A．研究目的

魚介毒の一斉分析・定量を行うにあたり LC/MS 分析によるフグ毒(TTX)およびTTX 誘導体の分離定量法の開発を行うことを目的とする。本年度においては、TTX関連物質のにつきニホンイモリから抽出、精製を行い、得られた毒についてはMS および NMR による同定を試みた。

近年、海産フグや淡水産フグからフグ毒以外に麻痺性貝毒（PSP）やドウモイ酸（DA）なども同時に検出されることがあり、これらフグの毒の分析・定量を行うにあたり、TTX 分析のみならず、PSP を目的とした分析も行う必要になる機会が増加してきた。現在、これらの成分分析には TTX、PSP それぞれで別々の機器分析法により定量を行っており、試料の分析には多大な時間と労力を必要としている。また、フグ毒や PSP においては異性体等の関連成分が多数発見されており、食品衛生上問題になる比毒性を示す成分も存在することから、毒の定量分析において各成分を分離定量することは重要事項であると考えられている。

このようなことから、まず TTX およびその関連成分について、LC/MS による分離定量法を確立することを目的とし、TTX および関連成分の標準品を TTX 保有生物から精製することを試みた。

TTX 標準品については市販されており、比較的入手しやすいが、それ以外の関連成分については、入手困難なため、精製する必要がある。

B．研究方法

1）標準毒の精製

試料を細切後、1% 酢酸-80% エタノールを 3 倍量加え、ホモジナイザー(10,000 rpm, 5 min)で均質化し、遠心分離（8,000 rpm, 20 min）に供した後、上清を分取した。残渣をこの操作に 3 回繰り返し供し、抽出液を合一した(63,000 MU)。この抽出液を減圧濃縮し、等量のジクロロメタンを加え脱脂した。水層画分につき pH を 6 に調整した後、試料と等量の活性炭（約 760 g）を加えてよく攪拌し毒を吸着させ、精製水で洗浄した後、1% 酢酸-20% エタノールで溶出し、ブフナー漏斗でろ過した。この際、洗浄液から毒が検出されたため、減圧濃縮後、上記の活性炭処理に再度供し、溶出液を得た。2 つの溶出液を合一し(53,700 MU)、減圧濃縮・凍結乾燥させた後、少量の純水に溶解し、Bio-Gel P-2 カラム(5×35 cm, Bio-Rad Laboratories)に付した。一晩カラムを精製水で洗浄後、毒を 0.1 M 酢酸で溶出した。有毒画分（51,800 MU）を減圧濃縮・凍結乾燥後、少量の純水に溶解し、Bio-Gel P-2(5×35 cm, Bio-Rad Laboratories)カラムに付し、0.03 M 酢酸を用いたクロマトグラフィーを行った。LC/MS 分析で有毒画分を確認後、これらを適宜合一し、Bio-Gel Fr.1, 2 とした。Bio-Gel Fr.1（15,600 MU）を減圧濃縮後、少量の精製水に溶解し、Bio-Rex 70 カラム(0.8×90 cm, $H^+$form, Bio-Rad Laboratories)に付した。その後、0-0.03 M 酢酸を用いたクロマトグラフィーを繰り返し行い、LC/MS 分析で有毒画分を確認した。Bio-Gel Fr.2（24,300 MU）においても同様にクロマトグラフィーを行い、カラムからの溶出順に T-I～VI の毒成分を

分離した(図１)。

分離した毒については、カラムに Puresil C18（4.6×250 mm, Waters）を、移動相に 60 mM ヘプタフルオロ酪酸を含む 1 mM 酢酸アンモニウム緩衝液を用いた LC/ESI-MS 分析法により分析した。

２）精製毒の同定

NMR のシステムは JEOL JNM-AL 400 MHz を使用した。また、T-VI についてはさらに Varian UNITYplus 500 MHz を使用し、NOEZY および COSY スペクトルを測定した。

T-I～VI をそれぞれ 4% $CD_3COOD$-$D_2O$ に溶解させ、NMR 試験管に注入し、$^1$H-NMR スペクトルを測定した。

C．研究結果

１)標準毒の精製

Bio-Gel P-2 によるカラムクロマトグラフィーにより得られた各フラクションを選択イオン *m/z* 320、304、302 でそれぞれ LC/MS 分析したところ図 2 のような溶出パターンとなった。Bio-Rex 70 による溶出パターンを図 3、4 に示す。粗抽出液（63,000 MU）から順次精製して最終的に 6 つの画分（T-I～VI）を得た。

T-II（総毒量 16,600 MU）では、LC/MS 分析において選択イオン *m/z* 320 で分析したところ、保持時間 7.07 分にピークを得、TTX 標準品のピークと保持時間が一致した(図 5)。また、このピークの MS スペクトルから、*m/z* 320 の水素付加イオン $(M+H)^+$ も確認された。以上のことから T-II が TTX であることが考えられた。

T-III（総毒量 931 MU）では、LC/MS 分析において選択イオン *m/z* 320 で分析したところ、保持時間 7.77 分にピークを得、6-*epi*TTX 標準品のピークと保持時間が一致した(図 5)。MS スペクトルを解析したところ、$(M+H)^+$ と考えられる *m/z* 320 のイオンピークが確認された。このことから T-II は 6-*epi* TTX であることが考えられた。

T-V（総毒量 252 MU）は、LC/MS 分析において選択イオン *m/z* 320 で分析したところ、9.82 分に出現するピークを主とし、7.00 分の TTX のピークも若干認められた。9.82 分のピークの MS スペクトルを解析したところ、*m/z* 320 の強いイオンピークが得られ、ピークの保持時間および MS スペクトルからこのピークは 4-*epi* TTX であることが推測され、T-V の主成分であった(図 6)。

