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COMPOUNDS PRODUCED BY *Bacillus thuringiensis* WITH ANTICANCER APPLICATION

Camila Cassia Silva Araujo
Universidade Federal de Pernambuco (UFPE)
Recife PE
http://lattes.cnpq.br/2278387392313875

Túlio Alexandre Freire da Silva
Universidade Federal Rural de Pernambuco (UFRPE)

Jose de Paula Oliveira
Instituto Agronômico de Pernambuco (IPA)
Recife PE
http://lattes.cnpq.br/3540150611094753

José Manoel Wanderley Duarte Neto
Instituto Agronômico de Pernambuco (IPA)
Recife PE
http://lattes.cnpq.br/0685998873631474

Ana Lucia Figueiredo Porto
Universidade Federal Rural de Pernambuco (UFRPE)
Recife PE
http://lattes.cnpq.br/4989617783837981

Abstract: In the year 2020, there was a notable increase in cancer in Brazil, totaling about 626,030 new diagnosed cases, and 128 million deaths. Despite the fact that conventional anticancer therapies such as (surgical procedure, radiotherapy and chemotherapy) are decisive in the management of cancer patients, they are, however, ineffective in approximately 50% of cases of malignancy. The use of new tools for the treatment of cancer cells makes studies with the gram-positive bacterium *Bacillus thuringiensis* (Bt) promising, as it is distinguished by its ability to produce, in addition to crystals, other entomotoxic compounds, such as Cyt, Vip proteins and polysaccharides. In this review the authors searched about 2 online scientific databases with the following descriptors *Bacillus thuringiensis* AND cancer. Scientific criteria adopted in this review to classify the articles found by score (0 to 10), after the exclusion criterion 31 articles were selected. None obtained the maximum score of 10 points defined by the methodology, which indicates a deficiency in studies that deal simultaneously with purification research, characterization, cultivation conditions, *in vitro* and *in vivo* tests, and description of characteristics of these anticancer compounds produced by Bt. There was a clear gap in the literature on research with these compounds for medical application, which prevents further development in the area and increases the need for further studies.
Keywords: Carcinogenic activity**;** compounds; *Bacillus thuringiensis.*

INTRODUCTION

Cancer is one of the main causes of death in the world population. It is estimated that by 2030, these numbers will continue to rise to an alarming 26.4 million new diagnosed cases and 17 million cancer-related deaths worldwide. In the year 2020, in Brazil there was a notable increase in cancer, totaling about 626,030 new diagnosed cases, and 128 million deaths (Aboul-Soud *et al*, 2019 and MS / INCA, 2020)

Despite the fact that conventional anticancer therapies (surgical procedure, radiotherapy and chemotherapy) are decisive in the management of cancer patients, they are, however, ineffective in approximately 50% of cases of malignancy. In addition, the synthetic cytotoxic drugs currently used in the treatment may be associated with a series of side effects that compromise the patient's recovery. Therefore, new compounds are gaining focus for the development of new natural alternative therapeutic techniques and effective treatment of tumors (Aboul-Soud *et al*, 2019).

Recently, new compounds have received a great deal of attention in the field of cancer research due to their specific toxicity to cancer cells (Chubicka *et al,* 2018). Among the natural sources of these compounds are microorganisms, such as bacteria of the species *Bacillus thuringiensis* (Bt). This gram-positive bacterium that occurs naturally in soil and in different environments belongs to the same genus as *Bacillus cereus,* and is distinguished by its ability to produce protein inclusions during its sporulation phase, called δ - endotoxins (Cry). These inclusions resemble crystals when observed through a phase contrast microscope, and the peptides that constitute them are responsible for transforming bacteria into the main biological control microorganism due to their potent entomopathogenic effect (Melo and Kitada, 2020). In addition to the crystals that show this activity, the species produces other entomotoxic compounds, such as Cyt, Vip proteins and polysaccharides (Ramamoorthy *et al*, 2018)

The different strains of Bt are producers of other active biomolecules of great importance

that can be applied in various fields including agriculture, environment and even in the process of developing new drugs with anticancer activity (Aberkane *et al*, 2020). cytotoxic and non-hemolytic, being classified as a family of proteins called "parasporins". Recently, parasporins have received a great deal of attention in the field of cancer research due to their specific toxicity to cancer cells, which may lessen the side effects of chemical treatment (Chubicka *et al*, 2018).

The anticancer property of Bt is attributed to two factors: Parasporins and cytolytic proteins (Cyt). Parasporins are Cry-like proteins with less than 25% amino acid sequence homology. Like Cry toxins, Parasporins also act specifically on their target cancer cell lines, as they require specific binding receptors on these cells for their activity to occur (Nair *et al*, 2018).

Therefore, the present systematic review reports the compounds produced by Bt that have already been isolated and characterized as non-hemolytic, and with antiproliferative activity against different cancer cell lines, in addition to comparing the mechanisms involved in the induction of cytocide-dependent cell death.

METHODOLOGY SEARCHES

The first step in the development of the article was to carry out searches on the PUBMED (https://pubmed.ncbi.nlm.nih.gov/) and SCIENCE DIRECT (https://www.sciencedirect.com/) platforms. The following descriptors were used to carry out the searches: *Bacillus thuringiensis* AND cancer. This procedure allowed us to select published articles on the applicability of compounds derived from *Bacillus thuringiensis* for the treatment of cancers.

Independent searches were carried out and the conformity of the selected works was validated by comparison, considering the inclusion and exclusion criteria described. In case of divergence between the selected works, all criteria were reviewed and discussed. When in the title of the article only *Bacillus thuringiensis* application was mentioned without terms related to cancer, the researchers proceeded to the abstract, seeking to carry out this application in the methodology or objectives. Works that did not report the process of anticancer activity were excluded. There was no limitation as to the year and date of publication, due to the lack of publications on the subject. There were no restrictions on the methodology used, types of analysis and quantification of results, in addition, there were no restrictions on the methodology of purification, culture conditions and *in vivo/in vitro assays*. There was a limitation regarding the type of microorganism studied, restricting only the Bt strains.

EVALUATION

The evaluation criteria of the scientific works selected in this review were organized according to those proposed by Greenhalgh. The parameters were classified in scale: adequate (score: 2), partially adequate (score: 1) and inadequate (score: 0) (Greenhalgh, 1997). The maximum score for achieving all criteria on the best scale is 10 points. Parameters such as production time, year of publication, satisfactory anticancer activity with mortality in different cell lines, among others, were not used in the scoring, but were taken into account, as they were relevant for further discussion.

The evaluation criteria determined for the present study were as follows. Amino acid sequencing: Articles that cited the sequencing method received a score of 2; Articles that did not mention the sequencing methodology performed receive a score of 1; And those that did not perform sequencing

received a score of 0. Purification: articles that performed purification and described the method received a score of 2; Those who did not mention the purification method received a score of 1; Those who made no mention of purification received a score of 0. Cultivation conditions: Those who described the entire cultivation process received a score of 2; Those who cited only part of the process, score 1; Did not mention the cultivation methodology, did not score. Structures and characteristics: Articles that performed multiple methods of compound characterization received a score of 2; Those who studied only one characteristic scored 1. Those who reported no characterization scored 0. Test models: Articles that tested activity *in vivo* and *in vitro* scored 2; Those who only tested one of the test modalities received a score of 1; And those who didn't test, didn't score. The evaluation parameters of the selected articles and the scores are summarized in Table 1.

ANALYSIS

All selected articles were analyzed in relation to the evaluation parameters and other aspects relevant to the discussion of the topic, such as: type of in *vivo/in vitro test,* sequencing, Bt subspecies, characteristics and structure of the compound and cultivation conditions. Each of these parameters was analyzed individually and constituted a topic of discussion throughout this article.

