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Review

Potential use of bacterial pigments as anticancer drugs and female reproductive toxicity: a review

Uso potencial de pigmentos bacterianos como drogas anticâncer e toxicidade reprodutiva feminina: uma revisão

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Abstract
Natural bioactive compounds obtained from microorganisms have awakened particular interest in the industry in recent years. This attention comes when the depletion of natural resources is pronounced, and the acquisition of new inputs and bioactive products of plant origin represents a challenge for the next generations. In this sense, prospecting for the large-scale production and use of bacterial pigments has represented a necessary strategy for the development of novel products. A wide variety of properties have been attributed to these substances, among them, the therapeutic potential against important diseases, such as cancer. There is consensus that available chemotherapy protocols are known to detrimentally affect the fertility of cancer patients. A considerable part of the deleterious effects of chemotherapy is related to the cytotoxicity of the drugs used for this purpose, which, in addition to cancer cells, affect normal cells. In this sense, the intrinsic properties attributed to bacterial pigments associated with low cytotoxicity and relevant cell selectivity certified them as potential anticancer drugs. However, little information is available about the reproductive toxicity of these new and promising compounds. Thus, the present review aims to address the main bacterial pigments, their potential uses as anticancer drugs, and their possible toxic effects, especially on the female gonad.
Keywords: cancer; chemotherapy; fertility; bioactive compounds; bacteria

Resumo
Os compostos bioativos naturais obtidos de microrganismos têm despertado especial interesse da indústria nos últimos anos. Esta atenção ocorre em um momento em que o esgotamento de recursos naturais é pronunciado, e a aquisição de novos insumos e produtos bioativos de origem vegetal representa um desafio para as próximas gerações. Neste sentido, a prospecção para a produção e uso em larga escala dos pigmentos bacterianos tem representado uma importante estratégia para o desenvolvimento de novos produtos. Uma grande variedade de propriedades foi atribuída a estas substâncias, entre elas, o potencial terapêutico contra doenças importantes, como o câncer. Existe um consenso de que os protocolos quimioterápicos disponíveis são conhecidos por afetarem negativamente a fertilidade de pacientes com câncer. Grande parte dos efeitos deletérios da quimioterapia está relacionado à citotoxicidade das drogas usadas para este fim, que além das células cancerosas, afetam as células normais. Nesse sentido, as propriedades naturais atribuídas aos pigmentos bacterianos associadas à baixa citotoxicidade e relevante seletividade, os qualificaram como potenciais drogas anticâncer. No entanto, pouco se tem de informação a respeito da toxicidade reprodutiva destes novos e promissores compostos. Dessa forma, a presente revisão tem o objetivo de abordar os principais pigmentos bacterianos, suas utilizações potenciais como drogas anticâncer, bem como os seus possíveis efeitos tóxicos, sobretudo, sobre a gônada feminina.
Palavras-chave: câncer; quimioterapia; fertilidade; compostos bioativos; bactéria

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Introduction

The mammalian ovary, as well as any organs that compound an organism, are continuously subject to several cytotoxic factors that may affect, and modify, its biological functions. One of the main consequences of the ovaries continuous exposure to cytotoxic agents is premature ovarian failure [POF[1,2]]. POF may be the direct consequence and the main side effect of chemotherapy drugs currently used for the cancer treatment[3].

Ovarian damage, with consequent permanent infertility, is one of the most common side effects during chemotherapy treatment in women with certain types of cancer, such as Hodgkin's lymphoma[4]. In order to mitigate chemotherapy harmful effects on female fertility, studies have investigated the influence of several anticancer potential substances, including natural bioactive compounds with low cytotoxicity and chemoprotective action, such as resveratrol[5]; lycopene[6–9]; fennel extract[10] and melatonin[11,12].

Natural bioactive compounds can be extracted from plant or animal sources[13]. However, some microorganisms represent the most important and promising sources of these compounds[14,15]. Natural bioactive compounds are characterized by their variety of therapeutic properties, which can be explored as alternative to the use of highly cytotoxic chemotherapeutics, or as adjuvant drugs in the chemotherapy. Microorganisms (fungi and bacteria) are rich sources of bioactive compounds with anticancer effects[13-15], being excellent pigments producers [pyocyanin[16,17]; prodigiosin[18,19]; carotenoids[20,21]]. Pigments generated by microorganisms, especially bacteria, can contribute to the development of new therapeutic approaches for cancer treatment and/or in the preservation of female fertility. Thus, this work aims to address the main bacterial pigments, their potential uses as anticancer drugs as well as their possible toxic effects on the female reproductive system.