T-I の LC/MS 分析によるクロマトグラムおよびスペクトルを図 6 に示す。11-oxoTTX のクロマトグラムのピークと保持時間が一致し、*m/z* 336 の水素付加イオン $(M+H)^+$ が確認できたため、T-I は 11-oxoTTX であることが推定された。

T-IV の LC/MS 分析から 5-deoxyTTX-std のクロマトグラムのピークと保持時間が異なり、11-deoxyTTX のピークと溶出傾向が近似していた。また、このピークの MS スペクトルから、*m/z* 304 の水素付加イオン $(M+H)^+$ が得られたため、T-IV は 11-deoxyTTX であることが推定された。

T-VI の MS スペクトル分析から、*m/z* 304 のイオンピーク が得られ、TTX から何らかの置換基が置き換わった誘導体であることが推測された。

2）精製毒の同定

T-Ⅱ、Ⅲ、Ⅴ、ⅥのNMRスペクトルチャートを図7に、ケミカルシフト値のデータを表1に示す。

(a)T-Ⅱ

ケミカルシフト値(σ) 2.35、3.95、4.01、4.03、4.07、4.25、4.29、5.50 ppmに、それぞれC4a-H、C9-H、C11-H、C11-H、C7-H、C5-H、C8-H、C4-Hに帰属し得るプロトンのシグナルが認められた。σ2.35 ppmとσ5.50 ppmのシグナルは結合定数 J = 9.6 Hzで互いにカップリングしており、TTXのスペクトル(Yotsu-Yamashita, 2001)の特徴とよく一致した。

(b)T-Ⅲ

スペクトルの解析により、hemilactal型とlactone型が混在していることが明らかとなった。ケミカルシフト値（σ）2.01、3.74、4.00、4.08、4.16、4.29、5.55 ppmに、hemilactal型のそれぞれC4a-H、C11-H、C9-H、C7-H、C8-H、C5-H、C4-Hに帰属し得るプロトンのシグナルが認められた。さらに、ケミカルシフト値(σ)2.13、3.68、3.69、4.02、4.26、4.58、4.62、5.55 ppmに、lactone型のそれぞれC4a-H、C11-H、C11-H、C5-H、C8-H、C9-H、C7-H、C4-Hに帰属し得るプロトンのシグナルが認められた。σ2.01 ppmとσ5.55 ppmのシグナルは結合定数 J = 9.2 Hz、J = 8.8 Hzで互いにカップリングしており、6-*epi* TTXのスペクトル(Yotsu-Yamashita, 2001)の特徴とよく一致した。

(c)T-V

ケミカルシフト値(σ) 2.74、3.87、3.91、3.97、4.17、4.20、5.04 ppmに、それぞれC4a-H、C5-H、C11-H、C7-H、C8-H、C9-H、C4-Hに帰属し得るプロトンのシグナルが認められた。σ2.74 ppmとσ5.04 ppmのシグナルは結合定数 J = 4.4 Hzで互いにカップリングしており、4-*epi* TTXのスペクトル(Nakamura と Yasumoto, 1985)の特徴とよく一致した。

T-IおよびT-IVの2画分ついては、精製により得られた重量が微小であり$^{1}$H-NMR スペクトルが測定不可能であった。

(d)T-VI

400 MHzで測定したスペクトルは形状が不明瞭であり解析が困難であったため、Varian UNITYplus 500 MHzを使用してより詳しい解析を行った。詳細なスペクトルの解析により、T-VIのシグナルは過去報告されているTTX誘導体のどのものとも一致しなかった。まず、C6-Hに帰属し得るシグナルが存在した。これは、C6に付属する水酸基（-OH）が水素（-H）に置換したことを示した。また、TTXに比べC11-Hのシグナルが高磁場にシフトしており、その積分比が2から3に変化していた。これはC11に付属するハイドロキシメチル基（$-CH_2OH$）がメチル基（$-CH_3$）に置換されていることを示した。一方、TTXでは存在したC5-Hに帰属し得るシグナルが消失していることが判った。これはC5に付属する水素（-H）が何らかに置換されていることを示唆した。

D．考察

T-IIは、TTXのスペクトルとよく一致したことからTTXであると同定された。

T-III は、hemilactal 型と lactone 型が混在しており、それぞれ 6-*epi* TTX のスペクトルとよく一致したことから 6-*epi* TTX であると同定された。

T-V は、4-*epi* TTX のスペクトルとよく一致したことから 4-*epi* TTX であると同定された。

T-VI については、C6 に付属する置換基が水素（-H）に、C11 に付属する置換基がメチル基（$-CH_3$）に、さらに、C5 に付属する水素が別の置換基に置き換わっていた。さらに、LC/MS 分析により、メインピークを *m/z* 304 とするスペクトルが得られたことから、Fig. 26 に示すような新規誘導体の存在が示唆された。これは、6,11-dideoxyTTX の lactone 型(Jang と Yotsu-Yamashita, 2007)に構造が似ているが、6,11-dideoxyTTX のメインピークは *m/z* 288 であることから、T-VI の C5 には分子量の合計が 17 となる置換基が存在することになる。さまざまな置換基の中で容易に想像し得るのは水酸基（-OH）である。しかし、C5 に水酸基が付属するとアセタール構造となり、非常に不安定な物質なのですぐに脱水された構造へと反応が進む。脱水された構造物の *m/z* は 286 となるはずであり、T-VI とは異なる。さらに、hemilactal 型で存在する可能性もあるが、lactone 型と互変異性体であるため、lactone 型に変換した直後に脱水されていくと考えると、hemilactal 型で存在できる可能性は低くなる。

以上のことより、T-VI は 6,11-dodeoxyTTX の lactone 型に近い構造を持ち、C5 に付属する置換基が分子量 17 のものに置き換わった、新規誘導体である可能性が示唆された。

## E．結論

ニホンイモリから TTX および関連成分を分離精製し、TTX、6-*epi* TTX 、4-*epi* TTX 、11-oxoTTX 、11-deoxyTTX の各 5 成分および 6,11-dodeoxyTTX の lactone 型に近い構造を持つ新規誘導体を得た。

## F．参考文献


Yotsu-yamashita Mari (2001): Chemistry of puffer fish toxin. *J.Toxicol.Toxin Reviews*, **20 (1)** , 51-66.