RESULTS AND DISCUSSION

Applying the search procedure established in the methodology, 116 articles were found in the PUBMED database, 1688 articles in the SCIENCE DIRECT database, totaling: 1084 articles. Based on the defined inclusion and exclusion criteria, 31 articles were selected for this review, distributed as shown in figure 1. The PUBMED platform was able to find most of the selected articles through the applied search methodology. 31 articles were

Score Determination Criteria	Punctuation		
	2	1	0
(A) Sequencing	Method described	Method not mentioned	There was no sequencing
(B) Purification	Purified compound and method described	Compound purified but not mentioned	I am not purified compound
(C) Growing conditions	Method described	Method with little information	Did not mention how the procedure was performed
(D) Structures and Characteristics	Citing two or more characteristics of the compound	Mention only one feature	Do not cite information
(E) Test model	In vitro and in vivo	In vitro or in vivo	Did not perform tests

Table 1-Score of parameters selected for critical evaluation of the systematic review.

Source: Authors.

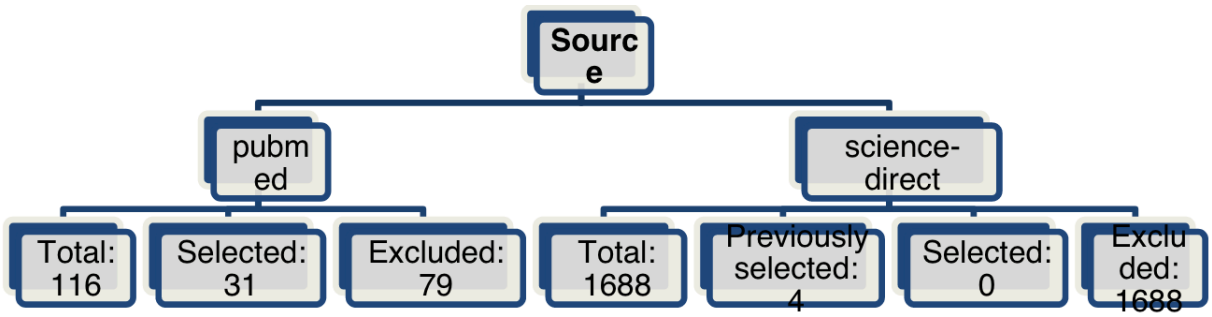
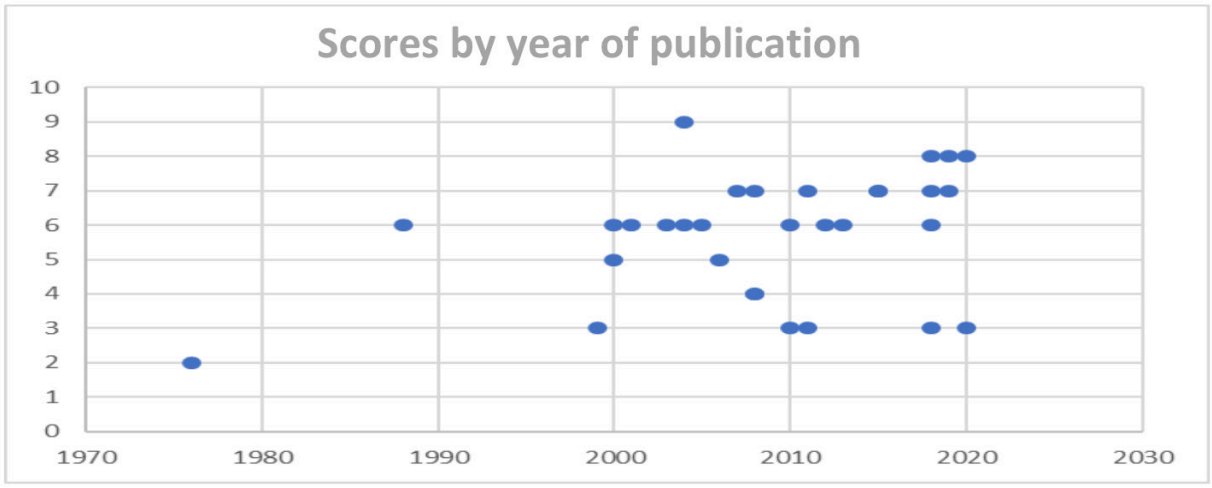


Figure 1. Total articles selected in four different databases using the described methodology.

Source: Authors.



Graph 01: Score of selected articles by year of publication.

Source: Authors.

found on the subject, however, in SCIENCE DIRECT, only 4 articles were found, among which all had already been found by the PUBMED platform, thus resulting in the exclusion of prototypes of the articles for this study.

After the evaluation and attribution of the scores of the selected articles, no article obtained a maximum of 10 points by the evaluation criteria defined in the methodology, thus indicating that this topic still requires more complete and in-depth studies.

Axis of the ordinates: Refers to the scores and their score, according to the years of publication.

As described in the methodology, no time interval was defined, however, only 15 articles were published in the last ten years. However, only 16 articles were published in the last 10 years. Of these 16 articles, only 10 were published in the last 5 years, indicating the need for further research related to the carcinogenic activity of compounds from *Bacillus thuringiensis*.

The complete distribution of the scores is available in Table 2, where it is observed that only 1 article published in 2004 scored 9 (3.23%), 3 articles in the years 2018, 2019 and 2020 scored 8 (9.68%), 7 articles from 2007. 2008, 2011, 2015, 2018 and 2019 scored 7

(22.58%), 10 articles from 1988, 2000, 2001, 2003, 2004, 2004, 2005, 2010, 2012, 2013, and 2018 simultaneously scored 6 (32.26%), 5 articles from 1999, 2010, 2011, 2018 and 2020 scored 3 (16.13%), 2 articles between 2000 and 2005 scored 5 (6.45%), 2 articles from 2008 scored 4 (6.45%), 1 article from 1976 scored 2 (3.23%) and no article scored 0. This indicates the deficiency of studies that do not simultaneously deal with purification, characterization, cultivation conditions, *in vitro* and *in vivo*, and description of characteristics of these anticancer compounds produced by Bt.

Among the 11 works that scored between seven and nine, all performed sequencing, purification, and described the cultivation conditions. Only 1 of the 10 performed *in vitro* and *in vivo tests* simultaneously and only 3 did not mention structures and characteristics. Of the articles that scored less than seven, 12 did not perform compound purification, 6 did not perform sequencing, 14 did not describe the methods and conditions of Bt cultivation, only 1 article performed *in vitro/in vivo tests* simultaneously, and 6 did not cite information on the structures and characteristics of the studied compound.

Table 3 gathers the most relevant information from the selected articles, data according to the analysis criteria adopted in the review and other relevant characteristics.

Next, each point in the table was discussed independently.

MICROORGANISM

Based on this systematic review, 31 articles were selected, all of which study the same species, *Bacillus thuringiensis.* Of these, 11 articles (35.48%) did not specifically mention the Bt strain used. Another 2 articles (6.45% d) tested the isolated Bt strain A1470, 1 article (3.23%) tested it on the Bt israelensis ONR 60 A strain, 1 article (3.23%) used *Bt serovar israelensis, kyushuensis and kurstaki,* 1 article (3.23%) used only Bt *serovar israelensis,* 1 article (3.23%) used *Bt serovar shandongiensis,* 1 article (3.23%) *used Bt coreanensis* A1519, 1 article (3.23%) *Bt serovar shandongiensis,* 1 article (3.23%) to *Bt serovar Dakota,* 1 article (3.23%) to *Bt A1462,* 1 article (3.23%) to Bt A1547, 1 article (3.23%) to *Bt serovar kurstaki HD-1 and serovar israelensis HD-500,* 1 article (3.23%) *Bt israelensis and morrisoni,* 1 article (3.23%) to *Bt B0195,* 1 article (3.23%) *Bt M019,* 1 article (3.23%) to *Bt B0462,* 1 article (3.23%) to *Bt 407,* 1 article (3.23%) to *Bt sorovar dakota* 4R2, 1 article (3.23%) *to Bt israelensis QBT229,* and 1 article (3.23%) *Bt Berliner A14d2.*

