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In vitro PROTEOLYTIC ACTIVATION OF CARCINOLYTIC PARASPORAL INCLUSIONS OF *Bacillus thuringiensis ssp. israelensis* BACTERIA

Abstract. Upon proteolytic activation, the parasporal inclusions of *Bacillus thuringiensis* bacteria break down into protein monomers (parasporins), some of which have a selective carcinolytic effect. The purpose of the study was to determine the possibility of proteolytic activation of parasporal inclusions under *in vitro* conditions of the culture medium with tumor cell lines in the presence of various concentrations of protease K. These experimental conditions were created to simulate the tumor nidus with regard to the increased concentration of proteolytic enzymes, which is characteristic of metastatic tumors and cancerous degradomes. The bacterial strain *Bacillus thuringiensis ssp. israelensis* was selected for the study as a producer of carcinolytic parasporal inclusions. The experiments were performed using monolayer tumor cell lines: Hep G2 (human hepatocarcinoma), MIA PaCa2 (human pancreatic carcinoma), RD (human rhabdomyosarcoma). Analysis of the cytotoxic effect was assessed by morphological changes in tumor cell cultures. The paper presents the results of determining optimal concentration of the protease K enzyme in tumor cell culture, at which activation of parasporal inclusions is possible without the effect of the cytotoxic action of the proteolytic enzyme itself. Current data can be used for further *in vivo* studies.

Keywords: cell lines, proteases, parasporal inclusions, bacteria.

Introduction. In 1999, Mizuki et al. first examined protein parasporal inclusions from a total pool of 1744 *B. thuringiensis* strains for cytocidal activity against human leukaemia T cells and hemolytic activity against sheep red blood cells. In general, *B. thuringiensis* strains did not have hemolytic activity (1684 strains), but 42 exhibited *in vitro* cytotoxicity against leukaemia T cells. These non-hemolytic, but toxic against tumor T cells strains, belonged to several H serovars, including *dacota, neoleonensis, shandongiensis, coreanensis* and other unidentified serogroups. The purified parasporal inclusions of the three selected strains designated as 84-HS-1-11, 89-T-26-17, and 90-F-45-14 exhibited no hemolytic activity and no insecticidal activity against dipteran and lepidopteran insects, but were extremely cytocidal against tumor T cells and other human cancer cells, demonstrating different toxicity spectra and varied levels of activity. In addition, the proteins from 84-HS-1-11 and 89-T-26-17 were able to discriminate between tumor and normal T cells, specifically killing leukaemia cells. The researchers concluded that these findings may lead to the use of *B. thuringiensis* protein inclusions for medical purposes (Mizuki E., 1999: 477).

Mizuki et al., while continuing to examine an unusual property to recognize human leukaemia cells, related to parasporal inclusions in *B. thuringiensis*, discovered a protein (named parasporin) responsible for this activity. They have subsequently cloned it (Mizuki E., 2000: 625). Parasporin, encoded by a gene 2169 bp long, was a polypeptide consisting of 723 amino acid residues with a molecular weight of 81 kDa. The parasporin sequence contained 5 conserved motifs commonly found in *B. thuringiensis* Cry proteins, but a low level of homology (<25%) was detected between parasporin and the existing classes of Cry and Cyt proteins. Parasporin exhibited cytocidal activity only when degraded by proteases into smaller molecules of 40 to 60 kDa.

The activity of parasporins as carcinolytic toxins of a protein nature has been confirmed in numerous screening studies resulting in the isolation of the most effective agents (Table 1) (Okumura S., 2013: 1889; Lee DW., 2000: 218; Okumura S., 2005: 6313; Poornima K., 2010: 348; Namba A., 2003: 29; Uemori A., 2005: 122; Yasutake K., 2005: 124; Kitada S., 2009: 121; Akiba T., 2009: 121).

Table 1 – Cytotoxic activity of various parasporins against tumor and normal human cells (Okumura S. et al.)