Shoji, Y., Yotsu-Yamashita, M., Miyazawa, T. and Yasumoto, T.（2001）: Electrospray Ionization Mass Spectrometry of Tetrodotoxin and Its Analogs; Liquid Chromatography/Mass Spectrometry, Tandem Mass Spectrometry, and Liquid Chromatography/Tandem Mass Spectrometry. *Analytical Biochemistry*, **290**, 10-17.

Nakamura, M. and Yasumoto, T.（1985）: Tetrodotoxin derivatives in puffer fish, *Toxicon,* **23**, 271-276.


## G．研究発表

今年度の研究成果については、残りのデータが出揃い次第、国内の学会発表を行う予定である。また、次年度以降のデータを加えて後日論文として公表する予定である。

H．知的財産権の出願・登録状況(予定を含む。)

特に予定していない。

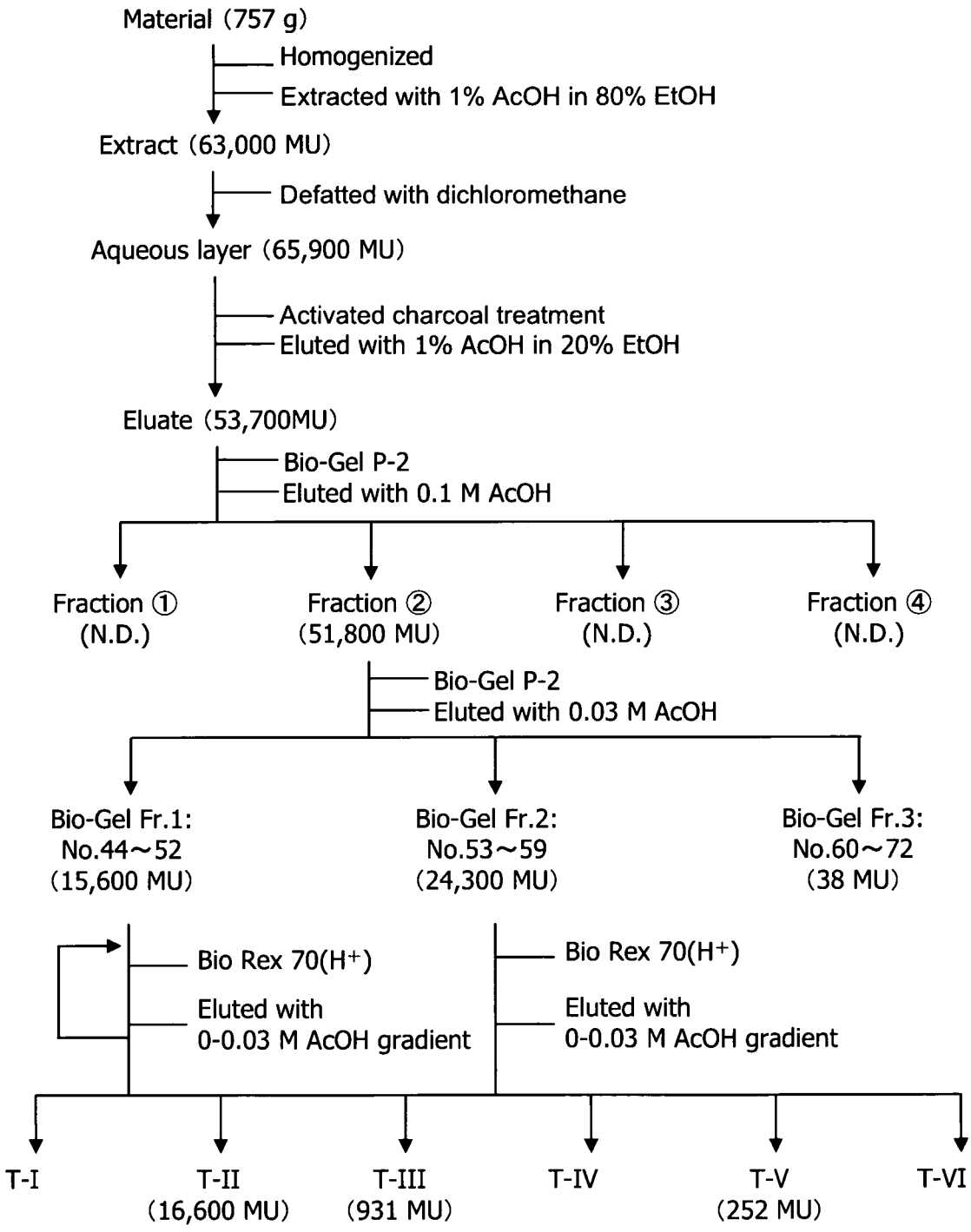