Chemotherapeutic agents and their anticancer mechanisms

Chemotherapeutic agents are drugs commonly used in therapeutic protocols for the treatment of the most varied cancer types. They are generally semi-synthetic or synthetic substances of different origins, such as plants and microorganisms[22]. In fact, there are four classes of plant-derived anticancer agents, namely: vinca alkaloids (vincristine, vinblastine, and vindesine), epipodophyllotoxins (etoposide and teniposide), taxanes (paclitaxel and docetaxel) and camptothecin derivatives [camptothecin and irinotecan[23]]. Other chemotherapeutic agents derived from anthracyclines and produced by microorganisms, such as doxorubicin (DOX), are highly effective in the treatment of different types of tumors[24]. These drugs, however, show high degree of toxicity to non-cancerous cells. This occurs because chemotherapeutic action mechanisms are not selective, causing normal cells depletion, especially those with a high degree of proliferative activity[25,26].

Specifically concerning the reproductive tract, drugs such as DOX cause apoptosis-induced damage to the primordial follicle pool, inducing double-strand breaks in DNA and causing stromal cell death, as well as microvascular damage that induces tissue hypoxia, contributing to the early loss of ovarian follicles[27]. Other chemotherapeutic drugs, such as cyclophosphamide, are metabolized in the liver and transformed into active alkylating metabolites[10]. These metabolites induce the activation of DNA-PK - γH2AX- checkpoint kinase 2 (CHK2), p53/TAp63α, protein kinase B (AKT), and forkhead box O3 (FOXO3a) isoforms in the oocyte nucleus. Such proteins are involved in DNA damage and repair, and in processes like apoptosis and cellular autophagy [28]. Table 1 presents the main mechanisms and the gonadotoxicity of the important chemotherapeutic agents.

Table 1. Main classes of chemotherapeutics and their anticancer and gonadotoxic mechanisms

Types of chemotherapy	Drugs	Mechanism of action	Gonadotoxicity
Alkylating agents	Carboplatin	Prevent cell division by cross-linking DNA strands. Their activity does not depend on DNA synthesis in the target cells. Cyclophosphamide also inhibits DNA synthesis	Induce double strand breaks in oocytes; DNA damage that interferes with transcription and cell replication, leading to oocyte death
	Cisplatin		
	Oxaliplatin		
	Cyclophosphamide		
	Ifosfamide		
	Altretamine		
Antimetabolites	5-Fluorouracil	Inhibit the metabolic processes necessary for the synthesis of purines, pyrimidines, and nucleic acids	Low gonadotoxic risk
	Methotrexate		
	Capecitabine		
	Gemcitabine		
	Pemetrexed		
Antitumor antibiotics	Dactinomycin	Acts via DNA intercalation, interfering with the synthesis of nucleic acids	Positively regulate p53 protein which induces apoptosis; induce DNA double-strand breaks leading to ATM activation, which initiates apoptosis
	Bleomycin		
	Doxorubicin		
Vinca Alkaloids	Vinblastine	Acts by altering the normal assembly, disassembly and stabilization of microtubules	Low gonadotoxic risk
	Vincristine		
	Vinorelbine		
Taxanes	Paclitaxel		Low and transient gonadotoxic risk
	Docetaxel		
Epipodophyllotoxin	Etoposide		Low gonadotoxic risk in non-pregnant women
Epothilone	Ixabepilone		N/A
Camptothecin Analogs	Topotecan	Inhibit topoisomerase I, inducing single-stranded DNA breaks	N/A
	Irinotecan		High gonadotoxic risk when combined with several chemotherapeutic agents

Source: Adapted[29–33]. N/A: not applicable.

Cytotoxicity of chemotherapeutic agents on the female reproductive system

Among the structures that conform the female reproductive system, the ovarian follicles and their respective oocytes are highly sensitive to the chemotherapy deleterious effects, which speeds up follicular atresia promoting the ovarian reserve depletion[34]. The gonadotoxic effect of chemotherapeutics can occur because of three known factors: 1- due to failures in DNA damage and repair mechanisms. These failures result in the Tap 73 protein activation, a p53 modulator that is upregulated in apoptosis, which also activates the pro-apoptotic protein p63. Tap 73 and p53 recruit and activate the pro-apoptotic proteins Bax and Bak, inducing apoptosis[33]; 2- Burnout effect; process that induces depletion of follicular reserve due to reducing the secretion of anti-Mullerian hormone (AMH), substance that inhibits primordial follicles activation and recruitment[34,35]. The reduced levels of AMH in the bloodstream culminate in amplifying follicular activation and depletion[36], and 3- related to vascular damage promotion. Studies indicated that chemotherapeutic agents such as DOX induce vascular damage[37]. This is reflected by a drop in ovarian arterial flow, inducing hypoxia and ovarian atrophy accompanied by cortical fibrosis, follicular loss, and a significant reduction in ovarian and, consequently, reproductive function[38]. The early reduction of ovarian function due to the use of chemotherapeutic drugs to treat cancer can characterize POF in cancer patients.