Cell line	Characteristics of cells	LD50 (мкг/mL)			
		Parasporin-1	Parasporin -2	Parasporin -3	Parasporin -4
MOLT-4	T-cell leukaemia	2,2	0,022	>10	0,472
Jurkat	T-cell leukaemia	>10	0,018	>10	>2
HL-60	T-cell leukaemia	0,32	0,019	1,32	0,725
T cell	Normal T cells	>10	–	>10	>2
HepG2	Hepatocellular carcinoma	3,0	0,019	2,8	1,90
HC	Normal hepatocytes	>10	1,1	>10	>2
HeLa	Cervical cancer	0,12	2,5	>10	>2
Sawano	Uterus cancer	>10	0,0017	>10	0,245
TCS	Cervical cancer	–	7,8	>10	0,719
UtSMC	Normal uterus cells	>10	2,5	>10	>2
Caco-2	Colon cancer	>10	0,013	>10	0,124

Studies on the cytotoxic effect of parasporin-2 have showed that unlike parasporin-1, it increases the permeability of the plasma membranes of tumor cells (Ohba M., 2009: 427; Petit L., 1997: 6480). Cytoplasmic lactate dehydrogenase flows out of the treated HepG2 cells, while extracellular propidium iodide enters the cytoplasm. The initial stage of the cytotoxic effect of parasporin-2 is the specific binding of the toxin to a putative receptor protein, not yet identified, which is located in a lipid raft of the plasma membrane of tumor cells susceptible to this protein. This is followed by the formation of oligomers of parasporin-2 in the plasma membranes, which leads to the pore formation and cell lysis (Petit L., 1997: 6480). Oligomerization occurs in the presence of membrane proteins, a lipid bilayer, and cholesterol. It should be noted that substantial homology exists in amino acid sequences between PS2Aa1 and *Clostridium perfringens* epsilon-toxin, whose cell action mechanism involves the toxin oligomerization in lipid rafts and pores formation in the plasma membrane (Petit L., 1997: 6480).

Abe et al. (Abe Y., 2005: 113), while examining the mechanism of action of parasporin-2, found that the toxin binds to the surface of target cells and increases the permeability of the plasma membrane. Subcellular fractionation and immunoblotting of cells treated with the toxin showed that the toxin is associated with lipid rafts and forms SDS-resistant oligomers. The binding and oligomerization of the toxin was inhibited by treating the cells with phosphatidylinositol-specific phospholipase C. The interaction of parasporin-2 with glycosylphosphatidylinositol proteins was therefore required to form an oligomeric toxin that could penetrate the plasma membrane (Abe Y., 2005: 113). Abe et al. (Abe Y., 2008: 269) examined the mechanism of action of parasporin-2 on the human HepG2 cell line (hepatocarcinoma) and showed that this Cry toxin targets lipid rafts and is assembled into oligomeric complexes in tumor cell membrane. The authors concluded that this protein is a pore-forming toxin that accumulates in lipid rafts of tumor cells. Recently, Bokori-Brown et al. (Bokori-Brown M., 2011: 4589) showed that the ε-toxin produced by *Clostridium perfringens* (the etiological agent of dysentery in newborn lambs, enteritis and enterotoxicity in goats, calves, and foals) forms heptameric pores in the membranes of the target cells in the same way as parasporin-2.

It has been found that Cyt1 protein with a molecular mass of 25 kDa isolated from parasporal inclusions of *Bacillus thuringiensis ssp. israelensis* (Bti) bacteria possesses cytotoxic activity against the L1210 murine leukaemia cell line (Yokoyama Y., 1988: 499); in later studies, this parasporin exhibited cytotoxic activity against human tumor cell lines, including MOLT-4, HeLa, and normal T-lymphocytes (Okumura S., 2004: 89; Okumura S. 2013: 1889). In *in vivo* experiments on a transplantable mouse tumor model, Cyt 1 protein turned out to be inactive on its own, but showed a strong potentiating effect in

combination with the cytostatic anticancer drug Bleomycin against Ehrlich sarcoma, B16 melanoma, and Meth A fibrosarcoma (Yokoyama Y., 1992: 1079).

Previous studies for determining the acute toxicity of the Bti unactivated parasporal inclusions evaluated LD_{50} equal to 1.0 mg per adult mouse when protein is dissolved in PBS and 0.1 mg if dissolved in 50 mM Na_2CO_3 with intravenous administration (Wendy E. 1983: 181). These findings enable further studies on a transplantable mouse tumor model using non-toxic concentrations of parasporal inclusions.

