacteria strains used in BMCT are pathogenic to human. A critical consideration after the *in silico* screening of bacterial prospects with aforementioned traits should be that of pathogenicity. For instance, *Clostridium histolyticum* spores preferentially germinate in hypoxic tumor regions, making it an excellent BMCT candidate. However, *Clostridium histolyticum* can also produce significant amount of exotoxins consequently causing pathophysiological changes to healthy tissue and organs. [31] In an attempt to circumvent some of the issues pertaining to toxicity while maintaining their efficacy, attenuated bacterial strains with less or no toxicity are being used in BMCT. *L. monocytogenes*, *S. typhimurium*, and *C. novyi* are some of the well-known examples of bacterial candidates that have been attenuated to improve safety. [173, 194, 195] For example, the *L. monocytogenes* cancer vaccine was rendered safer by the deletion of virulence factors such as ActA and Internalin B (Δ*actA*/Δ*inlB*), leading to >1,000-fold reduction in toxicity. [173] Similarly, *S. typhimurium* defective in the synthesis of guanosine tetraphosphate virtually resulted in an avirulent strain.[31] Thus, within the context of advancing BMCT, identifying non-pathogenic analogs to pathogenic strains of key importance is vital to BMCT. Nonetheless, pathogenic bacterial prospects with good BMCT potential cannot be disregarded since there is always the potential for attenuation. However, if issues pertaining to attenuation reversion and septicemia are to be permanently addressed, then identifying non-pathogenic bacteria with aforementioned anti-cancerous traits must be further explored.

Conclusion and perspective

In this review we (1) highlighted bacterial traits that make them good therapeutic candidates for the treatment of cancer, (2) suggested a four-step framework that can be used to identify bacteria with good cancer therapeutic potential, including uncultivable strains, and (3) touched on quantifiable attributes such as growth inhibition, cytotoxicity to normal cells,

and preferential accumulation ratio that can be used to compare and contrast important cancer therapeutic traits for BMCT.

The singular most important bacterial trait to cancer therapeutics is their ability to specifically target tumors or cancerous cells. The future of BMCT lies in being able to find bacteria that can target cancerous cells, secrete cytotoxic and/or immunogenic substances, and be tolerated and are stable in the targeted tissue and cancer environment. Recent advances in recombinant DNA techniques significantly advanced BMCT. Engineering a bacterium that targets tumors or cancerous cells, produces cytotoxic or immunogenic proteins, self-propels and responds to triggering signals, senses the local environment and produces externally detectable signals is not so far-fetched anymore.[196] However, despite the overall enthusiasm about the future of BMCT, using multi-layered genetically modified bacteria could result in stability issues. Identifying bacterial prospects with better cancer therapeutic potential, which require minimum genetic modifications, would go a long way to improve BMCT. Lastly, our analyses highlight the need to establish and standardize quantitative methods to identify and characterize bacteria with important cancer therapeutic traits.

Acknowledgement

This work was supported by Mitacs-Accelerate [Fund IT07941-Yuan_OGVG], Ontario Greenhouse Vegetable Growers (OGVG), Agriculture and Agri-Food Canada Growing Forward-II [project J-001589] and Natural Sciences and Engineering Research Council of Canada (NSERC) [Discovery grant RGPIN-2015-06052]. We also thank Michelle Bargel for help with literature search, Nanyikun (Nancy) Zhao and Michelle S.M. Li for critically reading and editing this manuscript.

Competing Interests

The authors have declared that no competing interest exists.

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