モモアカアブラムシに殺虫活性を示すBacillus thuringiensisサイトトキシン、Cyt2Aa

<table>
<thead>
<tr>
<th>項目</th>
<th>内容</th>
</tr>
</thead>
<tbody>
<tr>
<td>誌名</td>
<td>新潟大学農学部研究報告 = Bulletin of the Faculty of Agriculture, Niigata University</td>
</tr>
<tr>
<td>ISSN</td>
<td>03858634</td>
</tr>
<tr>
<td>著者</td>
<td>小山, 尚生&lt;br&gt;佐野, 義孝&lt;br&gt;田中, 未希&lt;br&gt;萩野谷, 功輔&lt;br&gt;Promodonkoy, B.&lt;br&gt;Angsuthanasombat, C.&lt;br&gt;三ツ井, 敏明&lt;br&gt;高屋, 朋彰&lt;br&gt;谷口, 正之&lt;br&gt;岡崎, 桂一#堀, 秀隆</td>
</tr>
<tr>
<td>巻/号</td>
<td>63巻2号</td>
</tr>
<tr>
<td>掲載ページ</td>
<td>p. 77-81</td>
</tr>
<tr>
<td>発行年月</td>
<td>2011年3月</td>
</tr>
</tbody>
</table>
Bacillus thuringiensis Cytotoxin, Cyt2Aa Killed Myzus persicae (Sulzer) (Hemiptera: Aphididae)

Naoki KOYAMA1, Yoshitaka SANO1, Miki TANAKA1, Kohsuke HAGINOYA1, Boonhiang PROMODONKOY2, Chanan ANGSUTHANASOMBAT3, Toshiaki MITSUI4, Tomoaki KOHYA4, Masayuki TANIGUCHI4, Kei-ichi OKAZAKI1 and Hidetaka HORI1

(Received January 14, 2011)

Summary

Aphid is a vector for various plant pathogenic viruses and gives severe damage to various economically important crops and vegetables. However, they are hardly controlled by biopesticides as they are living with phloem sap supplied by a sucking the liquid. We investigated the aphidical activity of Bacillus thuringiensis toxin Cyt2Aa. The green peach aphid, Myzus persicae (Sulzer), were collected from field and reared with leaves of radish in a plastic column. In the bioassay, 100 μl of Cyt2Aa was given with sucrose solution at 200 μg/ml as final concentration through Parafilm and significant kill effects were observed with 38% mortality at 72 h after the start of rearing. On the other hand, Cry1Aa, Cry1Ab and Cry1Ac as well as PBS showed no activity. Admire, chemical pesticide (Bayer CropScience, Monheim, Germany) which was used as positive control kill all aphids tested. This is the first report showing aphidical activity of Cyt2Aa. Kill mechanism are also briefly discussed.

Key words: Bacillus thuringiensis, Cyt2Aa, aphidical activity, Myzus persicae, Cry1Aa toxin

Dwarf disease or mosaic disease bring heavier inhibitory effects to various plants and these diseases are propagated from viruses. Soybean dwarf virus, sbDV (Ohto et al., 2005), and Watermelon mosaic virus, WMV (Yamamoto et al., 1982), are known to be plant pathogen giving severe damage, and these are propagated in the fields semi-permanently by aphid.

Aphid penetrates their rostrum into a vascular bundle to suck phloem sap. When aphid inserts its rostrum into plant tissues carrying viruses, the insect organ is contaminated with the viruses. The aphid becomes a carrier as a result and moves to healthy plants tissue and inserts their contaminated rostrum into vascular bundle (Gray, 1996).

Once virus infects plants, it is almost impossible to recover to ordinary healthy state, therefore, to avoid damage, control thoroughly agent insects or breeding virus resistant plants are strongly desired (Gao et al., 2008; Tougou, 2009).

To date nothing has been reported that B. thuringiensis insecticidal toxins kill aphid. On the other hand, it is well known that fungi and zygomyctota are pathogens against aphid (Hashimoto, 1996) and Dickeya dadantii was reported to be pathogen against M. persicae (Grenier et al., 2006). D. dadantii has been known to have a member of family of Cyt toxin which is homology to B. thuringiensis Cyt toxin therefore its pathogenicity to aphid was thought to be derived from cytotoxicity of Cyt toxin (Grenier et al., 2006). Generally, however, B. thuringiensis Cry toxin cannot kill the insects which are living by taking phloem sap such as aphid, various leafhoppers and so on. If we can express B. thuringiensis Cyt toxins in phloem sap the efficacy of the foreign proteins must increase significantly. B. thuringiensis Cry1A toxin has no activity against leafhoppers, but interestingly, when the toxin was expressed in rice, the rice was protected from a leafhopper.