POF and the use of animals as a model for the study of reproductive disorders

POF affects approximately 1% of women under 40 years of age, being the primary cause of reproductive disorders, such as anovulation and hypoestrogenism, primary or secondary amenorrhea, infertility, sex steroid deficiency and blood elevation levels of gonadotropin[39]. POF onset is usually idiopathic[40]. However, several non-physiological mechanisms may be associated with its development, which may include genetic, autosomal, autoimmune, metabolic and infectious diseases[41]. Anticancer treatments based on chemotherapy and radiotherapy, for example, are the best-known iatrogenic causes for the establishment and development of this disorder[41].

Also, POF is a human condition that has not been reported in animals. However, domestic animals, and especially ruminants, share important similarities with the human species in terms of reproductive aspects, such as duration of the ovulatory cycle (female: 24-30 days, cow: 17-24 days, sheep: 13-19 days), number of ovulations per cycle (female: 1, cow: 1, sheep: 1-2), duration of luteal phase (female: 14-16 days, cow: 15-18 days, sheep: 12-14 days), ovulatory follicle diameter (female: 18-20 mm, cow: 15-20 mm) and duration of gestation [female: ~9 months, cow: ~9 months[42]]. In addition, non-humans reproductive system may be equally susceptible to disorders that directly or indirectly promote reproductive dysfunction. Thus, due to the similarities they share with the human species in terms of reproductive aspects, some domestic animals can serve as experimental models for studies of fertility and female reproductive toxicology[2,42,43].

Bacterial pigments importance

Because of the cyto/gonadotoxicity of chemotherapeutics drugs, several studies have investigated the use of new therapies, based on new substances discovery to prevent, treat, and control several types of cancer. These studies seek substances that show a higher selectivity index for cancer cells or that can be used as adjuvants in the chemotherapy, attenuating the toxic effects of chemotherapeutics and helping to preserve healthy tissues[15,44-46]. In this sense, bacterial strains have ability to naturally produce an infinity of metabolites. Some metabolites produced by bacteria are classified as pigments because present some type of coloration in visible light spectrum[47]. These substances showed a variety of functions and properties that, generally, act as virulence factors (determining the pathogenicity of different bacterial strains), providing thermal and light resistance against ultraviolet radiation, and promoting redox balance [preventing against oxidative stress[48]].

Bacterial pigments have awakened the industry interest on several fronts: in human food sector, as dyes and natural antioxidant additives[49]; in the production of new generations of antibiotics and antifungals, due to the multiple resistance of microorganisms to substances routinely prescribed by the medical community[50]; in the cosmetic industry, aiming to create new photoprotective and antioxidant formulations[51]; in the animal food formulation and supplementation, with the purpose of increase animal performance and development[52–54]; and in the treatment of cancer, owing to its effectiveness against different cancer cells lines in animals and humans. Figure 1 illustrates the main and potential applications of bacterial pigments.

The use of bacterial pigments in biotechnology and pharmacology is trending worldwide. Pigments are highly bioactive compounds that exhibit many properties of social and economic interest. In addition, bacterial pigments production and acquisition represent several benefits that justify such interest, as well as the possibility of large-scale production, since the incubation of bacteria is relatively simple and stimulate the development of millions and millions of colonies[49]. Additionally, mastering the genetic manipulation of microorganisms allowed continuous improvement of bacterial origin pigments production, thanks to transgenesis and gene

editing. In this sense, chemotherapeutic agents of plant origin, such as the vinca alkaloids - vincristine and vinblastine - extracted from *Catharanthus roseus*, and taxane - paclitaxel - for example, were successful synthesized by yeasts[55], and biosynthesized by a variety of bacteria[56], respectively. Furthermore, thanks to recombinant DNA technology, non-violacein-producing *Escherichia coli* bacteria modified with plasmids, which expressed synthetic *vioABCDE* operons – involved in pigment synthesis – successfully produced this pigment, which has vast pharmacological potential[57].

Furthermore, pigments production derived from bacteria with diverse pharmacological potential, including anticancer, has a positive impact for environment, since bacterial cultivation eliminates the need to plant extensive areas of monocultures traditionally used to obtain vegetable origin chemotherapeutic drugs[55]. Therefore, reducing fertilizers and pesticides use, which are known to affect negatively human health and female fertility[58]. For these reasons, bacterial pigments production and use represent a window for promising advances in the coming decades. Hereafter, the main bacterial pigments with anticancer potential currently known will be discussed.