The acute toxicity and genotoxicity of parasporins has been sufficiently studied. The results of toxicity study in mice of one of the parasporins, namely PS4, were recently published (Okumura S., 2014: 2115). The LD_{50} for PS4 was 160 μg/kg in ICR mice after subcutaneous introduction. Although it is less toxic than the vast majority of bacterial toxins (Gill DM., 1982: 86), it would be rated as highly toxic substance according to the toxicity rating system of Gosselin et al. (Gosselin RE, 1987), since the LD_{50} is less than 1.0 mg/kg. Moreover, the concentrations of cations, creatinine, and urea nitrogen in urine and serum indicate that PS4 impaires kidneys function in mice. A histological evaluation of kidneys in PS4-treated mice showed that protein can cause damage to the renal proximal tubule (Okumura S., 2014: 2115). It should also be mentioned that the LD_{50} value of PS2 was found to be 0.42 mg/kg after intra-peritoneal injection in mice (Kyushu Institute of Technology, Japan, not published).

The tumor nidi are characterized by high vascular permeability, which is caused by the immunological process of local inflammation with the formation of pores in vessels having a diameter of up to 2 μm with the normal vascular endothelial pore width of 20 nm (Truskey GA, 2004: 427; Yuan F., 1994: 3352). This circumstance creates an opportunity for penetrating and making a local region of high concentration of crystalline parasporal inclusions having an average size of 0.4-0.7x1.2-1.5 μm (Naoya W., 2005: 988) in tumors after parenteral introduction.

At each stage, including tumor formation, growth, metastasis, and invasion into other tissues, a high concentration of proteases in these nidi is observed, the combination of which determines cancerous degradome, which is usually represented by 5 classes of proteases: serine (trypsin, protease K, etc.), cysteine, aspartic, threonine, and matrix metalloproteinases (Deu E., 2012: 10), thereby representing a natural environment for proteolytic activation of Bti parasporal inclusions.

Materials and Methods. *Bacterial strain and cultivation conditions*. The serotyped *Bacillus thuringiensis ssp. israelensis* (Bti) strain obtained from the collection at the Microbial Depository Center (Yerevan, Armenia), was used in the study. The bacterial strain was grown in MPA culture medium (pH 7) at 30 °C until sporulation was completed (approximately for 48-72 hours). To inactivate vegetative cells and stimulate spore germination, an inoculation loop full of sporulated *B. thuringiensis* bacteria was transferred to 0.5 mL of sterile deionized water, and then heated in a water bath at 75 ºC for 30 min. 0.5 ml aliquots of activated spores were placed in 250 ml of MPB culture medium (pH 7). The culture was incubated for about 24 hours at 30 °C under constant stirring at 250 rpm. The grown culture was stored in a refrigerator at 4 ºC for 4-5 days. By this time, more than 95% of the culture was presented as spores (Okumura S., 2004: 89).

Preparation of a spore-crystal mixture from a B. thuringiensis culture. Crystalline NaCl was added to a sporulated *B. thuringiensis* culture at a concentration of 1M to initiate lysis. The culture was centrifuged at 6,000 x g for 10 minutes at 4 °C, the obtained spore-crystal precipitate was then washed once with 1M NaCl and 2 times with distilled water; the resulting precipitate was further resuspended in an appropriate volume of Tris/KCl buffer (50 mM Tris/HCl, 10 mM KCl, pH 7.5). The spore-crystal mixture was aliquoted and stored at -20 °C until further use (Okumura S., 2004: 89).

Dissolution of protein parasporal inclusions. Parasporal protein inclusions were isolated from cultures by dissolving the spore-crystal mixture in 50 mM Na_2CO_3, 10 mM dithiothreitol (pH 10.5) for one hour at 37 °C under constant stirring at 1400 rpm. Insoluble spores and other artifacts were precipitated by centrifugation at 13,000 x g for 5 minutes. The resulting supernatant contained dissolved parasporal inclusions (Okumura S., 2004: 89).

Tumor cell lines and culture conditions. Tumor cell lines, including Hep G2 (hepatocarcinoma), RD (rhabdomyosarcoma), and MiaPaca-2 (pancreatic carcinoma), were grown in MEM, RPMI, and DMEM media, respectively, with the addition of 10% FBS in the presence of ampicillin (100 μg/mL), at 3 ºC in 5% CO_2. The stabilized cell lines at the 5^{th} passage were used in the experiment (Ohba M., 2009).

Proteolytic activation of parasporal inclusions in tumor cell culture. Activation of parasporal inclusions was carried out in 96-well plates with tumor cell cultures at a concentration of 20,000 cells per well by combined introduction with protease K (Sigma). The enzyme concentration ranged from 100 to 0.75 μg/mL with a 2-fold dilution step. The solvent of parasporal inclusions was used as a negative control, Triton X-100 served as a positive control.