Molecular size of active Cry toxin is generally 60 kDa, on the other hand Cyt toxin is around 25 kDa and the molecular size of Cyt toxin is suitable for stable expression in bacteria or plant. We have tried the expression of Cyt toxin in phloem tissue and we are expecting to soak into phloem sap. In initial stage of those challenging experiments, we checked the activity of Cyt toxin against the aphid, M. persicae. Here we evidenced the fact that Cyt2Aa toxin killed aphid and this is the first report showing aphidical activity of Cyt toxins.

MATERIALS AND METHODS

Production, purification and activation of Cyt2Aa

The expression vector, pGEM-Teasy harboring cyt2Aa

---77---
was gift from Dr. B. Promdonkoy, Biotec Central Research Unit, Thailand (Promdonkoy et al., 2003) and E. coli JM109 was transformed with pGEM-Teasy and were cultured as Koyama et al. (2011). Crystal of Cry2Aa was prepared from E. coli as Promdonkoy and Ellar (2000, 2003), and activation and purification of Cry2Aa was performed using DEAE Sepharose Fast Flow column (φ 1.5 x 20 cm GE Healthcare UK Ltd) as Koyama et al. (2011).

**Determination of protein concentration**

Protein concentration was determined with Bradford method (Bradford, 1976) using Bovine serum albumin.

**SDS-Polyacrylamide electrophoresis**

Analysis of protein with SDS-PAGE was done and stained with Coomassie brilliant blue as Koyama et al. (2011).

**Culture of Bacillus thuringiensis and preparation of Cry1A toxins**

*B. thuringiensis* was cultured as Suzuki et al. (1992). Cry1Aa was produced with *B. thuringiensis* subsp. *Sotto* strain T84A1 gifted from Professor M. Ohba, Kyushu University. Cry1Ac was produced from *B. thuringiensis* subsp. *kurstaki* strain HD-73 and Cry1Ab was from *E. coli* JM109 harboring expression vector of *pYD4.0* harboring cry1Ab gene gifted from Professor K. Kanda, Saga University and *E. coli* JM109 was cultured as Kim et al. (1998). Cry1A toxins were solubilized and purified as Intrusith et al. (1991) and Hossain et al. (2005).

**Rearing of aphid, Myzus persicae and bioassay of the insecticidal activity of Cry2Aa**

The green peach aphid, *Myzus persicae*, was reared with leaves of the radish, *Raphanus sativus* L. at 25°C in an incubator (LX-3200F; Taitec, Koshigaya, Saitama, Japan) with 12 h light and 12 h dark.

Purified Cry2Aa and as a control, Cry1Aa, Cry1Ab and Cry1Ac were placed into dialyzing cellulose tubes and thoroughly replaced with phosphate buffer saline (PBS) over night at 4°C. The each toxin was mixed with sucrose solution and adjusted to 200 µg protein/ml as final concentration. A hundred µl of sucrose/toxin mixture was sandwiched between two sheets of the Parafilm as shown in the figure superimposed below.

The toxin solution sandwiched was set in a plastic tube (5 cm long x 27 mm φ) as shown bellow and ten aphids were released into the tube. Yellow nylon tape was stick on the Parafilm opposite to the film facing to the room for aphids to attract the insects.

The apparatus containing aphids was placed in the chamber under the same condition as that for rearing of aphid and mortality was calculated from number of dead aphids at 72 h incubation. As a control, 1% water solution of Admire (Bayer Cropscience, Monheim, Germany) and PBS was used as positive and negative control, respectively.

**RESULTS AND DISCUSSION**

**Aphidicidal activity of B. thuringiensis Cry2Aa and various Cry1A toxins**

Purified Cry2Aa and various *B. thuringiensis* toxins were tested to kill the aphid, *Myzus persicae* and it was shown that no *B. thuringiensis* toxin had significant aphidicidal activity (Fig. 1). On the other hand, when Cry2Aa administered at 200 µg/ml, significant mortality with 38.3% was detected at 72 h incubation. Admire showed an admirable kill effects on the aphid and all aphids used were killed at 1% concentration (w/v).

**Mortality curve**

Mortality was monitored at every 24 h, with sucrose/PBS without toxin as control. During 72 h, in case of PBS, viable 50 aphids were reduced to 45 and in the case of

![Fig. 1 Aphidicidal activity of B. thuringiensis Cry toxins, various Cry1A and Admire to the green peach aphid, Myzus persicae. Five plastic columns containing each ten aphids were used for the bioassay. Ninety µl of toxin/sucrose solution at 200 µg/ml final concentration was sandwiched between two sheets of Parafilm as shown in the figure superimposed in the text and set in the plastic column. The mortality was calculated the dead aphid bodies observed during 72 h incubation. PBS without toxins and Admire, chemical pesticide from Bayer Cropscience (Monheim, Germany) were used as controls.](image)
sucrose/PBS/Cyt2Aa viable numbers were reduced to 32.