Of the 31 strains tested, 20 did not mention the Bt subspecies, only the code of the isolate. Among the studies that cited the subspecies, 6 articles cited the *Israeli subspecies* (19.4%) which showed significant results for studies with *in vitro tests* about its cytotoxic activity against murine leukemic cells (L1210), leukemic T cells (MOLT-4), cervical cancer cells (HeLa), uterine cancer (Sawano), uterine-cervical cancer (TCS), leukemic T cells (HL60), leukemic T cells (Jurkat), normal embryonic lung fibroblast (MRC-5), lung cancer (A549), normal hepatocyte (HC), hepatocyte cancer (HepG2), colon cancer (Caco2), normal monkey kidney epithelial cell (Vero animal cells), monkey kidney SV-40 (COS-7)) and normal mouse embryo fibroblast (NIH3T3). 2 cited subsp. *serovar shandongiensis*, (6.45%) had expressive results in *in vitro tests* for cell lines, (MOT-4) and (HeLa) inducing the apoptotic process. 2 of subsp. *serovar dakota,* (6.45%) *in vitro tests* provided cells (MOLT-4), (Sawano), (Caco2), (Jurkat), (HeLa), (TCS), (HepG2), (MRC-5), uterine normal cells (UtSMC), lung cancer cells (A549), normal hepatocytes (HC), normal T cells (T cell), leukemic T cells (HL-60) PC-3

Authors, years	(A)	(B)	(C)	(D)	(E)	Total Score
Prasad, S. S.S.V. et al, 1976	0	0	0	2	0	2
Youkoyama, Y et al., 1988	0	2	2	1	1	6
Mizuki, Eiichi et al, 1999	0	0	2	1	0	3
Yamashita, S. et al, 2000	0	2	0	1	2	5
Lee, Dae Weon et al, 2000	2	2	0	1	1	6
Lee, Dae Weon et al, 2001	2	0	2	1	1	6
Namba, Akitoshi et al, 2003	2	2	0	1	1	6
Ito, Akio et al, 2004	2	2	2	1	2	9
Okumura, S. et al, 2004	2	0	2	1	1	6
Yamashita, Satoko et al, 2005	2	2	0	1	1	6
Kitada, Sakae et al,2006	2	1	0	1	1	5
Jung, Y. C. et al, 2007	2	1	2	1	1	7
Okumura, Shiro et al, 2008	2	0	0	1	1	4
Uemori, Akiko et al, 2008	2	0	0	1	1	4
Nadarajah, Vishna Devi et al, 2008	2	1	2	1	1	7
Wong, Rebecca S.Y. et al, 2010	2	0	0	1	0	3
Nagamatsu, Yasunori et al, 2010	2	0	2	1	1	6
Okumura, Shiro et al, 2011	2	0	0	1	0	3
Gonzalez, Eric et al, 2011	2	1	2	1	1	7
Chan, Ko K.Keong et al, 2012	2	2	0	1	1	6
Kuroda, Shouta et al, 2013	2	2	0	1	1	6
Nair, Manoj S. et al, 2015	2	1	2	2	0	7
Brasseur, Kevin et al, 2015	2	1	2	1	1	7
Chubicka, Thomas et al, 2018	2	2	2	1	1	8
Moazamian, Elham et al, 2018	2	0	2	1	1	6
Ramamoorthy, Sathishkumar et al, 2018	0	0	2	1	0	3
Nair, Kavita et al, 2018	2	2	2	1	0	7
Souissi, Wided et al, 2019	2	2	2	1	1	8
Aboul-Soud, Mourad A.M. et al, 2019	2	2	2	1	0	7
Aberkane, Lila et al, 2020	2	2	2	1	1	8
Melo, André L.A. et al, 2020	0	0	2	1	0	3

(A) Sequencing: Sequencing performed in BT and description of the method (score 2); did not mention the sequencing methodology (score 1); did not perform sequencing (score 0).

(B) Purification: Compound of purified bt and description of methodology (score 2); Purified compound but did not mention methodology (score 1); Unpurified compound (score 1).

(C) Cultivation conditions: Description of the form of BT cultivation and preparation (score 2); Description with little information about the cultivation method (score 1); no information on how to grow bt (score 0)

(D) In vitro/vivo test : Studies that performed both tests (score 2); tested only one of the models (score 1); Did not perform tests (score 0)

(E) Structures and characteristics: Description of the characteristics of the tested compound (score 2); They only cited 1 characteristic (score 1); They did not mention the characteristics (score)

Table 02: Classification and scoring of selected articles.

Source: Authors .

prostate cancer cells (CRL- 1435), human uterine endometrial adenocarcinoma Hec-1A (HTB-112), human uterine endometrial adenocarcinoma cells KLE (CRL-1622), human breast adenocarcinoma MDA-MB231 (HTB-26) cells cancer MCF-7 (HTB-22), human non-tumorigenic epithelial cells MCF-10A (CRL-10317), human epithelial ovarian adenocarcinoma cells OVCAR-3 (HTB-161), and human ovarian epithelial adenocarcinoma cell line SKOV - 3 (HTB-77), with efficacy for apoptotic induction in up to 24 hours of treatment. 1 to subsp. *Coreanensis* (3.23%) their *in vitro study* with MOLT-4 cells show that within 3 hours of administration of the crystallized protein it causes an osmotic koiloid that later can cause cell lysis different from the Cry proteins, and Cyt which has faster activity, it is efficient. And 1 to subsp. Berliner (3.23%) evaluation of the tests in the *in vitro model* of the breast cancer lineage (MCF-7), managed to produce a cytopathic toxin with high toxicity.

From an industrial point of view, pathogenicity can negatively influence the choice of microorganisms for the development of agents that support cancer treatments. Interestingly, the Gram-positive soil bacterium, Bt, is a biological insecticide widely known in the scientific community, due to its attribution to crystals, which provide a lethal effect for certain types of insect larvae,

Much is attributed to compounds, including proteins that have been studied in recent years with great attention in the field of cancer research due to their toxicity to cancer cells (Yamashita *et al*, 2005). Species like this do not present pathogenic activity directly related to human health, but produce compounds, such as proteins and polysaccharides, which can act as adjuvants in the processes of oncological therapies.

GROWING CONDITIONS

The selection of culture medium conditions is of great importance for the fermentation of Bt, since this factor will directly affect the cost and the quality and quantity of the final compound for application. One of the benefits of working with microorganisms is the possibility of changing the composition of the culture medium, using alternative substrates, lower cost materials, as by-products that would be discarded by the industry.

Only 19 of the selected works presented the description of the applied cultivation conditions. Among these, 4 (21.05%) used the Nutrient Agar (commercial) medium, 4 (21.05%) used the Nutrient Agar, peptone and NaCl (Sodium chloride) medium, 3 (15.79%) used LB (Luria Bertani) culture medium, 2 (10.53%) used Nutrient Agar with NaCl, 2 (10.53%) used CSL (corn steep liquor), 1 (5.26%) used nutrient agar and meat extract medium, 1 (5.26%) used a medium based on glucose, peptone, MgOs (magnesium oxide) and (NaCl, 1 (5.26%) used nutrient agar medium CCY (supplemented with salts), and 1 (5.26%) used Nutrient Agar, peptone and NaCl.

Among the articles that described this part of the methodology, 17 used commercial culture media, already well described in the literature, with evidence of good production and yield in the formation of crystals, and Bt polysaccharides. The same 17 studies used supplements such as salts, peptone, NaCl, glucose, and meat extract, to potentiate the production of the compounds. Only 2 of the 19 articles used by-product in the composition of their culture medium, corn steep liquor.

However, when working with the by-product medium, such as CSL, it requires greater attention in the purification step, in view of its application in the medical industry. Purification of crystals by chromatographic method was extremely important to guarantee

a pure and efficient compound, with a cytotoxic effect, for oncological cells (Yumi Youkoyama, 1988).

