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Bacillus thuringiensis Parasporins and Their Use in Controlling Cancer Cells

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Abstract

Parasporins are Cry proteins produced by Bacillus thuringiensis (Bt) strains during sporulation processes and notable for targeting the cancer cells with their unique cytotoxicity mechanism without exerting haemolytic effect on normal cells. Parasporins are specifically produced by Bt strains with non-insecticidal effect. Although the studies on parasporins go back to the 1970s, comprehensive scan about the cytotoxicity of parasporins was performed by Mizuki and colleagues. Considering that these proteins exhibit selective toxicities on human cancer cell lines but not on normal cells, detailed studies about the mode of action of anti-cancer effect was investigated in several countries. Specificity and abundance of parasporin producing Bt species in nature brought them into an important position in terms of developing anti-cancer agents. Parasporins are classified into six groups by the committee of parasporin classification and nomenclature as parasporin-1 (PS1), PS2, PS3, PS4, PS5, and PS6 by taking the amino acid homology into account. Activated parasporins display cytotoxicity at varying degrees in different cancer cell lines. There are numerous studies about promoting the use of Bt parasporins as anti-cancer agent in human, but in depth studies should be carried out about their usability in model organisms. Researches should also be deepened especially in vivo due to production of different types of parasporins with different mechanism of action by different Bt strains. Clarification of the molecular mechanisms of toxicity for every candidate parasporin on cancer cell lines may ease the development of anti-cancer agents. Thus, the present study was conducted to provide a review about the cytotoxic impacts of Bt parasporins on human cancer cell lines

Key words

Bacillus thuringiensis, cancer cells, parasporin

1. INTRODUCTION

1.1. Bacillus thuringiensis and an Overview of Its Toxin Proteins

Bacillus thuringiensis (*Bt*) is an aerobic, Gram (+) and spore forming entomopathogenic bacterium belonging to *Bacilluscereus* group together with *Bacillus anthracis* [1], [2]. It was first discovered in diseased larvae of the silkworm, *Bombyxmori* by Ishiwata and characterized by its well-known insecticidal δ-endotoxin proteins [3]. It has a simple life cycle and under appropriate environmental conditions and nutrient supply their spores germinate and go into vegetative form. But, if one or more of compounds as carbohydrates, oxygen, amino acid or others are insufficient in their nutrient; they form parasporal bodies together with spore and delta endotoxins (Figure 1).

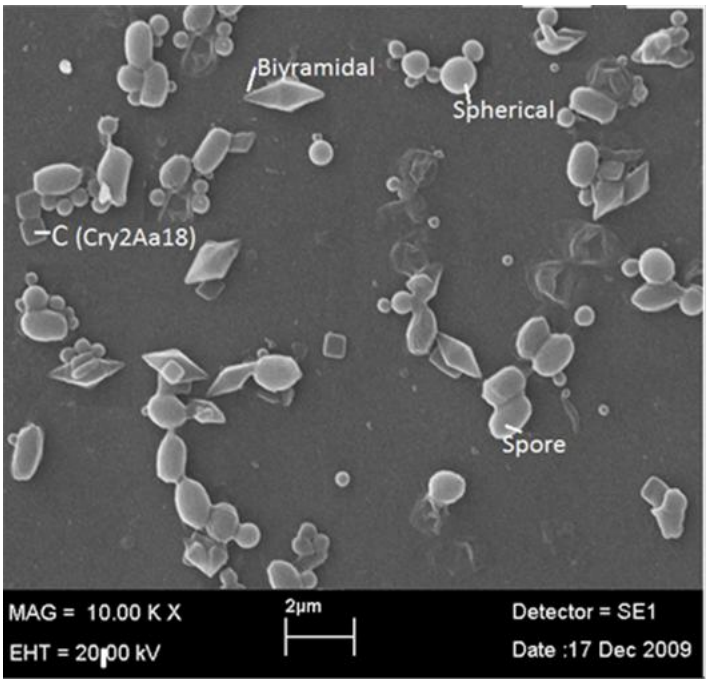

Figure 1. SEM view of Bt SY49.1 spore crystal mixture [4]

Cry proteins are widely used worldwide as insecticidal biopesticides in agricultural fields. But, it was also reported that the number of non-insecticidal strains of *B. thuringiensis* in natural environments are more widely distributed than insecticidal strains [5], [6]. Mizuki et al., [7] observed the first group of functional Cry proteins, known as parasporins, in non-insecticidal *Bt* stains. Parasporins are grouped into six family as PS1-PS6 by the parasporin nomenclature committee [8]. They specified as non-haemolytic and preferentially kill cancer cells [9]. *Bt* 89-T-34-22 isolate belonging to serovar*shandongiensis* revealed at least three potent toxicity on human leukemic T cells [10], [11].