Results and Discussion. During experiments for the determination of the carcinolytic effect of parasporal inclusions isolated from Bti in the presence of protease K, the possibility of activation of parasporal inclusions at non-cytotoxic concentrations of the proteolytic enzyme was revealed [A. Ilin, A. Okassov "The way of cancer cells damage by Bacillus bacteria origin crystal proteins" Patent application number: 2019/0685.1 from 17.09.2019]. Data on optimal concentrations of protease K in tumor cell lines are presented in table 2.

Table 2 – *In vitro* cytotoxic concentrations of protease K in the presence and absence of Bti parasporal inclusions

Cell line	Cytotoxic concentration of protease K in the absence of parasporal inclusions, μg/mL	Cytotoxic concentration of protease K in the presence of parasporal inclusions, μg/mL
Hep G2	6	3
MiaPaca-2	6	1,5
RD	6	1,5

The control smear of the Bti sporulating culture with visible parasporal inclusions in the apical parts of the cell is shown in figure 1.

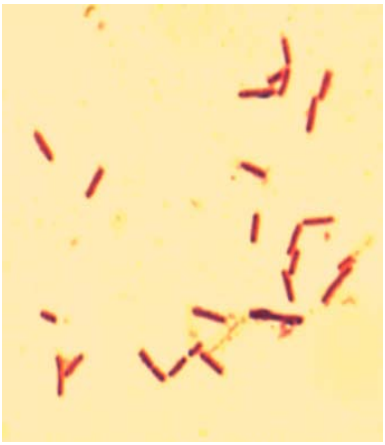


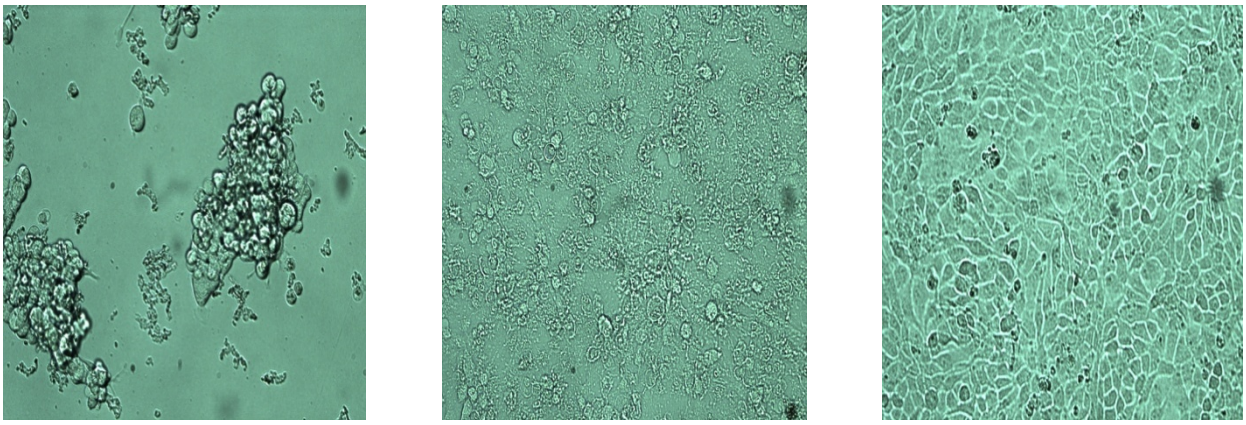
Figure 1 – Sporulating culture of *Bacillus thuringiensis ssp. israelensis*. The Gram staining method (100x)

The presence of dark inclusions in the apical parts of cells indicates the initial stage of sporulation, these inclusions are crystalline Cry proteins (parasporal inclusions), the monomers of which (parasporins) have a carcinolytic effect.

The morphological picture of the cytotoxic effect of protease K and protease K in the presence of parasporal inclusions against Hep G2, MiaPaca-2 and RD cell lines is shown in figures 2, 3 and 4, respectively.

