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Mosquitocidal Proteins from *Escheriachia coli* pSL 2-1 Clone and *Bacillus sphaericus* 1593

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Escherichia coli pSL2-1 클론과 *Bacillus sphaericus* 1593 균주가 생산한 모기치사 단백질

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A clone pSL 2-1, which is a recombinant plasmid believed to contain the mosquitocidal crystalline protein gene of the *Bacillus sphaericus* 1593, was expressed in *Escherichia coli* JM83 and the product of the clone was purified and identified. The unsolubilized mosquitocidal crystal proteins from the *B. sphaericus* had formed 43, 58, 64, 100, 113, and 130 Kd bands in the SDS-polyacrylamide gel, but the NaOH-solublized proteins at pH 12 formed 2 protein bands of 43- and 64 Kd in the gel because the larger protein (precursor) bands were cleaved. The products of the pSL 2-1 clone was purified by Sephadex G-200 and only the fractions having lethal activity to the 3rd instar larvae of mosquito *Culex pipiens* were analyzed by the gel. The only single protein band of 42 Kd toxic to the larvae was formed. The major toxic protein being produced from the *B. sphaericus* 1593 and the pSL 2-1 clone was found to be the 42 Kd.

The *Bacillus sphaericus* 1593 produces a crystalline protein that is highly toxic to mosquito larvae upon ingestion (1-5). Lee *et al.* (5) previously cloned the moquitocidal protein (MCP) gene in the chromosome of *B. sphaericus* 1593 by a shortgun digestion method. They cloned the MCP gene on the EcoRI DNA fragment(2 Kb) in the EcoRI site of pUC8 vector, and named the clone pSL2-1, which was transformed and expressed in *E. coli* JM83. The *E. coli* lysate was lethal to the 3rd instar larvae of *Culex pipiens* (5).

This time we wanted to find out the molecular weight and lethality of the mosquitocidal protein produced from the pSL2-1 clone, so we purified the mosquitocidal protein by gel filtration and measured the molecular weight of the toxic protein by SDS-polyacrylamide gel electrophoresis.

Materials and Methods

Organisms

The *Bacillus sphaericus* 1593 and *Escherichia coli* JM 83 containing pSL2-1 recombinant plasmid were used (5). *Culex pipiens* larvae were a gift from Mr. Shim, J.C. of the Korea National Institute of Health.

Preparation of lysates of *E. coli* clone and *B. sphaericus*

The *E. coli* clone and *B. sphaericus* were cultured in brain heart infusion broth at 30°C for 48 hours with shaking at 180 rpm, pelleted at 4,000 × g for 20 minutes, washed twice with 0.7% saline solution, resuspended in 1 M NaCl solution (to be 1 gram of cells per m*l*). Triton-X-100 was added to

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make a 0.01% concentration, and then the mixture was sonicated 50 times at intervals of 30 seconds. 5 m*l* of the lysate was loaded on a 48%(v/w) NaBr gradient (6), centrifuged at 17,000 × g for 3 hours, and then MCP bands or endotoxin crystals were collected. The MCPs or the crystals were washed three times with 0.7% saline solution, and resuspended in distilled water to be 10 mg per m*l*.

Solubilization of MCPs and crystals

The resuspended MCPs or the crystal solutions were adjusted to be pH 12 with 1 N NaOH for solubilization and then incubated at 37 °C for 5 hours with slight shaking. The NaOH-solubilized proteins were adjusted to be pH 8 with 1 M Tris·HCl buffer (pH 7.5) and then centrifuged at 40,000 × g and 4 °C for 30 minutes. The pellets were discarded, and then the supernatants were collected. The proteins were adjusted to be 500 μg per m*l* with the buffer and stored at -20 °C.

Gel filtration of the solubilized MCPs

The MCPs collections were solubilized with a 1 M NaOH solution (pH 12) and then filtrated. The solubilized MCP solution was fractionated through a Sephadex G-200 column (2.5 × 100 cm) running 20 mM phosphate buffer (pH 7.5), and then the fractions were bioassayed against the 3rd instar larvae of *Culex pipiensis* (5).

SDS-polyacrylamide gel electrophoresis

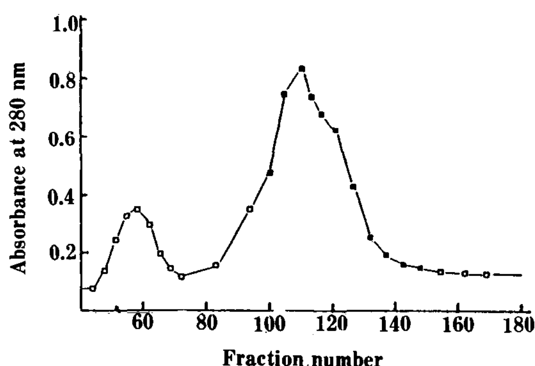

Fig. 1. Elution profile of the solubilized mosquitocidal protein (fractions 100 to 150) chromatographed on Sephaedex G-200.

The protein was produced by *Escherichia coli* pSL 2-1 clone. The black symbols (■) indicate the eluent having mosquitocidal activity.

Table 1. Larvicidal activity of the combined toxic fractions against 3rd instar larvae of *Culex pipiens*

[a]Final Conc. (μg/ml)	[b]No. of C. *pipiens* larvae killed 24 hours	48 hours
5	15	18
4	14	19
3	11	14
2[c]	10	12
1	8	10
0	20	20

[a]Each final concentration of the mosquitocidal fractions was triply tested.
[b]Twenty *Culex pipiens* larvae were placed in 100 m*l* of distilled water containing 5% tryptone and the various amounts of the mosquitocidal toxin, and then lethality was observed at 24 and 48 hours.
[c]LC_{50} at 24 hour treatment was 2 μg/m*l*.