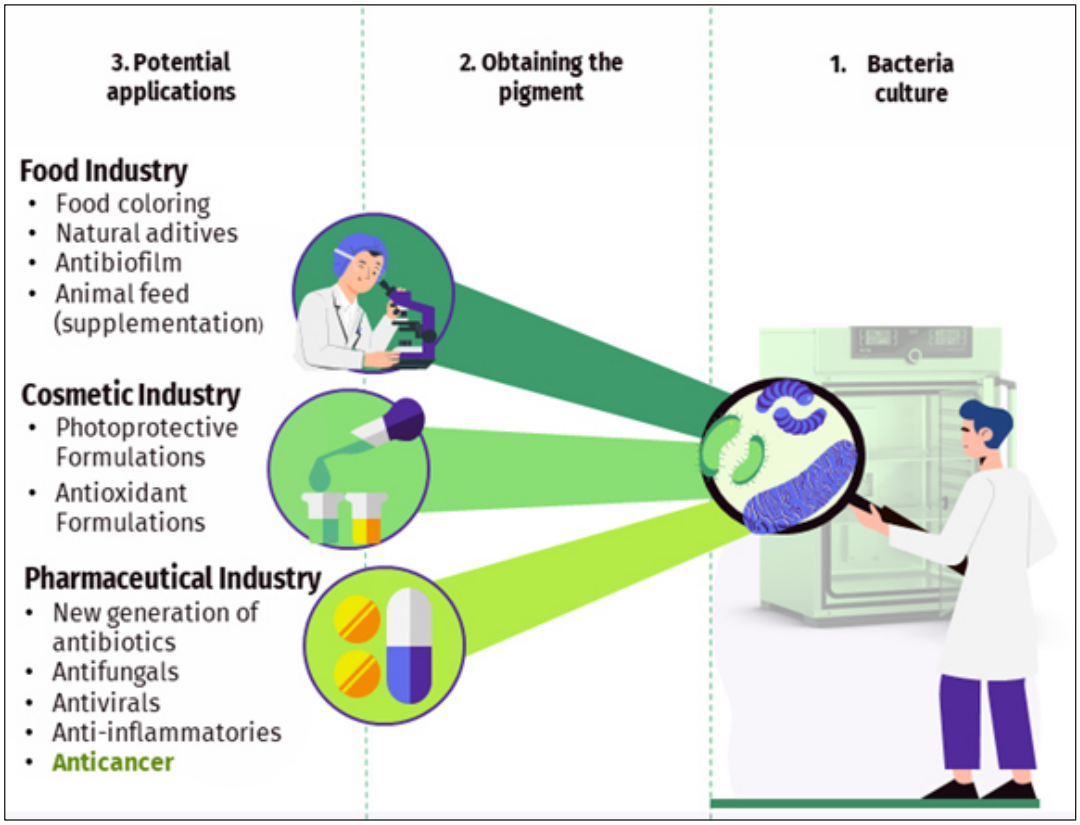

Figure 1. Potential applications for commercial use of bacterial pigments.

Carotenoids

Carotenoids are a wide variety of natural biomolecules produced by plants, algae, yeast, fungi and bacteria. They have different colours from red, yellow to orange, and belong to the isoprenoid subfamily[21]. These pigments are classified into two main groups: pure hydrocarbons, carotenes (α-carotene, β-carotene and lycopene), and oxygenated derivatives, xanthophylls [lutein, zeaxanthin, astaxanthin[59]]. In bacteria, carotenoids are secondary metabolites that play fundamental roles for cellular adaptability, protecting from ultraviolet radiation and oxidative damage, as well as acting in the mechanisms of maintenance of cell membrane fluidity[60]. Several bacteria genres have been reported to produce carotenoid pigments, such as astaxanthin, β-carotene, zeaxanthin, canthaxanthin and lycopene[21].

Carotenoids are widely known for their antioxidant capacity[52,61,62] and for being natural precursors of vitamin A (retinol), a fat-soluble vitamin involved in cell division and differentiation, bone development and reproductive function[62,63]. The antioxidant effects of carotenoids were investigated in several *in vivo* and *in vitro* tests involving structures of the female reproductive system of different domestic species. As an example, in an *in vivo* study evaluating the effect of β-carotene supplementation on ovarian function, goats supplemented with 50 mg/day of β-carotene in association with the diet, for 34 days pre and 17 days post ovulation, indicated increased ovarian activity characterized by increase of the follicles population, ovulation rate, and total number of corpora lutea[52]. *In vitro*, lycopene supplementation in the culture medium of ovarian fragments from aged hens, reduced oxidative stress through activation of antioxidants and the Nrf / HO-1 pathway, increasing cell proliferation and reducing apoptosis rates[7]. During bovine cumulus-oocyte complexes (COCs) *in vitro* maturation (IVM), the supplementation with lycopene, another carotenoid, reduced apoptosis rates and oocyte reactive oxygen species (ROS) levels, resulting in higher cleavage rates, as well as increased total trophectoderm cells and inner cell mass of embryos produced from *in vitro* fertilization[8].

Lycopene supplementation during IVM of bovine COCs also impacted blastocyst production with significantly higher rates and lower apoptotic cells ratio when compared to IVM groups treated without lycopene. In addition, lycopene-treated groups showed 296 differentially expressed genes after transcriptomic analysis, in which pathways associated with cell function, metabolism, DNA repair and anti-apoptosis were positively regulated in the lycopene group[9].