The development of the fermentation process is a very important factor that must be taken into account since the optimization of the culture conditions can promote an increase in protein yields and a reduction in production costs, thus being an important issue from the point of view of medical view. The initial pH of the culture medium influences other elements, such as enzymes and carbohydrates, which have a potentiating activity in the production of active compounds. The pH of the described culture media ranged from 6.0 to 10.5. While the temperature ranged from 27 ºC to 37 ºC. In most (7) of the works, the agitation was around 300 rpm. The fermentation time for crystal production took 2 to 4 days, reported in 11 articles, while (Ito *et al*, 2004) used 8 days to reach sporulation. 19 articles did not mention the cultivation time used.

No articles were found that studied different cultivation conditions. The lack of these studies, applied to fermentation processes, measuring the subsequent activity of the compounds generated, hinders the evolution of research in the microbiological scope and delays its therapeutic applications.

PURIFICATION

Among the selected articles, 12 (38.71%) did not perform purification and 8 (25.80%) did not mention the method used for the purification process, another 3 (9.30%) studies used separation by two-phase aqueous system, 2 (6.45) used an alkaline pH solution. Among the 6 articles that described chromatographic methods applied in their methodology, 2 (6.45%) used a density gradient with sucrose solution as a prechromatographic step by SDS PAGE, 2 (6.46%) used an ion exchange column Resource Q, 1 (3.23%) used a CM-cellulose ion exchange column, 1 (3.23%) was molecularly excluded by a cellulose column. The articles that did not mention the purification method made it difficult to distribute the scores in the table, making it difficult to compare data, in addition to reducing and limiting the results available in the literature.

Two-phase two-phase system methodology: an upper phase rich in spores captured by hexane (white) followed by a phase rich in aqueous cell debris and, finally, crystal-rich pellets. Purification of proteins by molecular exclusion chromatography revealed that peptide fractions obtained after activation of this proprotein appear on the producing strain, increasing yield levels by up to 40%, increasing the production of crystals with cytotoxic application (Aberkane *et al*, 2020) (Chubicka *et al*, 2018)

Second (Prasad and Shethna, 1976) Since the crystals obtained in the fermentation process undergo purification to provide higher degrees of purity and yield, these issues vary according to the methods applied to the process. The crystals that were extracted were not considered sufficiently pure on the basis of manual inspection by microscope, later, after being purified by binding to a CM-cellulose column, they resulted in the reproduction of satisfactory cytotoxic activity. It was not cited in the degree of yield of these proteins (Prasad and Shethna, 1976).

For the cytotoxicity assays, it is necessary to activate the crystals with the high pH methodology that affect the activity of proteases, enzymes responsible for degrading proteins, being an essential step to convert the protoxins of the crystals into toxins. This is how to promote greater income and potentiate the lethal effects (Nair *et al*, 2018).

The authors Okumura *et al.* (2008) and (Aboul-Soud *et al*, 2019) cite *B. thuringiensis* as the most efficient active compound after solubilization in an acidic solution, potentiating its lethal effect on proteins and

activating its toxicity against different tumor cell lines.

Among the articles that performed and described the purification, those that used liquid chromatography in their methodologies obtained a protein with a higher degree of purity, as described in (Wong *et al*, 2010), Chan *et al.* (2012), (Kuroda *et al.,* 2013), (Nair *et al.,* 2015) and (Souissi *et al.,* 2019).

DESCRIPTION

STRUCTURE AND CHARACTERISTICS

Among the selected articles, 26 (83.88%) did not describe the size and weight of the proteins and 3 (9.68%) cited characteristics and description of the compounds used. 2 articles (6.45%) described only one characteristic or description of the compound.

The multiplicity of factors that involve molecular planning with the application of new structures capable of presenting the desired effects and with adequate bioavailability for their therapeutic, safe and comfortable use requires that there is this detail about relevant information such as: Their size and weight of the compounds studied because it facilitates the understanding of its activity and allows to compare between its groups itself through databases.

PROTEIN COMPOUND

The vast majority of selected articles (96.78%) used Bt proteins to measure anticancer activity and only 1 (3.23%) used a polysaccharide compound. Of these 30, 21 (67.75%) used proteins of the parasporin type, which are predominant in the literature for having a greater anticancer activity, while 8 (25.80%) tested the Cry proteins, widely studied in procedures of insecticidal activity, and showed a positive result for apoptotic induction, while 1 article (3.25%) used Cyt proteins.

Studies that had parasporin proteins as active ingredient showed very promising results against cell lines (HeLa) 14 (45.17%) and (MOLT-4) (29.03%), and, among these, 2 (6.45%) studies simultaneously tested these two strains, which were the most cited cell groups among the articles found, and showed a good cytocidal response. Of the articles that work with the Cry protein, there was no predominance of cell lineage. Its cytocidal mechanism of action occurs by binding to receptors, and the transmembrane proteins of cancer cells inducing apoptosis. The Cyt protein has been shown to have amino acid substitutions that increase its cytotoxic activity against lung cancer cells through membrane adhesion that signals and acts as a receptor for anticancer drugs (Nair *et al*, 2018).

The properties described by the parasporin proteins are more complete. Its cytotoxic activity has a greater potential when associated with cells reaching active membrane agents affecting membrane integrity and functions, thus producing a rapid initial loss of high and low molecular weight metabolites from the cell's metabolic pool. The Cry and Cyt proteins, on the other hand, have a wide range of information regarding their activity in the insecticide field, being lethal to several species of insects and pests, and in the last 10 years only 5 works were published that had these proteins as an active principle for anticancer activity (Namba *et al.,* 2003) (Jung *et al.,* 2007) (Nair *et al.,* 2015) (Nair *et al.,* 2018) (Souissi *et al.,* 2019).

While 11 works explored parasporins for this purpose, and only 1 tested it with polysaccharides, which also showed to be a promising compound against cancer cells.

POLYSACCHARIDE COMPOUND

The only non-protein compound found was a polysaccharide. The purified polysaccharide is formed by: Fructose, galactose and glucose.

Regarding its anticancer properties, it showed prominent inhibition for lung cancer cell lines (A549) and liver cancer cells (HEp2) when compared to healthy cells (Vero). It also showed good free radical scavenging activity with reducing capacity, and high potential to act as an adjuvant in the production of oncological drugs (Ramamoorthy *et al,* 2018).

SEQUENCING OF GENES AND PROTEINS

Of the selected articles, 16 (48.39%) carried out sequencing of the genes referring to the compounds studied. 15 articles (51.61%) did not perform any sequencing. Among the articles that performed protein sequencing, the predominance of sequences from 24 kDa to 84 kDa was observed. 11 sequenced parasporin proteins, while 4 articles sequenced Cry proteins and only 1 sequenced a Cyt protein. (Table 02).

MECHANISM OF ANTIPROLIFERATIVE OR ANTINEOPLASTIC ACTION OF THE COMPOUNDS

In the vast majority of selected articles (96.78%), the active principle caused death by inducing cell apoptosis and only 1 article (3.25%) did not present cytotoxic effects to the cell lines tested.

The articles that showed significant results for the reduction of cancer cells, show variation in their form of action, with 22.6% being applied to a set with oncological drugs, these proved to be efficient acting in a synergistic way, facilitating the entry of therapeutic drugs to inside the cells. Already 77.4% have the induction of the apoptosis process as a mechanism, a natural preventive measure applied against cancer cells, thus causing their death, therefore, allowing the elimination of damaged or highly mutated cells, also preventing the emergence of a pathological state (inflammation) that can threaten tissues or even the entire body, further studies must be carried out to investigate the consequences in normal cells and tissues.

Parasporins

The mechanism of potentiation of its lethal activity is still unclear, but the interaction between the 25 kDa protein parasporin and cell membrane lipids may play an important role. Cytotoxic potentiation of anticancer drugs by some other compounds may be an important means of overcoming drug resistance of cancer cells (Yumi Youkoyama, 1988). The compounds with the best cytotoxic effect.