1.2. The Discovery of PSs

Parasporins are the type of δ-endotoxins produced as Cry proteins by *Bt* species. The most prominent feature of parasporins is that they don't exhibit insecticidal activity but prefer cancer cells to control. They are firstly discovered in non-haemolytic and non-insecticidal *B. thuringiensis* isolates A1519 and A1190 by Mizuki et al. [12] after an extensive scanning program and named as parasporin proteins. Studies revealed that they have cytotoxic activity on leukemic T cells and some other human cancer cells [7]. The first discovered protein was named as PS1Aa1 [8], and discovery of new proteins are followed by scientists in Vietnam [13], Canada [14], Malaysia [15] and Japan [16]. It was indicated that spore morphology and some other characteristic properties of parasporin producing *Bt* strains are different from their Cry producing counterparts [17]. Parasporins exert their cytotoxicity on cells without any serious deterioration on cell surface. It was reported that parasporins thought to have disruptive effect on cells via more specific mechanism other than known colloidal-osmotic swelling of cells and fragmentation caused by Cry proteins [18]. In a screening study carried out on sheep erythrocytes, potent proteins was obtained from more than 60 *Bt* isolates with strong haemolytic effect on organisms ranging from vertebrates to invertebrates [19]. Likewise, some of the parasporins with nonhaemolytic and non-insecticidal activity was found to have cytocidal effect against MOLT-4 (human leukemic T-cells) [12]. When PS1A1 was applied to cell lines from mosquito (*Aedesalbopictus*) cultures (NIASAeA1-2) and cells from *B. mori* (BMN), sensitivity was not observed [7]. However, in more detailed studies using parasporins high cytocidal effect was observed on HeLa, MOLT-4 and human lung cancer cells at various levels [12]. Such a situation revealed that parasporins have varying degrees of specificity on a wide variety of cancer cells.

1.3. Mode of Action and Target Specificity

High doses of parasporins result in cessation of respiratory activity in variety of cells (Caco-2, MOLT-4, HL60, Jurkat, HC TCS, HepG2, MRC-5, Sawano, HeLa and A549) following large morphological alterations and subsequently swell, begin to separate from holder and come apart before cell death is realized [20]. Due to death of the cells at high doses before the start of apoptosis, these morphological events are the indication that cytotoxicity is not an apoptotic result. The researchers also specified that every toxin protein have unique and specific target spectrum in mammalian cells because of their receptor recognition property. Wong et al. [21] studied the binding behavior of purified parasporins and put forth that product of *Bt* 18 has quite high affinity against CEM-SS (leukaemic cell line) cells. Heterologous competitive binding analysis revealed that parasporins attach to distinct positions in different cancer cells and thus may have disparate mode of action. PS1 group of proteins demonstrate their cytocidal effect through apoptosis [22]. PS1Aa3 and PS1Ab1 without any insecticidal and haemolytic effect, indicate a considerable toxicity on HeLa cells (human uterus cervix cancer cells), but, not on uterine smooth muscle cells (UtSMC) [23]. In studies with simultaneous application of parasporal proteins and commercial anti-cancer drugs as doxorubicin, cisplatin, navelbine, etoposide, and methotrexate on CEM-SS cell lines,

competitive relation was not reported. That's why it has great importance to clarify *in vitro* differential activity mechanism of every parasporin on different types of cancer cell lines.

Cytotoxic action was not evident upon application of 16 and 60 kDa parts of PS1Ac2 separately on HeLa and MOLT-4 cells; however, their mixed application resulted in cytotoxicy on HeLa cells but not on MOLT-4 cells [24]. Such a case has been attributed to refolding activity of proteins. These researchers suggested that depending on the lack of intracellular LDH release, the mode of action of PS1Ac2 was not associated with pore formation in cell membrane, but directly related with the start of apoptotic signal in susceptible cells through Ca^{+2} influx. While PS2Aa1, PS3Aa1 and PS4Aa1exhibit their cytotoxicity in 1h on susceptible cells, PS1Aa1 reveal in 8-10h on the same cell types [25], [26]. The situation shows that parasporal proteins can structurally be different from each other and reveals that mode of action of PS1Aa1was considerably different from other parasporins [7]. PS1Aa1 doesn't cause pore formation in cell membrane but rapidly increase the concentration of intracellular Ca^{+2}concentrations and physiologically result in a considerable decrease in DNA and protein synthesis [22]. PS2 structurally resemble to aerolysin-type β-pore-forming toxins [27] and exerts its cytocidal effect through lysing the cells [28]. But PS2 may also induce caspase activity at low doses [20]. The first step of PS2Aa1 in cytotoxicity is its specific connection with an undetermined putative receptor in cell membrane and increase in membrane permeability. With the production of PS2Aa1 oligomers, pores are formed and cells become lysed [9]. Despite the lack of detailed information about the mode of action of PS3Aa1, the 3-domain structure of this protein as in Cry proteins suggest that it can perform the cytotoxic effect on cancer cells through pore formation [26]. In some data it was stated that PS4 has pore forming activity similar to PS2 [29], but, these two proteins have different target cell spectrum and exhibit varied cytocidal activity mechanisms [30]. Sensitive CACO-2 and MOLT-4 cells and resistant HeLa cells were subjected to various concentrations of PS4 and seen that wide pore formation on target cell membranes were induced [29]. PS4Aa1 differs from other parasporins in many aspects and also thought that it has different mechanism in terms of mode of action [9]. However, there is no detailed information as in PS3.