Besides lycopene, the addition of carotenoids such as β-carotene and canthaxanthin was evaluated during IVM of murine and porcine oocytes, respectively. During murine IVM, β-carotene blocked the inhibition of oocyte maturation induced by Rosup, reagent that stimulates ROS production. Thus, β-carotene enhanced parthenogenetic activation of ROS-exposed mouse oocytes, reducing apoptosis levels, and restoring actin expression and cortical granule distribution in Rosup-exposed oocytes[64]. The addition of canthaxanthin, in turn, increased cleavage and blastocyst formation rates, from parthenogenetically activated porcine oocytes, increasing glutathione levels, a water-soluble antioxidant recognized as the most important non-protein thiol in living systems, and dramatically reduced the ROS levels[65].

Much of the positive effects achieved by the use of carotenoids in cell culture media or in animal feed, as nutritional supplements, is due to their antioxidant potential. It is well known that cancer, as well as chemotherapies and environmental pollutants, have been reported to promote a significant increase in EROS levels, acting as pro-oxidant factors for the rest of the body, affecting homeostasis and causing damage. In this sense, compounds such as carotenoids help to promote redox balance[66,67].

In addition to benefiting biological systems through their antioxidant potential, carotenoids have direct anticancer activity. In a study with an intraperitoneal model, tumor metastasis in murine with implanted ovarian cancer cells was attenuated by oral intake of lycopene, which significantly reduced the levels of pro-tumor factors such as ki67[68]. In another study, oral administration of astaxanthin promoted apoptosis in DMH-induced murine colon cancer by modulating the expressions of nuclear factor-κB (NFκB), cyclooxygenase-2 (COX-2), metalloproteinase (MMP) 2 and 9, proliferating cell nuclear antigen (PCNA), and serine/threonine protein kinase (ERK), factors related to carcinogenesis[69]. *In vitro*, lycopene induced apoptosis of human breast cancer cells[70] and reduced intracellular and mitochondrial ROS levels, as well as induced apoptosis of pancreatic cancer cells (Panc-1) by activation of caspase and increase of Bax[71].

Melanin

Melanin is a general term for a group of heterogeneous pigments, generally insoluble in water, aqueous acid and common organic solvents that usually appear in black or dark brown coloration, and can also produce reddish or yellowish colors[47]. These pigments are produced by organisms in all domains of living beings, from bacteria to mammals[72]. Melanin biosynthesis in bacteria occurs by oxidative phenolic polymerization compounds, predominantly by two pathways, 1,8-dihydroxynaphthalene and 3,4-dihydroxyphenylalanine, resulting in different types of melanin: eumelanin, pheomelanin, allomelanin, pyomelanin, and neuromelanin[73]. Melanins may exhibit a variety of functions in environmental and pathogenic bacteria, conferring adaptive advantages and increasing their suitability and survival under many stress conditions[73]. Among all melanin biological activities, we highlight: the conference of resistance to thermal stress caused by radiation and oxidative stress occasioned by redox imbalance, and resistance to toxic compounds and heavy metals[48]. These advantages confer to this substance, great potential of application as a natural antioxidant and anticancer compound.

Melanin is an antioxidant polymer that readily interacts with free radicals and other ROS, providing simple electrons transfer[46]. In an *in vitro* study, melanin produced by *Schizophyllum commune*, a yeast, showed a dose-dependent effect on the inhibition of proliferation of human laryngeal epidermoid carcinoma cell lines (HEP-2) and high activity in the elimination of 2, 2-diphenyl-1-picrylhydrazyl free radicals at a concentration of 50 μg/mL[47]. In another study, melanin produced by *Streptomyces glaucescen* demonstrated potent *in vitro* cytotoxic activity against proliferation and survival of skin cancer cell line (HFB4), showing a mortality rate of 81.3% of cancer cells when exposed to a concentration of 100 μg/mL for 24 hours. Furthermore, it was shown to be highly safe by exhibiting low cytotoxicity in normal cells

(human lung fibroblast and human amniotic cells) compared to usual chemotherapeutics such as 5-fluorouracil[46]. In addition to the anticancer effect, melanin extracted from *S. glaucescen* exhibited antioxidant effect comparable to that of ascorbic acid. Along the same lines, researchers recently extracted melanin produced by *Bacillus licheniformis* and tested its effect *in vitro* on several cancer cell lines. They observed a promising anticancer effect of the pigment against breast cancer cell line (MCF-7), human hepatocellular carcinoma cell line (HepG2), and colon carcinoma cell (HCT116). Additionally, low cytotoxicity of melanin has been observed when used *in vitro* at low concentrations on healthy cells such as human fibroblasts [HFB4[74]].