Different types of morphological changes in size and staining of cells tested with parasporins were also observed, when compared to control cells (treated only with solubilization solution), which can be observed under a phase contrast microscope, however, their selectivity mechanism must be most studied (Aberkane *et al*, 2020).

Cry

In the case of insecticidal and anticancer Cry toxins, they show that after the Nterminal cleavage alone is enough to activate Cry41Aa. This was demonstrated by the use of FLP40 which was cleaved only after the amino acid was activated against HepG2 cells. It was also only shown to be toxic to cancer cells after being proteolytically activated in the N-terminal region, resulting in the production of an active form consisting of polypeptides (Souissi *et al*, 2019).

Presumably, the crystal structure helps its protein cargo withstand the harsh, acidic environment of the gut. Given these properties, a possible application of Cry fusion protein crystals could be antigen delivery. Future studies will include elucidating the mechanism of uptake of Cry fusion protein crystals and exploring and developing new

uses for this novel protein delivery platform (Nair et al., 2015).

Cyt

From previous research, it is known that cytotoxicity by Bt is attributed both to anticancer proteins called Parasporins and to cytolytic proteins called Cyt. In the study by Nair et al (2018), it can be shown for the first time a new Cyt1A protein that has amino acid substitutions that increase its cytotoxic activity against lung cancer cells inducing the apoptotic process.

***IN VITRO* / *IN VIVO* TESTS**

Of the selected articles, 28 (90.33%) performed only *in vitro tests* and 3 (9.67%) performed *in vivo/in vitro tests.*

Of the 29 that tested *in vitro*, 20 were (64.5%) parasporin proteins, 6 (19.6%) were Cry proteins, 1 (3.23%) were polysaccharide compounds, and 1 (3.23%) were Cyt proteins. Of the studies that evaluated the activities simultaneously *in vitro/in vivo*, 2 (6.45%) tested Cry compounds, and 1 (3.23%) tested parasporin.

Parasporin-1 (81 kDa) was shown to be only toxic to cancer cells (HepG2, MCF7, KLE, Hec-1A, MDA-MB231 and PC-3) after being proteolytically activated in the Nterminal region, binding to receptors membrane causing pore formation and cell death by apoptosis (Souissi *et al*, 2019).

The results by Gonzalez *et al.* (2011), demonstrates that cry proteins, in the reduction of HepGpt2 and Hepa1-6 cells, have an action regardless of whether they were treated with trypsin or not, the protein binding with those cleaved by trypsin effectively decreased the viability of all cell lines tested.

The high uptake of proteins in cells suggests that the cry protein is an innovative tool to assist in the *in vivo delivery* of therapeutic proteins to animals and later humans diagnosed with cancer. The *in vitro* and *in vivo data* presented in the study clearly reveal the potent tumor inhibitory activity of the Cry protein. It also provides one of the most efficient (oral) routes to induce the immune response. The long lining of the mucosa (one of the largest areas of the body) exposes immune cells to foreign antigens, while secretory antibodies already present in the mucosa lead to rapid activation of known agents. One of the biggest challenges for drug delivery (Chubicka *et al*, 2018).

The effect of the purified anti-tumor parasporin protein in Ascites Sarcoma in the *in vivo tests in* rats provided an increase in the survival of the treated animals with 36 alive at the end of the experiment that used 48 animals, it resulted in the complete regression of the tumor. In comparison to the control model case that had the death of the 12 rats in the group. And with regard to the results observed *in vitro*, there was a decrease in the multiplication of cells in addition to helping the viability of anticancer drugs, allowing them to enter the cells and activate apoptosis, allowing the regression of the

Ascites Sarcoma tumor, since its mode of action is mainly in the cell membrane of tumor cells, binding to intramembrane proteins causing a loss of integrity and affecting membrane functions, thus producing a rapid initial loss of high and low molecular weight metabolites, causing ruptures, resembling the effect observed in the digestive system of the insect (Prasad and Shethna, 1976).

According to Nair *et al.* (2015), Cry proteins were tested in mutated cells of mice, which showed the ability to stimulate apoptosis by at least 10% more when compared to control cells. These crystals have a high potential to induce apoptosis, they are. Being explored as a tool to aid in the *in vivo delivery* of therapeutic proteins

to humans and animals, the crystals were studied and tested with therapy in C57BL albino mice. (Nair *et al*, 2015)

Since the parasporin toxin leads to the formation of pores, its contact causes specific cellular characteristics, such as swelling and cell rupture. Researches describe cell death with the reduction of size and structures that evaluated different types of exposed cells and found cytotoxicity through the activation of apoptosis (Melo and Kitada, 2020).

All the articles used in the production of the work cite the effect of toxicity as a factor directly linked to apoptotic induction by cellular rupture arising from the administration of crystals and polysaccharides, with largely positive results, as 30 of the 31 authors cite an increase in cell death in more than 20% when the treatment is associated or directly linked to compounds derived from Bt.

CYTOTOXIC EFFECT

Of the selected works, 30 (96.77%) showed significant anticancer activity and only 1 (3.23%) showed no cytotoxic effects against the cells tested.

However, (Yamashita *et al*, 2000) reports that, although the actual toxic portion is still unknown in strain 89-T-26-17, it is clear that the leukemic cell killing activities found in the two *B. thuringiensis isolates* are due to different toxins. Parasporins showed a preferential cytotoxicity effect for leukemic T cells. The occurrence of parasporal proteins with cytocidal activity against human cancer cells in three non-insecticidal isolates of *B. thuringiensis* kills leukemic T cells and HeLab cells, but not normal T cells and the cell receptor mutated cells are not yet described (Yamashita *et al. al.*, 2000) According to Lee *et al.* (2000) Cellular analysis revealed no evidence of apoptotic activity, including apoptotic body and DNA fragmentation in susceptible MOLT-4 cells. These results strongly support the hypothesis that protein-induced cytotoxic effects are closely related to necrosis. To understand the mechanism of cancer cell-specific cytotoxicity in these proteins, studies on the subject must continue.

The parasporin protein present in Bti, cytotoxic to tumor cells in mouse culture, can also potentiate the cytotoxic effects of some antitumor agents *in vitro*, for example, bleomycin. When tested with MOLT-4, HeLa and normal T cells, it exhibited indiscriminate toxicity against all three cell types (Yamashita et al., 2000). HeLa cells may have specific molecular receptor sites with which Bt isolates effectively react to produce a strong toxic response. There have been reports of parasporal Bt inclusions with non-hemolytic activity due to selective anticancer cytopathic activity. This led to the discovery of Cry31Aa, Cry46Aa, Cry41Aa and Cry45Aa (Mizuki et al, 2000; Ito et al, 2004; Yamashita et al, 2005).

Recently (Aberkane *et al*, 2020) reported that cytotoxicity tests against A549 cancer cells revealed a weak cytotoxic effect (IC50 > 10), even in normal Vero cells. The selective action against cell lines is probably due to the difference in membrane receptors that are specific for each cell line. This suggests that specific receptors are involved in toxicity. The result of the cytopathic effect confirmed that the cytocidal mode of action of parasporin-1 (BDzG) is based on membrane receptors, while the cytocidal activity of BDzB toxin is non-specific as it acts on both healthy and cancerous cells.

CANCER CELL LINES

The processed proteins and polysaccharide exhibited high cytotoxic activity against all cancer cells tested in 30 of the 31 selected articles. There was a wide diversity of cells tested with a high range of tissues of provenance.

The strains tested: (MOLT-4), (HeLa), Asciste sarcoma, murine leukemia cells (L1210), (Jurkat HL-60), (OVCAR-3 HTB-161), (KLE CRL-1622), (HC), (PC-3 CRL-

1435), (HepG2), (Sawano), (TCS), human uterine smooth muscle cells (UtSMC), (MRC5 A549), (A549), (CACO-2), (UtSMC, HC), normal lung cells (MRC-5), (Vero), monkey kidney cells (COCS-7), mouse embryos (NIH3T3-3), (T cell), (CEM-SS), (25CCRFSB), (MCF-7) and macrophage cells (RAW264).