1.4. Types and Specifications of Parasporins

Parasporins are characterized by their non-hemolytic but preferentially cancer killing properties. So far as noted in Table 1, a total of 19 parasporin proteins have been isolated from 17 *Bt* strains and classified into 6 groups as PS1-PS6 [8]. Parasporins need to be activated proteolytically for exhibiting toxicity on cancer cells [20]. PS1, PS3, and PS6 are three domain proteins with five block conserved sequences. PS1 is the most well studied protein with 81kDa precursor structure and 15-56 kDa active heterodimer [27]. PS3 is similar to PS1 with 88 kDa inactive and 64 kDa active forms [26]. PS6 is a protein with 84 kDa inactive form and 73kDa active forms with 14-56kDa heterodimers [31]. On the other hand, PS2 is a low molecular weight (37 kDa precursor, 30kDa active forms) protein with unconserved three-domain structure [27]. PS4 is the protein with molecular weight of 30 kDa inactive form and 27 kDaproteolytically activated form [32]. PS5 is also low molecular weight protein which lack five block conserved sequences [27].

Table 1: Known parasporin proteins

Type of Parasporin		Corresponding Cry No.	*Bt* source strain	Reference
Parasporin 1	PS1Aa1	Cry31Aa1	A1190	[7]
	PS1Aa2	Cry31Aa2	M15	[14]
	PS1Aa3	Cry31Aa3	B195	[33]
	PS1Aa4	Cry31Aa4	Bt 79-25	[34]
	PS1Aa5	Cry31Aa5	Bt 92-10	[34]
	PS1Aa6	Cry31Aa6	CP78A, M019	[31]
	PS1Ab1	Cry31Ab1	B195	[33]
	PS1Ab2	Cry31Ab2	Bt 31-5	[34]
	PS1Ac1	Cry31Ac1	Bt 87-29	[34]
	PS1Ac2	Cry31Ac2	B0462	[24]
	PS1Ad1	Cry31Ad1	CP78B, M019	[31]
Parasporin-2	PS2Aa1	Cry46Aa1	A1547	[20]
	PS2Aa2	Cry46Aa2	A1470	[35]
	PS2Ab1	Cry46Ab1	TK-E6	[36]
Parasporin-3	PS3Aa1	Cry41Aa1	A1462	[26]
	PS3Ab1	Cry41Ab1	A1462	[26]
Parasporin-4	PS4Aa1	Cry45Aa1	A1470	[37]
Parasporin-5	PS5Aa1	Cry64Aa1	A1100	[27]
Parasporin-6	PS6Aa1	Cry63Aa1	CP84, M019	[31]

Available at: http://parasporin.fitc.pref.fukuoka.jp/list.html

1.5. Haemolytic and Cytotoxic Effects of PSs

There are large number of isolates without haemolytic activity and high selective toxicity to a wide variety of mammalian cells lines. For example, while PS2Aa1 selectively kill liver and colon cancer cells, it didn't exert cytotoxicity on non-neoplastic cells, chronic inflammatory cells or blood vessels in the same organism [20]. Cytotoxicity spectrum of parasporins from these isolates exhibit heterogeneity. While some of them have toxicity over a wide range of human cells, others are strictly specific to few cells [38]. Ito et al. [20] revealed that cytotoxicity levels of recombinant parasporin vary from cell to cell. Among the cells that they examined, while the highest toxicity was observed on MOLT-4, Jurkat, Sawano, and HepG2 with 10-40 ng/ml concentrations, MRC-5, HC, TCS, A549, and HeLa cells were reported to be resistant. Nevertheless, a clear common characteristic among susceptible and resistant cell lines couldn't identified, but, it was clear that tumor cells are more sensitive compared to normal cells. Different anti-cancer cytotoxicity spectrum and activity levels of parasporins on human cell lines are their most striking features. Some PS2 and PS4 types reported to have broad spectrum of activity through indicating lethal effect on six of the nine cancer lines [20], [22], [26], [32]. However, PS3 exhibits moderate cytotoxicity with narrow spectrum in a limited number of cancer cells. It is also interesting that Jurkat, TCS and HeLa cell lines were reported to have monosensitivity against one of PS1Aa1, PS2Aa1 and PS4Aa1[20].

In a study carried out with recombinant PS5, while quite high toxicity (EC50<0.1 µg/mL) were evidenced on HepG2, COS7, HeLa, MOLT-4, TCS, Vero, and Sawano cells, weak toxicity (EC50, 0.1 to 1 µg/mL) was seen on Jurkat, CACO-2, NIH3T3, MRC-5, CHO-K1, and UtSMC cells. On the other hand, cytocidal activity was not observed on U937 and HC cells (EC50>10 µg/mL) [27]. Therefore, a determined specific toxicity mechanism was not proposed for PS5.

2. CONCLUSIONS

Bacterial parasporins provide promising results for decelerating or preventing the proliferation of cancer cells. In this respect, parasporal proteins with different activity spectrum on cancer cell lines can be obtained from a variety of bacterial species. Thus, screening studies should be conducted to find new putative parasporins and their mechanisms of cytotoxicity have to be elucidated on variety of cancer cell lines.

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