Violacein

Violacein is a violet-colored bacterial pigment synthesized from tryptophan through a pathway involving the sequential action of five different enzymes (encoded by the genes vio A, B, C, D, and vio E). It is produced by the species *Chromobacterium violaceum* and *Janthinobacterium lividum*[75], which are gram-negative bacteria found in terrestrial and aquatic environments, such as Negro River in Brazil[44]. *In vitro* studies have indicated that violacein is characterized by its antibiotic[76], antiprotozoal[75], and antiviral[77] therapeutic effects. This pigment has been reported to induce apoptosis in several cancer cell lines, indicating its use as a potential anticancer agent. The cytotoxicity of violacein for Ehrlich Ascites Tumor cells, for example, is mediated by a rapid (8-12 h) production of ROS and a decrease in intracellular glutathione levels, probably due to oxidative stress[44]. In a human leukemia cell line (HL-60) violacein cytotoxicity was shown to be preceded by caspases activation, nuclear factor kappa-B (NF-kappaB) target genes transcription, and mitogen-activated protein kinase [MAP[78]]. In human colon cancer cell lines (Caco-2), violacein mediates ROS production, followed by caspase-3 activation, cytochrome C release, and calcium release into the cytosol, leading to Caco-2 cell death via the apoptosis pathway[79]. Another study found that the pigment produced by the species *Janthinobacterium lividum* was able to inhibit, *in vitro* and *in vivo*, head and neck carcinoma cell lines growth. In this study, violacein inhibited cell growth and induced autophagy and apoptosis, and its effect on the inhibition of cell proliferation pathways (ERK1 and ERK2), as well as increasing the Bax/Bcl-2 ratio linked to apoptosis, induction of p53 degradation, accumulation of NF-kappaB, and ROS production[80] was observed. In an *in vivo* study in BALB/c mice, the intratumoral injection of 0.75 mg/kg violacein dissolved in DMSO and diluted in PBS, as well as the injection of 1 mg/kg during 35 days were safe and did not alter the hematometric levels[44].

Besides inducing apoptosis in cancer cells, violacein promoted morphological alterations in brain tumor cell lines (U87) regulating their migratory capacity, and interfering in the metastatic process[81]. Finally, an *in vitro* study found that violacein acts synergistically with the chemotherapeutic 5-fluorouracil, increasing cytotoxicity and apoptosis induction, as well as interfering with Akt-mediated signal transduction in human colorectal cancer cell lines[45].

Prodiginines

Among the class of bacterial pigments discovered and widely studied over the years, prodiginines can be considered the most remarkable, curious and important of this category. The name "prodiginine", associated with "prodigy, miracle", has its origin in its supposed connection with reports dating back to the year 322 BC, when Macedonian soldiers reported the appearance of supposed drops of blood inside the bread used for food, an event considered prophetic by the clairvoyants of Emperor Alexander the Great. The knowledge and study of these pigments also sought to justify miraculous events that occurred more than 700 years ago, such as the Miracle of Bolsena, origin of the Corpus Christi celebration, when supposed drops of blood were observed on the host during a mass celebrated by a priest who was struggling with a lack of faith[82].

In contrast to the rumors involving this class of pigments, which are formed by bacterial colonies resembling blood droplets, the prodiginines class comprises a group of red pigments, alkaloids, structurally characterized as heterocyclic tripyrroles, i.e., they contain three interconnected pyrrole rings [A, B, and C[49]]. This group of pigments includes prodigiosin, metacycloprodigiosin, undecylprodigiosin, nonylprodigiosin, cycloprodigiosin, cyclonylprodigiosin, and butylcycloheptylprodigiosin[83,84]. Conveniently, due to the isoforms similarity of these substances, it has become a consensus to use the term "prodiginine" to characterize the class of alkaloids, and "prodigiosin" to refer to the particular names of the red or magenta pigments[85]. Prodiginines are secondary metabolites that were first extracted and characterized from the gram-negative *Serratia marcescens*[84]. In addition to *S. marcescens*, the pigment has also been isolated from several gram-positive and gram-negative species[83,86]. Between this pigment-producing species, S. marcescens stands out for being easy to cultivate, providing massive production of this bioactive product[87].

Prodigiosins are pH-sensitive, photosensitive, insoluble in water, sparingly soluble in alcohol and ether, and soluble in chloroform, methanol, acetonitrile, and DMSO[88-90]. Although the mechanism by which these compounds act, is complex and probably multifactorial, a wide variety of studies have demonstrated important pigment-associated properties. Prodigiosins have exhibited varied properties, namely: 1- antiviral, specifically inhibiting at least the NF-kappaB and Akt signaling pathways, which promotes accelerated cell death in cells infected with Herpes simplex virus[91]; 2- antimicrobial activity, with 30% inhibitory activity for *Candida albicans*, *Escherichia coli*, and *Staphylococcus aureus*[92]; 3- anti-inflammatory activity, identified as a potential COX-2 protein inhibitor[93]; and 4- antioxidant activity, with a DPPH radical scavenging

potential of ~78% and ABTS radical scavenging potential of ~71% at a concentration of 500 µg/mL[87].