The pattern of lineages with the highest repetition was that of cells (MOLT-4), was present in 11 articles and in all of them showed a potentiated effect of up to 30% when associated with other methods of treatment for cancer cells. Followed by cells (HeLa), which was cited in 14 articles and had an effect of more than 35% toxicity. The other lineages were present in a lower form than the citation number when compared to the previously mentioned lineages.

Tested cell lines: Most of the selected articles, 23 (74, 20%), tested only on human cell lines. Another 3 (9.68%) tested on rodents, humans and primates, 2 (6.45%) were tested on rodent strains only, 2 (6, 45%) tested on humans and monkeys simultaneously 1 (3.25%) did not perform tests.

Control cells: Only 12 (38, 70%) work did not mention control cells for test validation. 12 (38.70%) used T cell under normal conditions, 2 (6.45%) used vero cells, 1 (3.25%) used in addition to T cell, HC, MRC-5 and UtSMC, while 1 (3.25%) used normal liver HC cell, 1 (3, 25%) used Bt M15, 1 (3.25%) and 1 (3.25%) used MCF.

CONCLUSION

This work of systematic review approaches the main compounds produced by *Bacillus thuringiensis* isolated that present activities of inducing cell death in cancer cells, comparing their mechanism of action and toxic effect. A predominance of proteins was observed through the analysis, while only one of the articles cited the use of polysaccharides. Among the protein compounds studied, the best studied parasporins stand out, followed by the Cry and Cyt proteins. The results obtained in the studies show that some of the compounds are highly selective for certain cancer cell lines. However, some studies still show proteins with cytocidal activity in healthy cells. These results demonstrate the potential pharmaceutical use of *Bacillus thuringiensis strains*, but more studies are needed to confirm and better understand the mechanism of action and its cytotoxic activity, in addition to more studies that include *in vivo tests*. However, compounds from *Bacillus thuringiensis* already present themselves as a promising alternative in the medical field for the treatment of cancer.

DECLARATION OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that may have influenced the work reported in this article.

THANKS

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Year	Authors	Titles	cancer cell lineage	Methodology	Mechanism of action	Structure and characteristics	Compound	Subspecies	sequencing	growing conditions	Purification	Species tested	control cells	Toxicity in healthy cells	COUNTRY	LINEAGE OF CELLS
1976	Prasad, S. S.S.V. et al	Mode of action of a purified antitumor protein from the proteinaceous crystal of Bacillus thuringiensis subsp. thuringiensis on Yoshida ascites sarcoma cells	Ascites Sarcoma Cells	Action is mainly on the cell membrane of tumor cells, exact site of action of this purified protein in the tumor cell membrane.	Dye exclusion method to analyze the protein binding ability	X	Protein	Bacillus thuringiensis	X	X	Lyophilized Phosphate Buffer cold Krebs at pH 7.4	In vitro and in vivo	X	Significant effects on the tested strain	India	rodents
1988	Youkoyama, Y et al.	Potentiation of the cytotoxic activity of anti-cancer drugs against cultured L1210 cells by Bacillus thuringiensis subsp. israelensis toxin	murine leukemic cells L1210	Cytotoxicity potentiator associated with anti-cancer drugs (Bleomycin; Tegafur; Vincristine; 5-Fluorouracil; Vinblastine; Methotrexate; Doxorubicin; Thio-TEPA; Neocarzineostantin; UNCA; Mintomycin C.)	To test the protein binding capacity of 25 kDa to the lipids that make up the membrane allowing the entry of drugs	X	Proteins, for porin 25 kilodaltons (kDa)	Bacillus thuringiensis Israelensis ONR-60A	X	Cells were inoculated into 5 ml of CSL medium (2% corn steep liquor and 1% glucose in H20; the pH was adjusted to 7.0 with aqueous NH4OH) stirring at 30° for 6 to 12 h until the OD570 of the cell suspension becomes 3 to 4. One milliliter of cell suspension obtained was added to 50 ml of CSL medium in a 150 ml flask and aerated by shaking at 30 °C for 90 h.	Cellulose chromatography	In vitro	X	Significant effects on the tested strain	Japan	human
1999	Mizuki, Eiichi et al	Unique activity associated with non-insecticidal Bacillus thuringiensis parasporal inclusions: In vitro cell-killing action on human cancer cells	Human leukemia T cells (MOLT-4)	Adhesion of proteins to the membrane	CPE and MTT	0.9 to 1.2 μm	Parasporal proteins (84-HS-1-11, 89-T-26-17 and 90-F-45-14)	Bacillus thuringiensis Serovar israelensis, kyushuensis e kurstaki	X	To prepare sporulating cultures, strains were grown on nutrient agar, pH 7.6, at 28° C for 4 days. Nutrient agar consisted of meat extract (10 g), polypeptone (10 g), NaCl (2 g), agar (20 g) and distilled water (1000 ml).	X	In vitro	Cell t normal	Significant effects on the tested strain	Japan	Humans
2000	Lee, Dae Weon et al	Noninsecticidal parasporal proteins of a Bacillus thuringiensis serovar shandongiensis isolate exhibit a preferential cytotoxicity against human leukemic T	MOLT-4, leukemic T cell; HeLa, cervical cancer cells;	cell necrosis	X	6 μg	89T-34-22 proteins	Serovar israelensis	160 (MNQKKYHNDEYQVND) 16 (MYTEIKDVIELFSTYAAK) 34 (AIINLLNELAIVAK) 32 (AIINLLRELEIY	X	Discontinuous centrifugation in a gradient of sucrose density	In vitro	Cell t normais	effects significant the strains tested	Japan	humans

		cells														
2000	Yamashita, S. et al	Characterization of the anticancer-cell parasporal proteins of a Bacillus thuringiensis isolate	MOLT-4 (cell human leukemic T) and HeLa (human cervical cancer cell)	Adhesion of the proteins to the membrane of the tested cells	CPE And MTT	X	parasporal proteins (180, 150, 120, 100, and 88 kDa)	Bacillus thuringiensis Isolado 89-T-26-17	120 (MNYNVTKAR EAVQAL) 100 (MNQNVTKAR EAVQAL MIR R MNQNYNN) 80 (MNQNYNN C)	These strains were grown on nutrient agar, pH 7.6, at 28 °C for 4 days. the nutrient agar consisted of meat extract	biphasic separation of Goodman et al. (1967)	In vitro	Cell t normais	effects significant the strains tested	Japan	humans
2001	Lee, Dae Weon et al	A 28 kDa protein of the Bacillus thuringiensis serovar shandongiensis isolate 89-T-34-22 induces a human leukemic cell-specific cytotoxicity	leukemic cells MOLT-4 and HeLa	Signaling of membrane adhesion receptors	CPE and MTT	X	parasporal proteins	Bacillus thuringiensis serovar shandongiensis	AIINLLRE-LEIYGMQ (nterminal)	It was cultivated at 28°C in nutrient agar (pH 7.6), composed of beef extract	X	In vitro	Cell t normais	effects significant the strains tested	Japan	humans
2003	Namba, Akitoshi et al	The cytotoxicity of Bacillus thuringiensis subsp. coreanensis A1519 strain against the human leukemic T cell	MOLT-4	induce cell death apoptotic (through binding to membrane)	MTT	X	Cry A1519	Bacillus thuringiensis subsp. coreanensis A1519	X	X	ultracentralização através de um gradiente gradual de	In vitro	Cell t normais	Significant effects of the tested strains	Japan	humans
2004	Okumura, S. et al	Bacillus thuringiensis serovar shandongiensis strain 89-T-34-22 produces multiple cytotoxic proteins with similar molecular masses against human cancer cells	MOLT-4	INDUCTION TO APOPTOSIS	Anion exchange chromatography, determination of proteins, SDS-PAGE and immunoblot	27–28 ug	89-T34-22 proteins	serovar shandongiensis	QSTTDVIREY (N terminal)	It was grown at 28 °C on nutrient agar (pH 7Æ6), composed of meat extract (10 g), polypeptone (10 g), NaCl (2 g), agar (15 g) and distilled water (1000 ml).	X	IN vitro	x	effects significant the strains tested	Japan	humans