To date no *B. thuringiensis* Cry toxins have been reported to kill aphid which is giving a harsh damage to various vegetables and crops by propagating viruses (Yamamoto et al., 1982; Ehara, 2000). Therefore, to control aphid only chemical pesticides have been used. However though, chemical pesticide such as admire effectively control aphid, must be small as a few sheets of Parafilm, therefore the concentration to give 38% mortality should be about 1 µl corresponding to 0.2 µg toxin.

The toxin must be cytotoxic to the cell in gut tissue, therefore after uptake the appropriate volume of toxin solution, the feeding will be stopped at early in the sucking. Therefore, if this is the case, volume of the sucrose solution taken by the aphid could be significantly smaller than that volume we estimated. By using radioactive Cyt2Aa, the volume taken will be calculated with specific radioactivity of the toxin inside a whole body of aphid.

The concentration of the toxin applied in the bioassay was high, but this is the first report to show the kill effect of Cyt2Aa against the aphid, *Myzus persicae*. Cyt2Aa is cytotoxic to various cells including human, therefore the concentration of the toxin must be precisely determined to avoid side effects against non target cells. But as long as the target strictly limited to ornament flower such as rose, gene modification with Cyt2Aa gene will bring significant benefits to Integrated Pest Management.

Recently we showed the binding between PC liposome and Cyt2Aa and thereby, the reaction to release calcein was reached to plateau in short period compared to the reaction between PC-Lipo and Cry1A toxins (Koyama et al., 2011). Cry toxin of *B. thuringiensis* has cytotoxicity and Cyt2Aa is thought to bind lipid of BBM of midgut of aphid. On the other hand, Cry1A toxin of *B. thuringiensis* has been thought to bind to receptor(s) on the BBM (Schnepp et al., 1998), such as cadherin like protein, aminopeptidase N, and alkaline phosphatase. At this moment nothing is clear why Cry1A tested did not show any aphidicidal activity, but there must be three reasons, i.e., [1] aphids midgut BBM has no receptor for the Cry1A toxins, [2] aphid midgut contains substance(s) to inhibit the binding, [3] Cry1A toxins used could not pass the rostrum duct of aphid due to unknown reason(s).

More precise researches will provide evaluation for the usefulness of the Cyt2Aa as foreign source for the gene modification.

**REFERENCES**


モモアカアブラムシに殺虫活性を示す *Bacillus thuringiensis* サイトトキシン、Cyt2Aa

小山尚生1・佐野義孝1・田中未希1・森野谷功輔1・ブーピアン プロモドンコイ2・
チャナン アングスタナソンバット3・三ツ井敏明1・高屋朋彰4・谷口正之4・岡崎桂一1・堀 秀隆1

（平成23年1月14日受付）

要 約

アブラムシは様々な植物病原ウィルスを媒介し、経済的に重要な作物や野菜に深刻な損害を与えている。持続的発展可能な農薬の開発のために生物農薬による防除体系の構築が求められるが、師管液を吸汁して生息するアブラムシに対して、生物農薬による有効な防除手段は報告されていない。そこで我々は、*Bacillus thuringiensis* が生産する Cyt2Aa をアブラムシに吸汁させ、その殺虫活性について調査した。フィールドから採取したモモアカアブラムシ、*Myzus persicae* (Sulzer) をプラスチック製の柱状容器内で飼育し、Cyt2Aa の毒性試験を行なった。スクロース溶液と混合して最終濃度が200 µg/ml になるよう調整した100 µl の Cyt2Aa を2重のパラフィルムで挟み、パラフィルム越しにアブラムシに吸汁させたところ、24時間で88% の有意な殺虫活性が示された。一方、Cry1Aa や Cry1Ab、Cry1Ac は、ネガティブコントロールである PBS 同様、アブラムシに対して殺虫活性を示さなかった。これに対し、化学農薬アドマイヤーは、用いた全アブラムシを殺虫した。アブラムシに吸汁された量を推論すると約0.2mg 以下のトキシンで殺虫されていると考えられた。本論文は、Cyt2Aa のアブラムシ殺虫活性を示した初めての報告であり、その殺虫メカニズムについても簡単に考察した。

キーワード：*Bacillus thuringiensis*、Cyt2Aa、アブラムシ殺虫活性、*Myzus persicae*、Cry1Aa トキシン

---

1 新潟大学大学院自然科学研究科
2 タイ王国バイオテック中央研究ユニット
3 タイ王国マヒドン大学サラヤキャンパス分子生物化学研究所
4 新潟大学工学部
*代表著者：hide-hri@gs.niigata-u.ac.jp