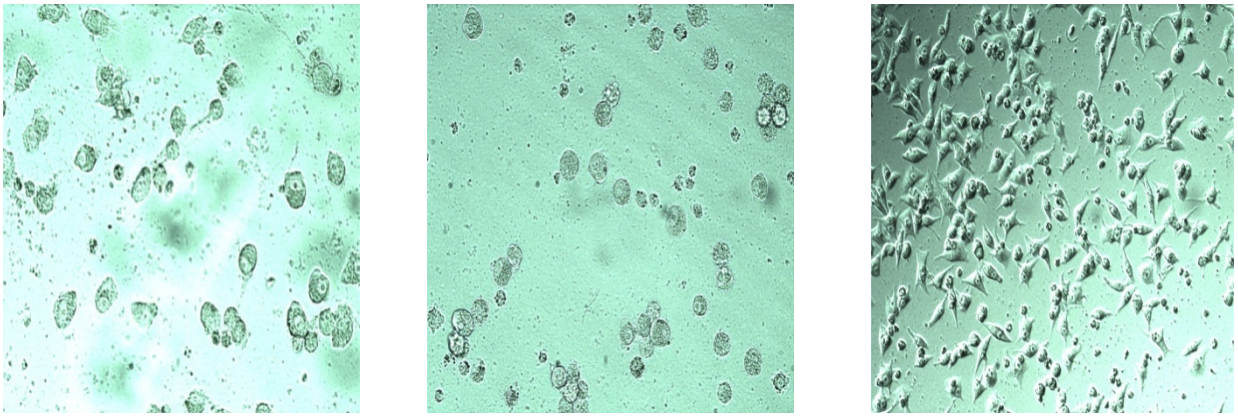
The observed pattern of changes in the morphology of tumor cells when parasporal inclusions operated in combination with protease K is characteristic of parasporins (Kim H., 2000: 16), which indicates the successful activation of parasporal inclusions by protease K. The similarity of changes in cell cultures indicates the universality of the action of activated parasporins irrespective of the cell line.

It can be seen from the above results that morphological changes in cells under the action of parasporal inclusions in the presence of a proteolytic enzyme differs sharply from the pattern of the cytotoxic effect of protease K. The rounding of cells in experimental samples is characteristic of the action of parasporins, which carry out their cytotoxic effect by changing the intracellular osmotic pressure through oligomerization in cell membranes, followed by the formation of transmembrane pores in raft domains (Yuich A. 2017: 71).



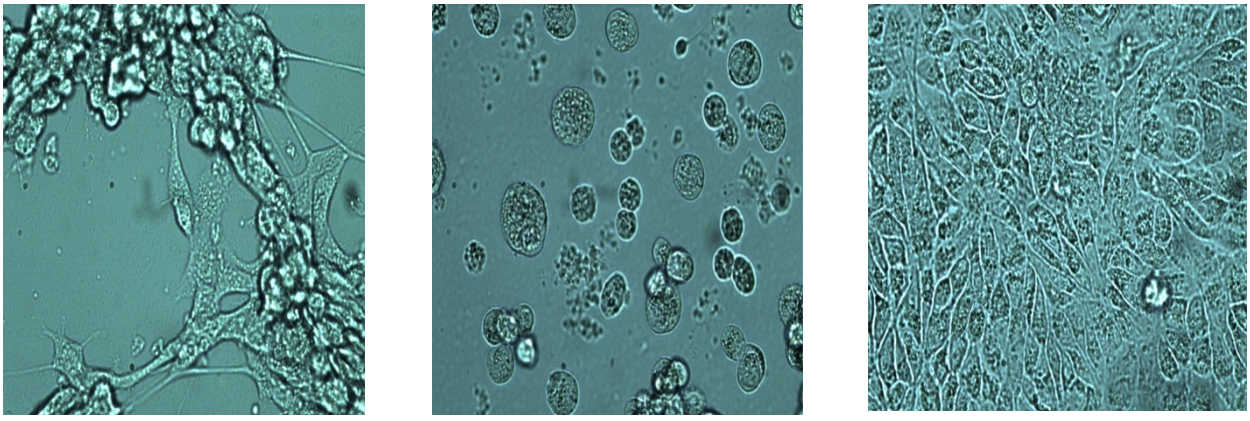
A B C

Figure 2 – Morphological picture of Hep G2 cell lines (40x):
A – cytotoxic effect of protease K (6 μg/mL) against Hep G2 cell line; B – cytotoxic effect of parasporal inclusions in the presence of protease K (3 μg/mL) against Hep G2 cell line; C – control Hep G2 cell line



A B C

Figure 3 – Morphological picture of Mia Paca-2 cell lines (40x):
A – cytotoxic effect of protease K (6 μg/mL) against Mia Paca-2 cell line; B – cytotoxic effect of parasporal inclusions in the presence of protease K (1.5 μg/mL) against Mia Paca-2 cell line; C – normal Mia Paca-2 cell line



A B C

Figure 4 – Morphological picture of RD cell lines (60x): A – cytotoxic effect of protease K (6 μg/mL) against RD cell line;
B – cytotoxic effect of parasporal inclusions in the presence of protease K (1.5 μg/mL) against RD cell line;
C – normal RD cell line

A four-fold decrease in the cytotoxic concentration of protease K when combined with parasporal inclusions to manifest the cytotoxic effect of parasporal inclusions was observed against Mia Paca-2 and RD cell lines, and a two-fold decrease was recorded for Hep G2 cell line, as compared with the cytotoxic concentration of the proteolytic enzyme of 6 μg/mL.