Year	authors	Titles	cancer cell lineage	Methodology	Mechanism of action	Structure and characteristics	Compound	Subespécie	sequencing	growing conditions	Purification	Species tested	control cells	Toxicity in healthy cells	COUNTRY	LINEAGE OF CELLS
2004	Ito, Akio et al	The Bacillus thuringiensis crystal protein with selective cytocidal action to human cells	MOLT-4 Jurkat HL-60 T cell HC HepG2 HeLa Sawano TCS UtSMC MRC-5 A549 CACO-2 Leukemic	cell lysis	MTT	2-8 kb	Proteinas parasporais A1547	Bacillus thuringiensis serovar dakota	DVIREYLMF	The bacteria were cultivated in a nutrient medium containing 0.1% beef extract, 0.1% and 0.2% NaCl, pH 7.6, at 27°C for 8 days	biphasic separation	in vitro	Cell t normais	effects significant the strains tested	Japan	humans
2005	Yamashita, Satoko et al	Typical three-domain cry proteins of Bacillus thuringiensis strain A1462 exhibit cytocidal activity on limited human cancer cells	MOLT-4, HL60, HeLa, TCS, Sawano, HepG2, A549, CACO-2, T cell Normal, UtSMC, HC, MRC-5,	cell lysis	MTT and LDH	X	64KDA	Bacillus thuringiensisA1462	50-TCCGGTAGATAT-TACGCTGAATC-30, 50-TCACAACATTTTCTT-	X	biphasic separation of Goodman et al. (19)	In vitro	Cell t normais	No toxic effect	Japan	humans
2006	Kitada, Sakae et al	Cytocidal actions of parasporin-2, an anti-tumor crystal toxin from Bacillus thuringiensis	colon cancer cells, MOLT-4, Jurkat, HL-60, T ceel, HC, HepG2, HeLa, Sawano, TCS, UtSMC, MRC-5, A549, CACO-2.	apoptosis induction	MTT and LD50	X	parasporina-2	Bacillus thuringiensis A1547	C-terminal	X	Yes	In vitro	x	presents significant effects to all lineages	Japan	Rodents, Humans, and Apes
2007	Jung, Y. C. et al	Isolation and characterization of a novel Bacillus thuringiensis strain expressing a novel crystal protein with cytocidal activity against human cancer cells	HeLa, TCS, Sawano, UtSMC, HL60, MOLT-4, Jurkat, HepG2, T cell, HC, A549, MRC5, CACO-2, Vero, COS-7, NIH3T3.	apoptosis	MTT and SDS page	X	Cry31	Bacillus thuringiensis serovar kurstaki HD-1 e serovar israelensis HD500	5¢-GARCARAARTAYCCNGAY-3¢. This	Cultivated in T3 broth for 5 days at 30°C on a rotary shaker	Yes	In vitro	X	presents significant effects to all lineages	Japan	Rodents, Humans, and Apes
2008	Nadarajah, Vishna Devi et al	Selective cytotoxic activity against leukemic cell lines from mosquitocidal Bacillus thuringiensis parasporal inclusions	CEM-SS e HeLa.	apoptosis	SDS page and BLAST	X	Proteina parasporais	Bacillus thuringiensis subs israelensis subsp morrisoni	X	These isolates were cultured in nutrient agar, pH 7 and 30°C until sporulation was completed. (approximately 48-72 hours)	Yes	In vitro	Cell t normais	presents significant effects to all lineages	X	human

2008	Okumura, Shiro et al	Identification and characterization of a novel cytotoxic protein, parasporin-4, produced by Bacillus thuringiensis A1470 strain	MOLT-4, U-937 DE-4, HL60, Jurkat, K562, HeLa, TCS, Sawano, HepG2, A549, CACO-2, Vero,COS-7, PC12, CHO, NIH 3T3.	Cell membrane binding biosensor	SDSPAGE and exchange chromatography ionic	X	Parasporinas	Bacillus thuringiensiss A1470	IINLANELA (N-terminal)	X	acid solution	In vitro	CELL T, HC, MRC-5, UtSMC	has effects significant in all cancerous languages	Japan	humans and apes
2008	Uemori, Akiko et al	Parasporin-1Ab, a novel Bacillus thuringiensis cytotoxin preferentially active on human cancer cells in vitro	sHeLa, UtSMC	Induction of cytopathy	SDS page and MTT	X	Parasporinas	Bacillus thuringiensis B0195	p1B0195 gene (AB250922)	X	X	In vitro	X	Present activity cytotoxic in cancer cells	Japan	human

Year	Authors	Titulos	cancer cell lineage	Methodology	Mechanism of action	Structure and characteristics	Compound	subspecies	sequencing	Growing conditions	Purification	Species tested	control cells	Toxicity in healthy cells	COUNTRYS	LINEAGE OF CELLS
2010	Nagamatsu, Yasunori et al	Three cry toxins in two types from bacillus thuringiensis strain M019 preferentially kill human hepatocyte cancer and uterus cervix cancer cells	HepG2, CACO-2, HeLa,	Induction of cytopathy	SDS page, Ion exchange chromatography	X	Parasporinas	Bacillus thuringiensis strain M019	GAATGAAAGT CATAACAATA TTTTGAA-TATGTT	Bacteria were cultured at 30 °C for 4 d on 2% agar plates containing 1% polypeptone, 1% meat extract and 0.4% NaCl medium (pH 8.5) supplemented with 4:5? 10? 4M MnSO4.1)	X	In vitro	Normal liver HC cell	present activity cytotoxic in cancer cells	Japan	Humans
2010	Wong, Rebecca S.Y. et al	Characterisation of the binding properties of bacillus thuringiensis 18 toxin on leukaemic cells	CEM-SS, CCRFSB, MCF-7	Binding to cell membrane receptor inducing apoptosis	SDS page, MTT, Microscopy with focal	X	bt 18	Bacillus thuringiensis	X	X	FPLC connected to AKTA System	In vitro	Ceel t normais	present activity cytotoxic in cancer cells	X	Humans
2011	Okumura, Shiro et al	Mode of action of parasporin-4, a cytocidal protein from	CACO-2, HeLa, MOLT-4, and K562	apoptosis	contrast microscopy phase, CYTOMETRY FLOW	X	parasporin-4	A1470	ECTGLAWEWW R C terminal	X	X	In vitro	X	present activity cytotoxic in cancer cells	Japan	Humans

		Bacillus thuringiensis														
2011	Gonzalez, Eric et al	Parasporins from a Caribbean Island: Evidence for a globally dispersed Bacillus thuringiensis strain	HepG2, Hep3B, Hepa1-6, NIH3T3, HeLa, Caco-2 e HT-29	apoptosis	PCR	X	paraporin	Bacillus thuringiensis Strain	94F1/94R1	Bacteria were recovered from storage at -80°C, grown on agar YT supplemented with CCY salts to promote sporulation and examined for purity using a stain and light microscopy	yes	In vitro	Bt strain, M15	Present activity Cytotoxic in cancer cells	X	
2012	Chan, Ko K.Keong et al	Bacillus thuringiensis parasporal proteins induce cell-cycle arrest and caspase-dependant apoptotic cell death in leukemic cells	CCRF-SB, CCRF-HSB-2, e CEM-SS	apoptosis	Electron microscopy, Western blot	X	Bt 18 parasporal	Bacillus thuringiensis	X	X	liquid chromatography	In vitro	Cel t normais	Present activity Cytotoxic in cancer cells	X	Humans
2013	Kuroda, Shouta et al	Parasporin 1Ac2, a novel cytotoxic crystal protein isolated from Bacillus thuringiensis B0462 strain	HeLa, MOLT-4	apoptosis	MTT, LDH, SDS page	X	Parasporin 1Ac2	Bacillus thuringiensis B0462 Strain	XEPPST(Nterminal)	X	sucrose solutions	In vitro	X	No toxic effect	Japan	Humans
2015	Nair, Manoj S. et al	Cry protein crystals: A novel platform for protein delivery	Células de macrófagos (RAW264.7)	X	Microcopiaeletronica, SDS page	X	Cry3Aa	Bacillus thuringiensis 407	X	X	CM-cellulose	In vitro e in vivo	GFP protein	X	X	Rodents