Parallel to the properties quoted above, prodigiosin exhibited relevant and potent anticancer action against several cancer cell lines, with distinct mechanisms. In leukemia cell line (K562), it inhibited proliferation, increased the rate of ROS, induced apoptosis, probably by inducing an increase of pro-apoptotic proteins, caspase-3-cleaved, 8 and 9, and inhibited autophagy. It was also identified that the activated ERK cascade plays a primary role in prodigiosin-induced apoptosis in K562 cells[89]. In hematopoietic cancer cell lines (Jurkat-derived T lymphocyte leukemia cells, NOS-derived murine myeloma model cell line, HL-60, RAMOS-derived Burkitt lymphoma cells), prodigiosin affected cell proliferation rates and apoptosis, which was characterized by dose-dependent decrease in the number of viable cells, and increase in apoptotic cells in all cancer cell lines studied[94].

Another cancer cell line studied was human mucoepidermoid lung carcinoma (NCHI-292), HEP-2, MCF-7 and HL-60, in which the pigment isolated from *Serratia marcescens* produced significant cytotoxic effects in all cell lines, with an inhibitory concentration (IC50) of 3.6, 3.4, 5.1 and 1.7 µg/mL, respectively[95]. In cervical cancer cell line (HeLa), prodigiosin inhibited proliferation and induced apoptosis, thanks to upregulation of Bax and caspase-3, with an IC50 of 2.1, 1.2 and 0.5 µg/mL after 24, 48 and 72 h of exposure[96]. Additionally, the association of prodigiosin with different substances was also effective. For example, in association with Zelavespib (PU-H71), an experimental Hsp90 chaperone inhibitor, prodigiosin alone or in combination up-regulated the expression of Bax without affecting that of Bcl-2. The combination also increased the expression of caspase-3, 8 and 9, inducing apoptosis and inhibiting adhesion of breast adenocarcinoma cell lines [MDA-MB-231[15]]. Similar effects were obtained with the association between prodigiosin and cisplatin, in which prodigiosin increased the sensitivity of cisplatin-resistant urothelial carcinoma cell lines[97]. Interestingly, despite being effective against a wide variety of cancer cells, prodigiosin has little, if any, cytotoxicity against normal cells[15,19,94].

Pyocyanin

Pyocyanin is a secondary redox active metabolite and an important virulence factor of gram-negative bacteria *Pseudomonas spp*[98]. It is a bluish pigment that composes a family of tricyclic compounds, phenazines, and may exist in oxidized or reduced form, the latter being unstable and highly reactive with molecular oxygen[17]. Unlike other pigments with antioxidant action, pyocyanin seems to induce oxidative stress in cellular systems and, for this reason, may induce cytotoxicity in cancer cells through the generation of ROS and, then, progressive cellular oxidative damage[99].

There is a consensus that balanced ROS levels are involved in the processes of tumor formation, maintenance, and progression[100]. ROS disbalance, characterizing an oxidative stress induced by chemotherapeutic drugs, is associated with tumor depletion[66], since oxidative stress induces lipid peroxidation, generating numerous electrophilic aldehydes that can attack several cellular targets[101]. Hence, the unbalanced increase in ROS associated with the accumulation of DNA damage, senescence, and cell death induced by agents such as pyocyanin may be a strategy for the depletion of growing tumors[102]. On the other hand, the pro- or antioxidant effect of pyocyanin may be more related to its concentration and bioavailability, since in one study the *in vitro* free radical scavenging activity of pyocyanin was higher than that of ascorbic acid. In the same study, the substance isolated from *Pseudomonas aeruginosa*, did not significantly affect the viability of human fibroblasts, even at high concentrations (100 µg/mL) indicating, for example, safety of its use for food manufacturing, since it has antibiofilm activity against food pathogens such as *Salmonella enteriditis* and *Vibrio diabolicus*[103].

The low cytotoxicity of pyocyanin in normal cells, combined with its anticancer property, places it in the Hall of bacterial pigments acting as natural anticancer compounds. The dose-dependent *in vitro* cytotoxic effects of pyocyanin were first reported in human hepatoma cells [HepG2[104]] and against the Panc-1[99] cell line. Similar to other bacterial pigments, pyocyanin induces cell apoptosis, probably due to increased ROS, DNA damage, activation of caspase-3, and acceleration of cell senescence and apoptosis[104,105]. A brief summary with the main pigments addressed in this review, including pyocyanin, diverse pharmacological potential, as well as anticancer potential in distinct cancer cell lines is presented in table 2.

Future prospects and limitations

This review highlights the relevance to the study and practical applications of bacterial pigments. Microorganisms, and more specifically bacteria, are endless sources of these and other little-known bioactive compounds with vast pharmacological potential. Therefore, these pigments may contribute to the development of new therapeutic approaches in cancer treatment, as an alternative to reduce the negative effects of highly cytotoxic chemotherapeutics, or as adjuvants to chemotherapies. In addition, the domain of microorganisms manipulation associated with advances in genetic engineering allows considerable advances today, as well as for the future.