Conclusion. To date we have not found published data on the precise concentrations of each of protease classes in the tumor nidus and similar studies on modeling tumor nidi. But due to one of the reasons for conditionality of the degradation of the tumor stroma and subsequent process of metastasis caused by a high concentration and cytotoxic effect of proteases (Cao H., 2016: 1099), it can be assumed that the protease concentration values are close to those necessary for activation of *in situ* parasporal inclusions.

The resulting data are the basis for further *in vivo* experiments using inactivated Bti parasporal inclusions by intravenous introduction at concentrations below toxic in order to create a local high concentration of activated parasporins in the tumor nidus without distribution of the latter among organs and tissues, thereby reducing the overall toxic effect.

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ПРОТЕОЛИТИКАЛЫҚ *Bacillus thuringiensis ssp. Israelensis* БАКТЕРИЯНЫҢ ПАРАСПОРАЛДЫ БІРІГУЛЕРІН БЕЛСЕНДІРУ *in vitro*

Аннотация. *Bacillus thuringiensis* бактерияларының параспоралды бірігуі, протеолитикалық белсендіру арқасында ақуыз монемерлерыне (параспорины) ыдырайды, жоғарыдағылардің кейбіріне селективті канцеролитикалық әрекет бар. Жұмыстың мақсаты: *in vitro* жағдайында ісік жасушаларының әр түрлі концентрациядағы протеза К қатысуымен, параспоралды бірігуідің протеолитикалық белсенділігін анықтау. Бұл тәжірибелік жағдайлар метастаздық ісіктерге және қатерлі тозу процестеріне тән протеолитикалық ферменттердің шоғырлануының жоғары бөлігінде ісік қалыптасуының фокусын модельдеу үшін жасалған. Зерттеу үшін канцеролитикалық параспоральдық қосындылардың алдын-ала расталған өндірушісі ретінде *Bacillus thuringiensis ssp israelensis,* бактерия штамдары таңдап алынды. Тәжірибе моноқабатты ісік сызығы: Нер G2 (адамның гепатокарциномасы), МАА ПаСа2 (адамның асқазан безінің қатерлі ісігі), RD (адамның рабдомиосаркомасы) ды қолдану арқылы жасалды. Мақалада протеолитикалық ферментердің цитотоксикалық әрекетсіз параспоралды бірігуі арқылы ісік жасушаларындағы протеаз К ферментерінің оңтайлы концентрациясын анықтау нәтижелері көрсетілген. Цитотоксикалық әсерді талдау ісік жасушаларының морфологиялық өзгерістерімен бағаланды. Зерттеу нәтижелері болашақ *in vivo* эксперименттерде қолдану мүмкіндігі бар.

Түйін сөздер: клеткалық қыралар, протеазалар, параспоральдық қосындылар, бактериялар.

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ПРОТЕОЛИТИЧЕСКАЯ АКТИВАЦИЯ КАНЦЕРОЛИТИЧЕСКИХ ПАРАСПОРАЛЬНЫХ ВКЛЮЧЕНИЙ БАКТЕРИЙ *Bacillus thuringiensis ssp. israelensis in vitro*

Аннотация. Параспоральные включения бактерий *Bacillus thuringiensis* при протеолитической активации распадаются на белковые мономеры (параспорины), некоторые из которых имеют избирательное канцеролитическое действие. Целью работы явилось определение возможности протеолитической активации параспоральных включений в *in vitro* условиях культуральной среды с линиями опухолевых клеток в присутствии различных концентраций протеазы К. Данные условия эксперимента создавались для моделирования очага опухолевого образования в части повышенной концентрации протеолитических ферментов, характерной для метастазирующих опухолей и раковых деградомов. Для исследования был выбран продуцент канцеролитических параспоральных включений бактериальный штамм *Bacillus thuringiensis ssp.*

israelensis. Эксперименты проводились с использованием монослойных опухолевых линий: Hep G2 (гепатокарцинома человека), MIA PaCa2 (панкреатическая карцинома человека), RD (рабдомиосаркома человека). Анализ цитотоксического действия оценивался по морфологическим изменениям культур опу-холевых клеток. В статье представлены результаты исследования определения оптимальной концентрации фермента протеазы К в культуре опухолевых клеток, при которой возможна активация параспоральных включений без влияния цитотоксического действия самого протеолитического фермента. Текущие данные могут быть использованы для проведения дальнейших исследований *in vivo.*

Ключевые слова: клеточные линии, протеазы, параспоральные включения, бактерии.