2015	Brasseur, Kevin et al	Parasporin-2 from a new bacillus thuringiensis 4r2 strain induces caspases activation and apoptosis in human cancer cells	HepG2 (HB-8065), PC-3 (CRL-1435), Caco-2 (HTB-37), HeLa (CCL-2), Hec-1A (HTB-112), KLE (CRL-1622), MDA-MB231 (HTB-26), MCF-7 (HTB-22), OVCAR-3 (HTB-161), SKOV-3 (HTB-77),	apoptosis	MTT, SDS page, Western blot	X	Parasporinas	B. thuringiensis sorovar dakota cepa 4R2	GGGGGCTTCAAG TAGATAATCAATT AGTGGAAACATT TCATTTCAAT	Bacterial cells were cultured at 30°C on Sigma-Aldrich nutrient agar (St-Louis, MO, USA) at pH 7.1.	yes	In vitro	MCF-10A (CRL10317),IOSE-144	were highly cytotoxic to cells HepG2, MCF-7, KLE, Hec-1A, MDA-MB231 and PC-3,	X	Humans

Year	Authors	Titles	cancer cell lineage	Methodology	Mechanism of action	Structure and characteristics	Compound	subspecies	sequencing	Growing conditions	Purification	Species tested	Control cells	Toxicity in healthy cells	COUNTRY	LINEAGE OF CELLS
2018	Nair, Kavita et al	The replacement of five consecutive amino acids in the cyt1a protein of bacillus thuringiensis enhances its cytotoxic activity against lung epithelial cancer cells	NCIH1975 [H1975, H1975] (ATCC® CRL5908 ™)	apoptosis	MTT and PCR	X	cyt1A	Bt subsp. israelensis QBT229	accession number: MG708177	They were cultivated in Luria Bertini (LB) medium rich in nutrients and low in sodium at 30 ◦C	High ph solution	In vitro	X	present activity cytotoxic in cancer cells	X	Humans
2018	Moazamian, Elham et al	Anti-cancer Parasporin Toxins of New Bacillus thuringiensis Against Human Colon (HCT-116) and Blood (CCRF-CEM) Cancer Cell Lines	HCT-116 e CCRF-CEM	apoptosis	MTT, SDS page, Microscopy	X	Parasporinas	Bacillus thuringiensis	ATC AAG AAT TTT CCG ATA ATC	A: 0.25 g of sample was grown in Nutrient Broth with 0.25 mol of sodium acetate at 37 °C for 24 h	X	In vitro	Normal t cells	present activity cytotoxic in cancer cells	Irã	Humans

2018	Ramamoorthy, Sathishkumar et al	Structural characterization and anticancer activity of extracellular polysaccharides from ascidian symbiotic bacterium Bacillus thuringiensis	A549, células HEp-2,	Apoptosis	HPLC, espectrofotometro, radicais DPPH, MTT, SEM	X	polysaccharides extracellular (EPS)	Bacillus thuringiensis RSK CAS4	X	Serially diluted homogenates were streaked onto Zobell marine agar (Himedia, Mumbai) and incubated at 37 °C, pH 8 for 72 h. O	Acid hydrolysis	In vitro	true cells	present activity cytotoxic in cancer cells	X	Humans
2018	Chubicka, Thomas et al	A parasporin from Bacillus thuringiensis native to Peninsular India induces apoptosis in cancer cells through intrinsic pathway	L929, Jurkat, HeLa, MCF7, Kato III, HT29, Caco2, Hep2, AGS.	Apoptosis	SDS page, MTT, LDH, Microscopia de constrate	X	parasporins	Bacillus thuringiensis	4 PS4F 50TGGTGTGCTG CAAGGGGATA3	10 mL of LB broth (Luria-Bertani) and incubated with 0.25 M sodium acetate for 4 h at 30 °C	Biphasic aqueous	in vitro e in vivo	true cells	Present activity Cytotoxic in cancer cells	X	Humans, Rodents and Apes
2019	Souissi, Wided et al	Differential proteolytic activation of the Bacillus thuringiensis Cry41Aa parasporin modulates its anticancer effect	HepG2, MCF-7, KLE, Hec-1A, MDA-MB231 e PC-3	Apoptosis	Western blot, SDS page	X	Cry41Aa	Bacillus thuringiensis	DVRDA N terminal	Bt were cultured on LB agar plates containing 5 mg/ml chloramphenicol for 3 days at 30°C.	ÄKTA PurifierFPLC	In vitro	X	Present activity Cytotoxic in cancer cells	X	Humans
2019	Aboul-Soud, Mourad A.M. et al	Specific cytotoxic effects of parasporal crystal proteins isolated from native saudi arabian bacillus thuringiensis strains against cervical cancer cells	HT-29, HeLa	Apoptosis	SDS page, Microscopia de contrate e varredura , MTT, RT-qPCR)	X	Parasporinas	Bacillus thuringiensis	GAGTTTAATCGA CAAGTAGATAAT TT	Medium supplemented with Nutrient Agar (NAS).	High ph solution	In vitro	X	Present activity Cytotoxic in cancer cells	Arabia saudita	Humans
2020	Aberkane, Lila et al	In Vitro Cytotoxicity of Parasporins from Native Algerian Bacillus thuringiensis Strains Against Laryngeal and Alveolar Cancers	HEp2, A549, Vero	Apoptosis	SDS page, MTT e microscopia de flurorecencia	X	Parasporinas	Bacillus thuringiensis	5′-GGG CAC ATA AAT AAA ATT ATTG-3′)	Cultivation conditions were performed as described by Djenane et al 4	two-phase separation	In vitro	Cell t normais	present activity cytotoxic in cancer cells	Argelia	humans and apes

2020	Melo, André L.A. et al	Selection of the Bacillus thuringiensis Berliner strain to produce a parasporin with cytotoxic activity against MCF-7 breast cancer cells	MCF-7	Apoptosis	MTT e Espectrofotometro	X	Parasporinas	Bacillus thuringiensis Berliner A14d2	X	heated to 65 °C for 30 min and seeded on nutrient agar. Nutrient agar (pH 7.8) consisted of beef extract (10 g/L), polypeptide (10 g/L), NaCl (2.5 g/L) and agar (20 g/L).	X	In vitro	X	They showed cytotoxic activity	Japan	Humans 24

Authors, Year: Authors responsible for the article and their year of publication. **Cancer Cell Lines:** Type of cancer cell used. **Methodology:** Method of action that compounds developed for anticancer activity in cells. **Structures and characteristics:** Information on weight, size of compounds. **Compound:** The type of material used to act with cytocidal effects (proteins and polysaccharides). **Bt subspecies:** Isolates from the bacterium. **Sequencing:** Method applied to sequence the compounds. **Cultivation conditions:** Information about the fermentation process of Bt. **Purification:** Methodology used to purify. **Model:** Type of application of experiments at the cellular and animal level. **Control cells:** Cells tested standard for evaluation of the real cytotoxic effect. **Toxicity:** The degree of anticancer effect on cancer cells. **Species:** Realization in lines of different species already mentioned above.

Table 02: Summary of the relevant data of the articles selected according to the criteria adopted in the review.

Source: Research data, Authors.

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