Identification, isolation and study of these and other bacterial pigments and bioactive metabolites are important alternatives for the generation of new anticancer drugs that present fewer side effects, i.e., greater selectivity against cancer cells and less cytotoxicity for normal cells. Among the side effects of chemotherapeutic protocols currently used for the treatment of cancer, we can highlight reproductive disorders, such as premature ovarian failure. However, despite advances in studies for the potential use of bacterial pigments as anticancer drugs, few have tested the effect of

these pigments on the female reproductive system, gonads, gametes and embryos. In this sense, the use of animal models to study the reproductive toxicity of these substances is a valuable alternative, since the available biotechnologies enable to obtain most of the necessary biological material, following ethical research principles. Studies of *in vitro* culture of ovarian follicles, *in vitro* gametes maturation and *in vitro* embryos production can be used as tools to verify the pigments effects on females reproduction processes.

Table 2. Diverse pharmacological potential and anticancer potential of the main categoris of bacterial pigments

Pigments	Producing bacteria	Pharmacological potential	Anticancer potential		
			Cell lineage/Tumor type	Experimental system	Animal model (*in vivo*)
Carotenoids	*Flavobacterium spp., Agrobacterium spp., Micrococcus spp., Pseudomonas aeruginosa, Serratia marcescens, Chromobacterium spp., Rheinheimera spp. e Arthrobacter spp*[21]	Antioxidant[52,61,62], anti-inflammatory[69], anticancer[68-71]	OV-MZ-6 ovarian cancer cells[68], MDA-MB-468 breast cancer cells[70], Panc-1 pancreatic cancer cells[71]	*In vitro and in vivo*	Murine
Prodiginines	*Serratia marcescens*[84], *Hahella chejuensis, Pseudomonas magnesiorubra, Vibrio spp., Streptomyces spp, Streptoverticillium rubrireticuli, Actinomadura madurae, Saccharopolyspora sp., Actinomadura pelletieri, Alteromonas rubra, Pseudoalteromonas denitrificans e Hahella chejuensis*[83,86]	Antiviral[91], antibacterial, antifungal [92], anti-inflammatory[93], antioxidant[87], anticancer[15,89,94-96]	K562 leukemia cells[89], Jurkat cells, NOS, HL-60, RAMOS of hematopoietic cancer[94], NCHI-292 lung carcinoma cells, HEP-2 cells of laryngeal carcinoma, MCF-7 breast cancer cells[95], HeLa cells from cervical cancer[96], MDA-MB-231 breast adenocarcinoma cells[15]	*In vitro*	N/A
Pyocyanin	*Pseudomonas aeruginosa*[98,103]	Antibacterial[103], pro-oxidant[17], antifungal[98], anticancer[99,104-105]	HepG2 hepatocellular carcinoma cells[104], Panc-1 pancreatic cancer cells[99]	*In vitro*	N/A
Melanin	*Streptomyces glaucescen*[46], *Bacillus licheniformis*[74], *Pseudomonas aeruginosa*[48], *Burkholderia xenovorans, Legionella pneumophila*[73]	Antioxidant[46,48], anticancer[46-47,74]	HEP-2 cells of laryngeal carcinoma[47], HFB4 skin cancer cells[46], MCF-7 breast cancer cells, HepG2 hepatocellular carcinoma cells, HCT116 colon cancer cells[74]	*In vitro*	N/A
Violacein	*Chromobacterium violaceum*[75], *Janthinobacterium lividum*[80]	Antibacterial[76], antiviral[77], antiprotozoal[75], antioxidant[44], anticancer[44-45, 78-81]	Ehrlich tumor cells[44], HL-60 leukemia cells[78], Caco-2 colon cancer cells[79], CAL-27 tongue carcinoma cells, FaDu pharyngeal carcinoma cells[80], U87 brain tumor cells[81], HCT116 colon cancer cells[45]	*In vitro and in vivo*	Murine

N/A: not applicable.

Conclusion

The prospection for large-scale production of natural bioactive compounds extracted from microorganisms, such as bacterial pigments, is one of the great alternatives for obtaining substances with high potential for industrial and pharmaceutical application. In this sense, given the scarcity of *in vitro* and *in vivo* studies evaluating the effects of these substances on the female reproductive system, new investigations regarding the impact of these promising pigments on the development of ovarian follicles, gametes and embryos, are of great importance.

Conflict of interests

The authors declare no conflict of interest.

Author Contributions

Conceptualization: A. L. da C. Santos. *Data curation*: A. L. da C. Santos, A. C. A. Ferreira and J. R. de Figueiredo. *Formal analysis*: A. L. da C. Santos, A. C. A. Ferreira and J. R. de Figueiredo. *Methodology*: A. L. da C. Santos, A. C. A. Ferreira and J. R. de Figueiredo. *Supervision*: J. R. de Figueiredo. *Revisão de textos e edição*: A. L. da C. Santos and J. R. de Figueiredo.

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