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REFERENCES

Abe Y, Kitada S, Kuge O, Ohba K, Ito A. (2005) Oligomerization of Parasporin-2, a New Crystal Protein from Non-Insecticidal Bacillus thuringiensis, in Lipid Rafts. *In: Côté JC, Otvos IS, Schwartz JL, Vincent C (Eds): 6th Pacific Rim Conference on the Biotechnology of Bacillus thuringiensis and its Environmental Impact, Érudit, Montréal,* pp. 113-116.

Abe Y, Shimada H, Kitada S. (2008) Raft-targeting and oligomerization of Parasporin-2, a bacillus thuringiensis crystal protein with anti-tumour activity. *J Biochem*., vol. 143, pp. 269-275.

Akiba T, Abe Y, Kitada S et al. (2009) Crystal structure of the parasporin-2 Bacillus thuringiensis toxin that recognizes cancer cells. *Journal of Molecular Biology*, vol. 386, pp. 121-133

Bokori-Brown M, Savva CG, Fernandes da Costa SP et al. (2011) Molecular basis of toxicity of Clostridium perfringens epsilon toxin. *FEBS J.*, vol. 278, pp. 4589-4601.

Cao H, Eppinga R, G L Razidlo, E W Krueger, J Chen, L Qiang and M A McNiven (2016) Stromal fibroblasts facilitate cancer cell invasion by a novel invadopodia-independent matrix degradation process. *Oncogene* vol. 35, pp. 1099-1110

Deu E, Martijn V, Matthew B (2012). New approaches for dissecting protease functions to improve probe development and drug discovery. *Nature Structural Mole. Biol.,* vol. 19, pp. 10-16.

Gill DM. (1982) Bacterial toxins: a table of lethal amounts. *Microbiol Rev.,* vol. 46, pp. 86-94.

Gosselin RE, Smith RP, Hodge HC (2000) Clinical toxicology of commercial products (5th Edn). Williams and Wilkins, Baltimore, US, 1987.

Kim H., Yamashita S., Akao T., Saitoh H., Higuchi K., Park Y., Mizuki E., Ohba M. (2000) In vitro cytotoxicity of non-Cyt inclusion proteins of a Bacillus thuringiensis isolate against human cells, including cancer cells. *Journal of Applied Microbiology,* vol.89, pp.16-23

Kitada S, Abe Y, Maeda T & Shimada H. (2009) Parasporin-2 required GPI-anchored proteins fro the efficient cytocidal action to human hepatoma cells. *Toxicology,* vol. 264, pp. 80-88

Lee DW, Akao T, Yamashita S et al. (2000) Noninsecticidal parasporal proteins of a Bacillus thuringiensis serovar shandongiensis isolate exhibit a preferential cytotoxicity against human leukemic T cells. *Biochem Biophys Res Commun*., vol. 272, pp. 218-223.

Mizuki E, Ohba M, Akao T, Yamashita S, Saitoh H, Park YS. (1999) Unique activity associated with non-insecticidal Bacillus thuringiensis parasporal inclusions: in vitro cell-killing action on human cancer cells. *J Appl Microbiol*., vol. 86, pp. 477-486.

Mizuki E, Park YS, Saitoh H et al. (2000) Parasporin, a human leukemic cell-recognizing parasporal protein of Bacillus thuringiensis. *Clin Diagn Lab Immunol*., vol 7, pp. 625-634.

Namba A, Yamagiwa M, Amano H et al. (2003) The cytotoxicity of Bacillus thuringiensis subsp. coreanensis A1519 strain against the human leukemic T cell. *Biochim Biophys Acta*, vol. 1622, pp. 29-35.

Naoya Wasano, Hiroyuki Saitoh, Minoru Maeda, Akira Ohgushi, Eiichi Mizuki, Michio Ohba (2005) Cloning and characterization of a novel gene cry9Ec1 encoding lepidopteran-specific parasporal inclusion protein from a Bacillus thuringiensis serovargalleriae strain. *Canadian Journal of Microbiology*, vol. 51, pp. 988-995.