図1 ニホンイモリからの毒の精製手順

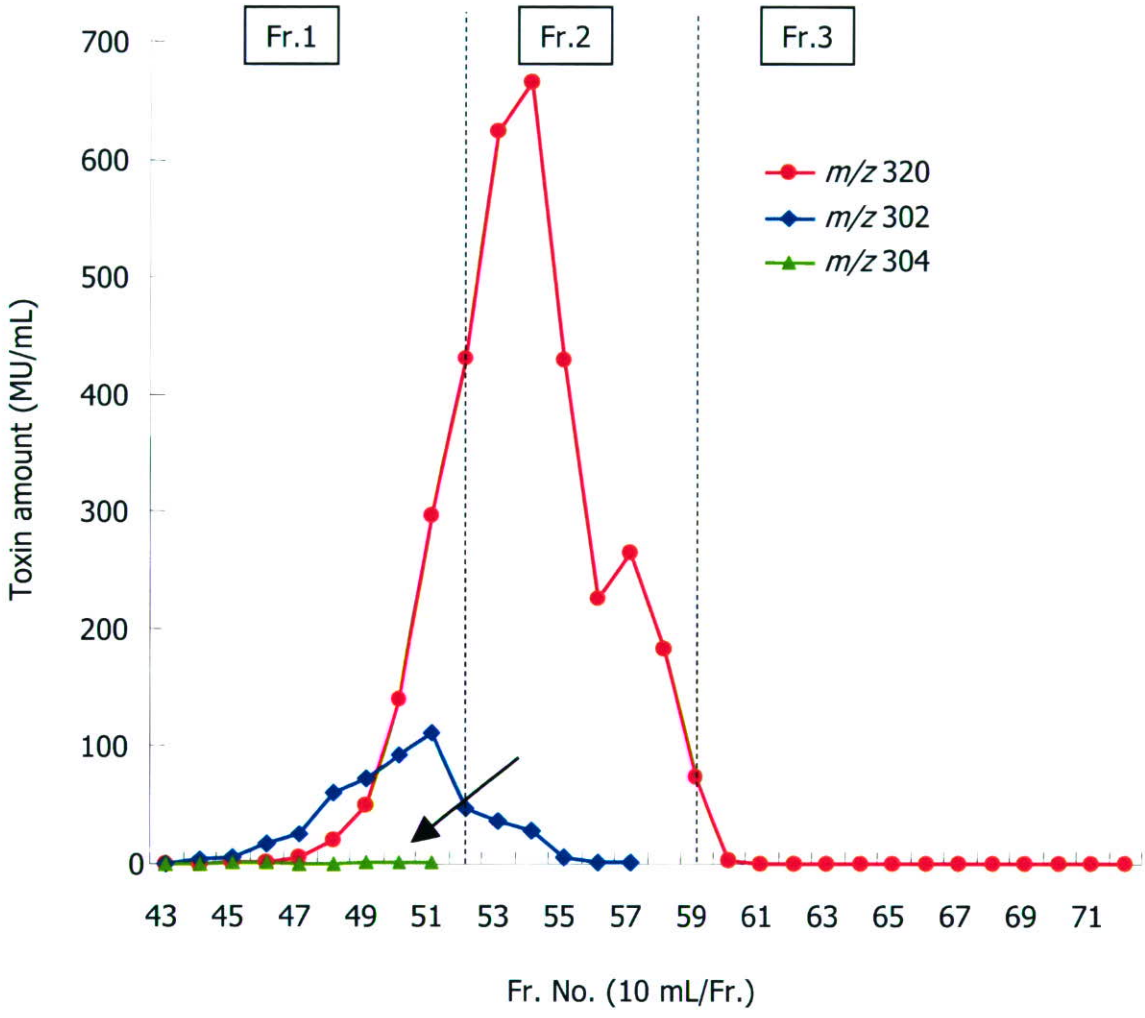


図2　Bio-Gel P-2によるカラムクロマトグラフィーでのTTX関連成分の溶出

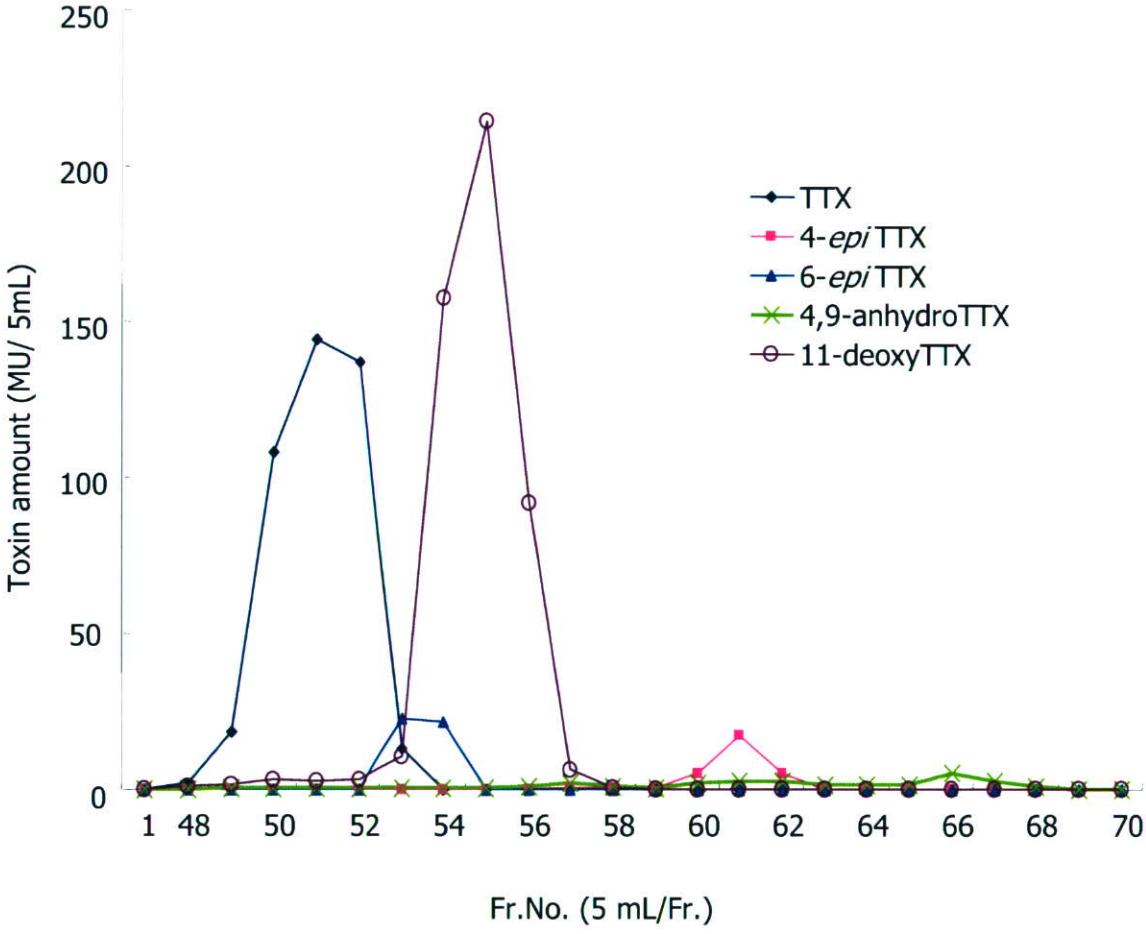


図3　Bio-Rex70($H^+$)によるカラムクロマトグラフィーでのTTX関連成分の溶出（Fr.1）

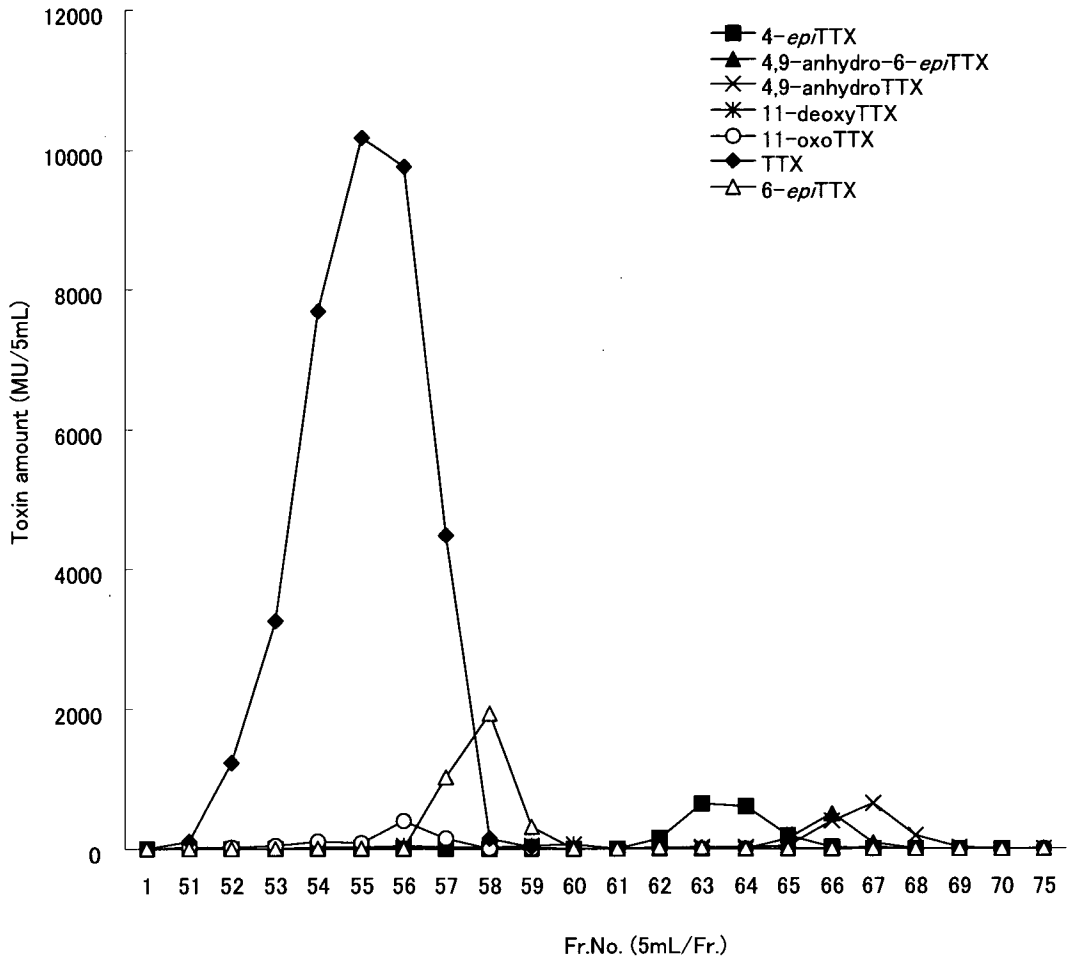


図4 Bio-Rex70($H^+$)によるカラムクロマトグラフィーでのTTX関連成分の溶出(Fr.2)