SDS-polyacrylamide gel electrophoresis was carried out through a little modification of the O'Farrel method (7). Gel size was 18 × 13 × 1.5 cm. 10% of polyacrylamide for separating gel and 5% for stacking gel were used, and the gel was run for 8 hours at 25 mA to 30 mA.

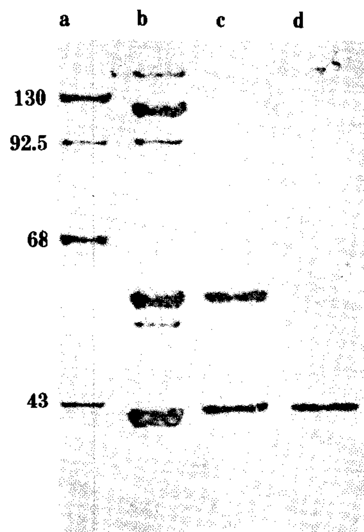

Fig. 2. SDS-polyacrylamide gel electrophoresis of mosquitocidal proteins (MCP) produced by *E. coli* pSL 2-1 clone and *B. sphaericus* 1593.

Lane a, proteins for standard molecular weights (130 Kd, β-galactosidase; 92.5 Kd, phosphorylase; 68 Kd, bovine serum albumin; 43 Kd, ovalbumin); lane b, MCPs produced by *B. sphaericus* 1593; lane c, the MCPs solubilized at pH 12; and lane d, MCP produced by *E. coli* pSL 2-1 clone which was purified through Sephadex G-200 column chromatography.

Protein determination

Protein was determined by the Lowry procedure (9).

Molecular weight determination

Molecular weight was determined by the known standard proteins (Pharmacia Fine Chemicals Kit) running together on the gel.

Bioassay

Mosquitocidal proteins were bioassayed against *Culex pipiens* larvae (5).

Results and Discussion

Mosquitocidal proteins from *E. coli* pSL 2-1 clone and *B. sphaericus* 1593

B. sphaericus 1593 strain produces a highly mosquitocidal toxic crystal (1-5). The toxin crystals are soluble at pH 12, which were analyzed with SDS-polyacrylamide gel to find out major proteins having the lethality to mosquito larvae (6, 10). Lee *et al.* (5) cloned the toxin gene in the vector pUC8 and then expressed the recombinant plasmid pSL2-1 clone in *E. coli* JM83 strain. They found the lysate of the *E. coli* showed strong lethality to mosquito *Culex pipiens* larvae. But the molecular weight and lethality of the mosquitocidal proteins produced by the *E. coli* pSL2-1 clone were not measured in the previous report (5).

The mosquitocidal protein produced by the *E. coli* pSL2-1 clone was fractionated through the Sephadex G-200 column and the results are illustrated in Fig. 1. The fractions were bioassayed against the 3rd instar larvae of *C. pipiensis,* out of which the fraction number 100 to 150 showed strong mortality to the larvae, and a LC50 value was about 2 μg per ml at 24 hours treatment (Table 1).

The unsolubilized crystal complex proteins from the *B. sphaericus* 1593 were formed at 43, 58, 64, 100, 113 and 130 kilodalton bands in the SDS-polyacrylamide gel (Fig. 2 lane b), but the NaOH-solubilized proteins at pH 12 were formed 2 protein bands at 43 and 64 Kd in the gel because certain linkages in the larger molecular proteins might be cleaved at pH 12 (Fig. 2 lane c). These results indicate that the larger proteins may be precursors of the 43 and 64 Kd peptides. There were similar reports. Baumann *et al.* (6) reported that the proteins in the crystal of *B. sphaericus* 2362 strain migrated in positions corresponding to 43, 63, 98, 110, and 125 Kd; solubilization of the crystal at pH 12 with NaOH eliminated all but the bands at 43-and 63 Kd. The only 43 Kd protein was toxic to mosquito larvae, whereas the 63 Kd protein was not. The 43 Kd protein also persisted for the duration of the experiment upto 4 hours (6). Broadwell and Baumann (10) reported that larger proteins of the crystals from *B. sphaericus* 2362 strain were precursors of 43- and 63 Kd peptides. The proteins in the fractions having lethal activity of the pSL 2-1 clone (Fig. 1) were formed the only single protein band of 42 Kd (Fig. 2 lane d). The results strongly indicate that the major toxic products of the pSL 2-1 clone and *B. sphaericus* toxin gene would be 42 Kd protein.

요 약

Escherichia coli pSL2-1 clone은 *Bacillus sphaericus* 1593의 모기살충 유전자를 클로닝한 재조합 DNA이다. 이 클론이 생산하는 살충독소 단백질의 분자량을 SDS-polyacrylamide gel을 이용하여 측정했다. *B. sphaericus* 1593균이 생산하는 독소결정체를 분리하여 전기영동을 한 결과는 6개의 단백질 밴드(43, 58, 64, 100, 113, 130 Kd)가 형성되었으나, 독소결정체를 알칼리 pH로 용해하여 전기영동을 하면 2개의 단백질 밴드(43과 64 Kd)만이 나타났다. 그러나 대장균 pSL2-1균이 생산하는 독소단백질을 Sephadex G-200으로 정제하여 모기유충에 살충력이 있는 단백질을 전기영동한 결과는 42 Kd만이 나타났다. LC50은 2 μg/ml이었다. *B. sphaericus*와 pSL2-1 clone 생산하는 살충단백질은 42 Kd 단백질인 것으로 생각된다.

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