Ohba M, Mizuki E, Uemori A. (2009) Parasporin, a new anticancer protein group from Bacillus thuringiensis. *Anticancer Res.,* vol 29, pp 427-433.

Okumura S, Akao T, Higuchi K et al. (2004) Bacillus thuringiensis serovar shandongiensis strain 89-T-34-22 produces multiple cytotoxic proteins with similar molecular masses against human cancer cells. *Lett Appl Microbiol*., vol. 39, pp. 89-92.

Okumura S, Ishikawa T, Saitoh H, Akao T, Mizuki E. (2013) Identification of a second cytotoxic protein produced by Bacillus thuringiensis A1470. *Biotechnol Lett*, vol. 35, pp. 1889-1894.

Okumura S, Koga H, Inouye K, Mizuki E. (2014) Toxicity of parasporin-4 and health effects of pro-parasporin-4 diet in mice. *Toxins* (Basel), vol. 6, pp. 2115-2126.

Okumura S, Saitoh H, Ishikawa T et al. (2005) Identification of a novel cytotoxic protein, Cry45Aa, from Bacillus thuringiensis A1470 and its selective cytotoxic activity against various mammalian cell lines. *J Agric Food Chem.,vol.* 53, pp. 6313-6318.

Petit L, Gibert M, Gillet D et al. (1997) Clostridium perfringens epsilon-toxin acts on MDCK cells by forming a large membrane complex. *J Bacteriol* vol. 179, pp. 6480-6487.

Poornima K, Selvanayagam P, Shenbagarathai R. (2010) Identification of native Bacillus thuringiensis strain from South India having specific cytocidal activity against cancer cells. *J Appl Microbiol.,* vol. 109, pp. 348-354.

Truskey GA, Yuan F and Katz DF: (2004) *Transport Phenomena in Biological Systems. Part II Chapter 9; Pearson, Prentice, Hall, ed, Upper Saddle River, New Jersey* pp. 427-447.

Uemori A, Maeda M, Yasutake K et al. (2005) Characterization of cancer cell-killing activity associated with parasporal proteins of novel Bacillus thuringiensis isolates. *In: Côté JC, Otvos IS, Schwartz JL, Vincent C (Eds): 6th Pacific Rim Conference on the Biotechnology of Bacillus thuringiensis and its Environmental Impact, Érudit, Montréal*, pp 122-124.

Wendy E. Thomas and David J. Ellar (1983) Bacillus thuringiensis var israelensis crystal 6-endotoxin: effects on insect and mammalian cells in vitro and in vivo *J. Cell Sci*, vol. 60, pp. 181-197

Yasutake K, Binh ND, Kagoshima K et al. (2005) Occurrence of Bacillus thuringiensis Producing Parasporin, Cancer Cell-Killing Cry Proteins, *In: Côté JC, Otvos IS, Schwartz JL, Vincent C (Eds): 6th Pacific Rim Conference on the Biotechnology of Bacillus thuringiensis and its Environmental Impact, Érudit, Montréal,* 2005, pp 124-126.

Yokoyama Y, Ohmori I, Kohda K & Kawazoe Y. (1988) Potentiation of the cytotoxic activity of anticancer drugs against cultured L1210 cells by Bacillus thuringiensis subsp israelensis toxin. *Chemical & Pharmaceutical Bulletin* (Tokyo), vol. 36, pp. 499-504.

Yokoyama Y., Ohmori, I., Suzuki, H., Kohda, K., Kawazoe, Y. (1992) Hyperthermic potentiation of bleomycin cytotoxicity in the presence of Bacillus thuringiensis subsp. israelensisδ-endotoxin . *Anticancer Research,* vol. 12, pp. 1079 1082.

Yuan F, Leunig M, Huang SK, Berk DA, Papahadjopoulos D and Jain RK: (1994) Microvascular permeability and interstitial penetration of sterically stabilized (stealth) liposomes in a human tumor xenograft. *Cancer Res.,* vol 54, pp. 3352-3356.

Yuich Abea, Hiroshi Inouea, Hisashi Ashidab, Yusuke Maedab, Taroh Kinoshitab, Sakae Kitada (2017) Glycan region of GPI anchored-protein is required for cytocidal oligomerization of an anticancer parasporin-2, Cry46Aa1 protein, from Bacillus thuringiensis strain A1547. *Journal of Invertebrate Pathology,* vol. 142, pp. 71–81

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