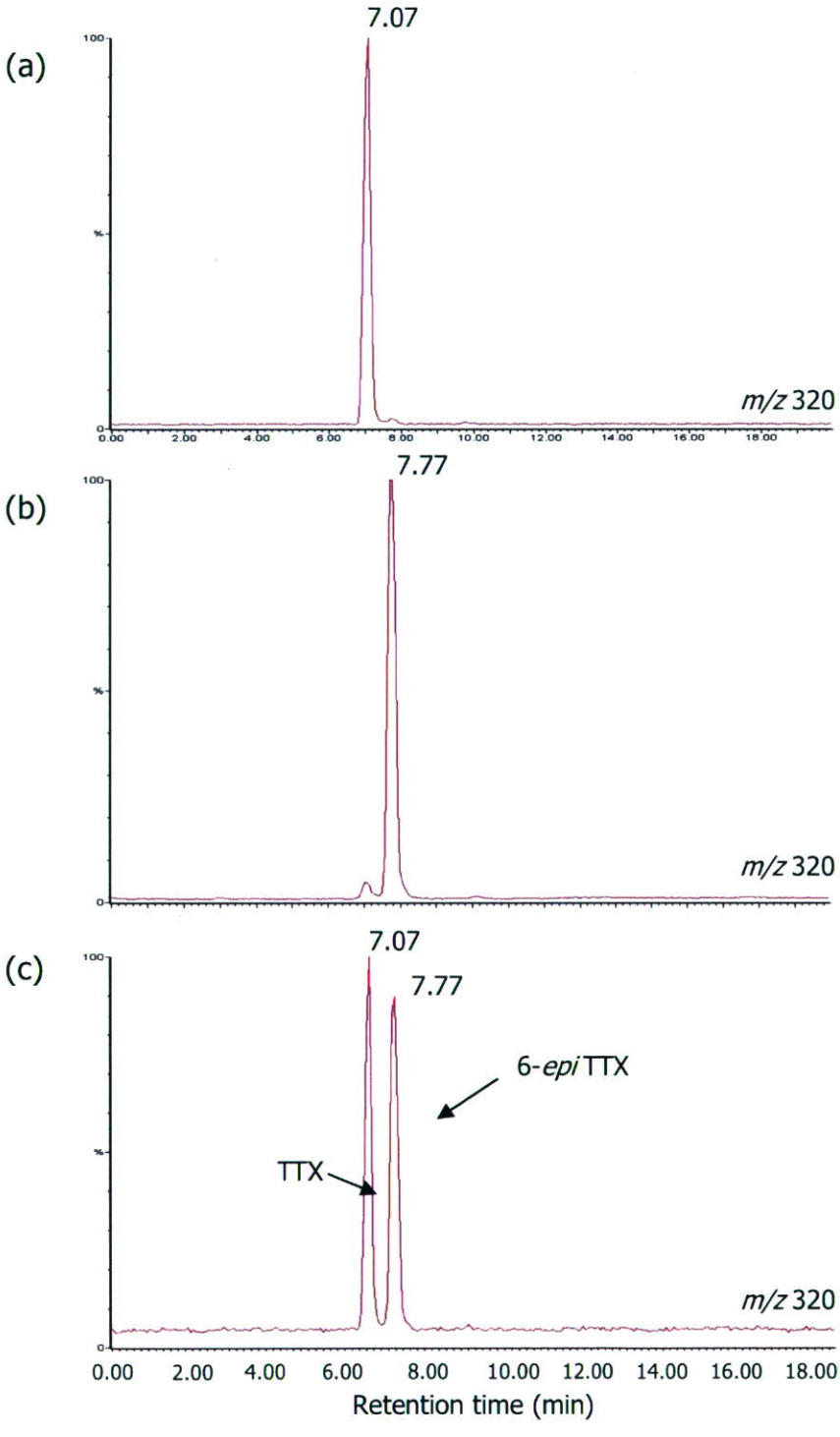


図5　各フラクションのLC/MSクロマトグラム（a: T-Ⅱ; b: T-Ⅲ; c: TTXs-std.）

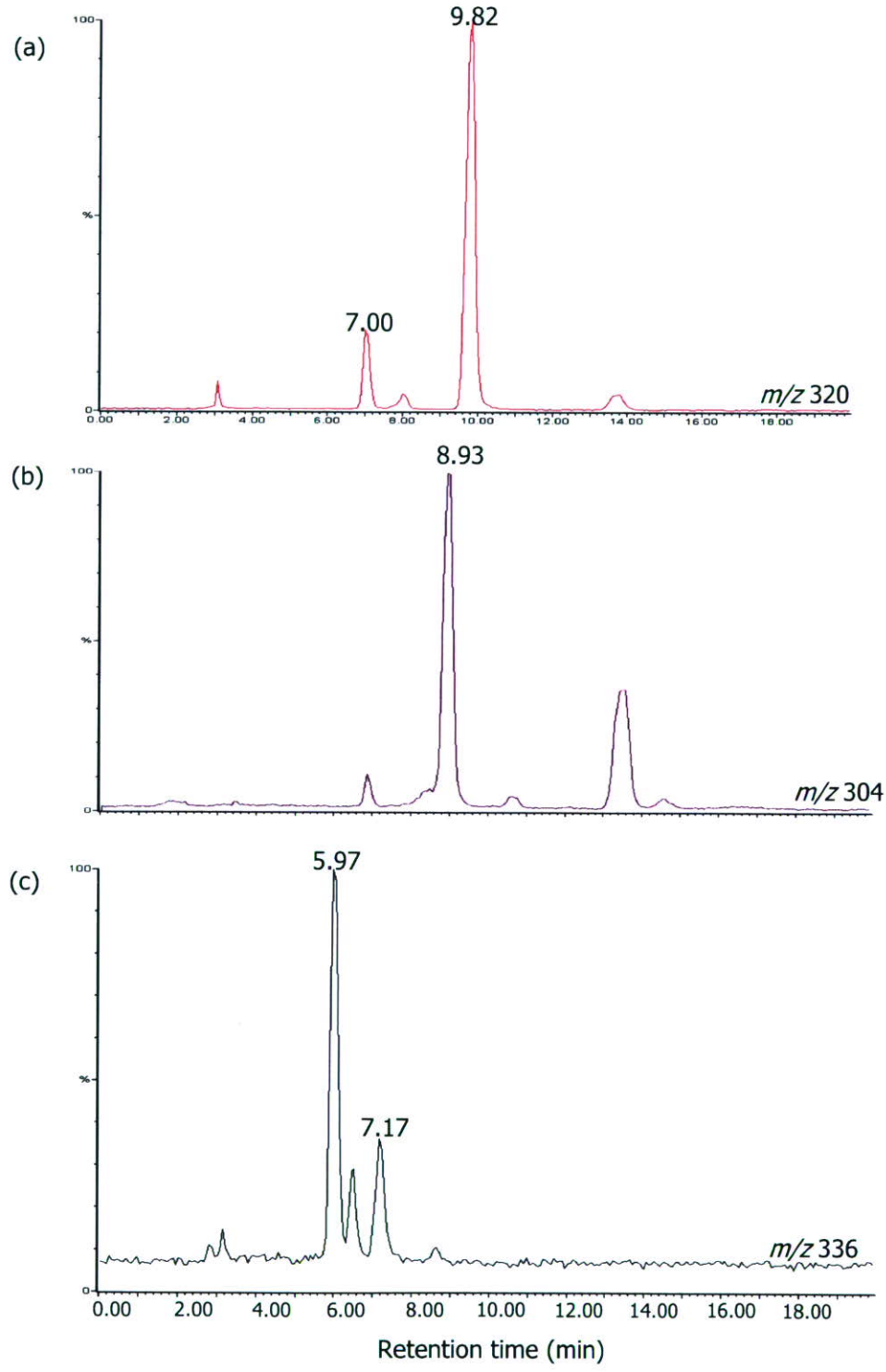


図6　各フラクションのLC/MSクロマトグラム（a: T-Ⅴ; b: T-Ⅳ; c: T-Ⅰ.）

研究成果の刊行に関する一覧表

Beppu, R., Nojima, K.; Tsuruda, S.; Gomez-Delan, G.; Barte-Quilantang, M.; Taniyama, S.; Sagara, T.; Nishio, S.; Takayama, H.; Miyazawa, K.; Asakawa, M. Occurrence of PSP-producing dinoflagellate *Alexandrium tamiyavanichii* in Bingo-Nada, the central coastal water of the Seto Inland Sea, Hiroshima Prefecture, Japan. Marine Pollution Bulletin, 2008; 56(4): 758-763.

相良剛史, 谷山茂人, 江戸 梢, 橋本多美子, 西堀尚良, 浅川 学, 西尾幸郎. 西表島産イワスナギンチャク*Palythoa tuberculosa* の毒性について. 四国大学紀要, 2008, 26(B), 9-12.

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# Occurrence of PSP-producing dinoflagellate *Alexandrium tamiyavanichii* in Bingo-Nada, the central coastal water of the Seto Inland Sea, Hiroshima Prefecture, Japan

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**Abstract**

During surveillance of the distribution of the paralytic shellfish poison (PSP)-producing dinoflagellate in 2003, 2004 and 2005 along the coastlines of the Seto Inland Sea, Hiroshima Prefecture, Japan, some species of toxic phytoplankton were isolated from the eastern coasts, Bingo-Nada, the central regions of the Seto Inland Sea. It was rather unexpectedly revealed from the basis of the morphological characteristics that they were unambiguously identified as *Alexandrium tamiyavanichii* and *Alexandrium catenella*. Two strains (ATY041106, ATY051018) of *A. tamiyavanichii* showed a specific toxicity of $38.7 \times 10^{-6}$ and $111.5 \times 10^{-6}$ MU/cell, respectively. These values seemed to be several times or much higher than that of *A. catenella* (AC030816, AC040614), having a specific toxicity of $4.5 \times 10^{-6}$ and $4.1 \times 10^{-6}$ MU/cell, respectively, isolated in the same area. From the results of HPLC-furuorometric analysis, it revealed that the toxins in ATY041106 exist almost exclusively as β-epimers (C2, GTX3, GTX4), which accounted for 72.7 mol%. The toxin profiles of this strain are featured by the presence of a large amount of GTX3 (59.1 mol%) and a small amount (20.6%) of C1 and 2 in comparison with the PSP compositions of *A. tamarense*, which is isolated as the main responsible species in Hiroshima Bay, a western part of coastal sea in Hiroshima Prefecture. On the other hand, it revealed that the toxin profiles of two strains (AC030816, AC040614) of *A. catenella* exist almost exclusively as β-epimers (C2, GTX3, GTX4), which accounted for 81.8 and 56.5 mol%, as the same manner. The toxin profiles of these two strains are featured by the presence of a large amount of C2 (80.5 and 46.3 mol%) in comparison with the PSP compositions of *A. tamiyavanichii*.

To our knowledge, this is the first record to show the distribution and harmful influence of *A. tamiyavanichii* and *A. catenella* in Bingo-Nada in Hiroshima Prefecture. Though contamination of bivalves with these PSP-producing planktons in this area has not occurred yet so far, attention should be paid to this species as well as the other causative dinoflagellate from the stand point of public health and food hygiene.


*Keywords:* Paralytic shellfish poison; Dinoflagellate; *Alexandrium tamiyavanichii*; *Alexandrium catenella*; *Alexandrium tamarense*; Gonyautoxin; Hiroshima prefecture

## 1. Introduction

The occurrence of harmful algal blooms (HABs) reportedly has been spreading on a global scale in recent years.

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Especially, dinoflagellates species from the genus *Alexandrium* such as *A. tamarense* and *A. catenella* have been known well known as producers for the potent neurotoxins of paralytic shellfish poison (PSP), which is one of the notorious marine toxins known (Hashimoto and Noguchi, 1989). This toxin produced can accumulate in filter-feeding shellfish that feed on the dinoflagellates, resulting in illness to humans at higher trophic levels in the food chain, mainly along with paralysis in parts of the body, followed by death in severe cases. When infestation of bivalves with PSP toxins occurred, secondary intoxication of edible gastropod such as *Rapana venosa* inhabiting there through the food web is also pointed out as an important problem from the view of food hygiene and public health as well as fishery (Ito et al., 2004). Hence, marine pollution due to PSP-producing dinoflagellate and subsequent contamination of shellfish with PSP may cause serious economic losses in the shellfish culture and its related industries, negatively impacting a public health in many coastal countries throughout the world.

Hiroshima Bay, Hiroshima Prefecture is one of the largest oyster culture areas in Japan. Many oyster culture rafts produce 50–70% of the total amount oysters consumed in the country. The first infestation of shellfish with PSP was reported in 1992 (Asakawa et al., 1993). Since this episode, subsequent monitoring for toxins contained in commercial shellfish by mouse bioassay showed that short-necked clams, mussels and oysters were contaminated with PSP with appearance of *A. tamarense* in Kure Bay, which is a part of Hiroshima Bay, almost every year (Asakawa et al., 1995, 2005).

In Hiroshima Bay, the main dominant species responsible for PSP is *A. tamarense*, which is one of the most harmful taxa. However, in a global scale, species belonging to other genera, such as *Gymnodinium catenatum* (Oshima et al., 1987; Ikeda et al., 1989; Takatani et al., 1998a,b) and *Pyrodinium bahamense* var. *compressum* (Harada et al., 1982) have also been found to be responsible for shellfish toxicity, indicating that contaminated sea is spreading along with the increase of the number of the causative species. For these reasons, a strict and constant monitoring focused on understanding the distribution of these toxic dinoflagellates along the coastlines not only in Japan but also in many other countries in the world will be needed more often from now on.

These situations prompted us to undertake the present study, which is an extension of previous studies in which the authors participated, to focus on the distribution and spread of toxic dinoflagellate in the coastal water of Hiroshima Prefecture and to assess potential health risks to human shellfish consumers with marine pollution due to toxic dinoflagellate. In this paper, we report for the first time the detection of *A. tamiyavanichii* in Uchiura Bay, which is located in the east coastal sea of Hiroshima Prefecture, Bingo-Nada, the central coastal water of the Seto Inland Sea, and report that this species is the dinoflagellate paid attention to in connection to toxification of bivalves in Hiroshima Prefecture.

## 2. Materials and methods

### 2.1. Dinoflagellate and shellfishes

Fig. 1 shows Uchiura Bay which belong to Bingo-Nada, central region of the Seto Inland Sea, in the eastern coasts in Hiroshima Prefecture and Kure Bay, a part of the Hiroshima Bay, in the western coasts. The Seto Inland Sea has many islands and narrow waterways, and divided into several basins called "Nada". Seawater samples were collected from 0 m depth in spring, summer and fall from 2003 to 2005, simultaneously with the trial to isolate the toxic dinoflagellate. The 20 μm mesh-screen net hauling seawater was concentrated properly. Each vegetative cell in the net hauling samples was isolated by capillary pipet method. Total six clonal cultures of dinoflagellate were established and used for their toxicities and/or toxin analysis. These six strains were mass-cultured using 3 L Fern-bach flasks. Cells were harvested at late-exponential phase. The culture method, toxicity assays and toxin composition analysis of these strains were almost the same as reported previously (Asakawa et al., 1995). On the other hand, specimens of bivalves such as oysters *Crassostrea gigas* and mussels *Mytilis edulis* adherent to the rocks and short-necked clams *Tapes japonica* in the mud were collected simultaneously from the same area. These live specimens were transported to our laboratory in ice, and shucked. Some of them were immediately assayed for toxicity as described below.

### 2.2. Assay of toxicity

In the assay for toxicity of the dinoflagellates, cells were suspended in 0.1 mol/L acetic acid and ultrasonicated for 10 min. The lysate was centrifuged at 2000*g* for 20 min and the supernatant was obtained. A series of test solution was prepared by dilution with a small amount of distilled water and assayed for PSP toxicity by an official Japanese method (Kawabata, 1978). The PSP toxicity of the shellfish samples was measured by the same method, using 0.1 N hydrochloric acid as the extraction solvent. The activity was expressed in mouse units (MU), in which 1.0 MU is defined as the dose of toxin required to kill a 20 g ddY strain male mouse in 15 min after i.p. injection.

### 2.3. Purification of toxins from dinoflagellate

The acetic acid extract of cultured cells was concentrated and loaded on to a Sep-Pak Plus C18 Environmental Cartridge (Waters). The unbound portion was collected and concentrated to dryness *in vacuo*. The residue was dissolved in a small volume of water and injected into the HPLC-fluorometric system (Asakawa et al., 1993, 1995). The content of each toxin was estimated by comparing the peak area of each toxin with that of each toxin standard and calculated as mol%. The reference standards of PSP used in this study were prepared from the digestive glands of PSP-infested scallops *Patinopecten yessoensis* in Ofunato

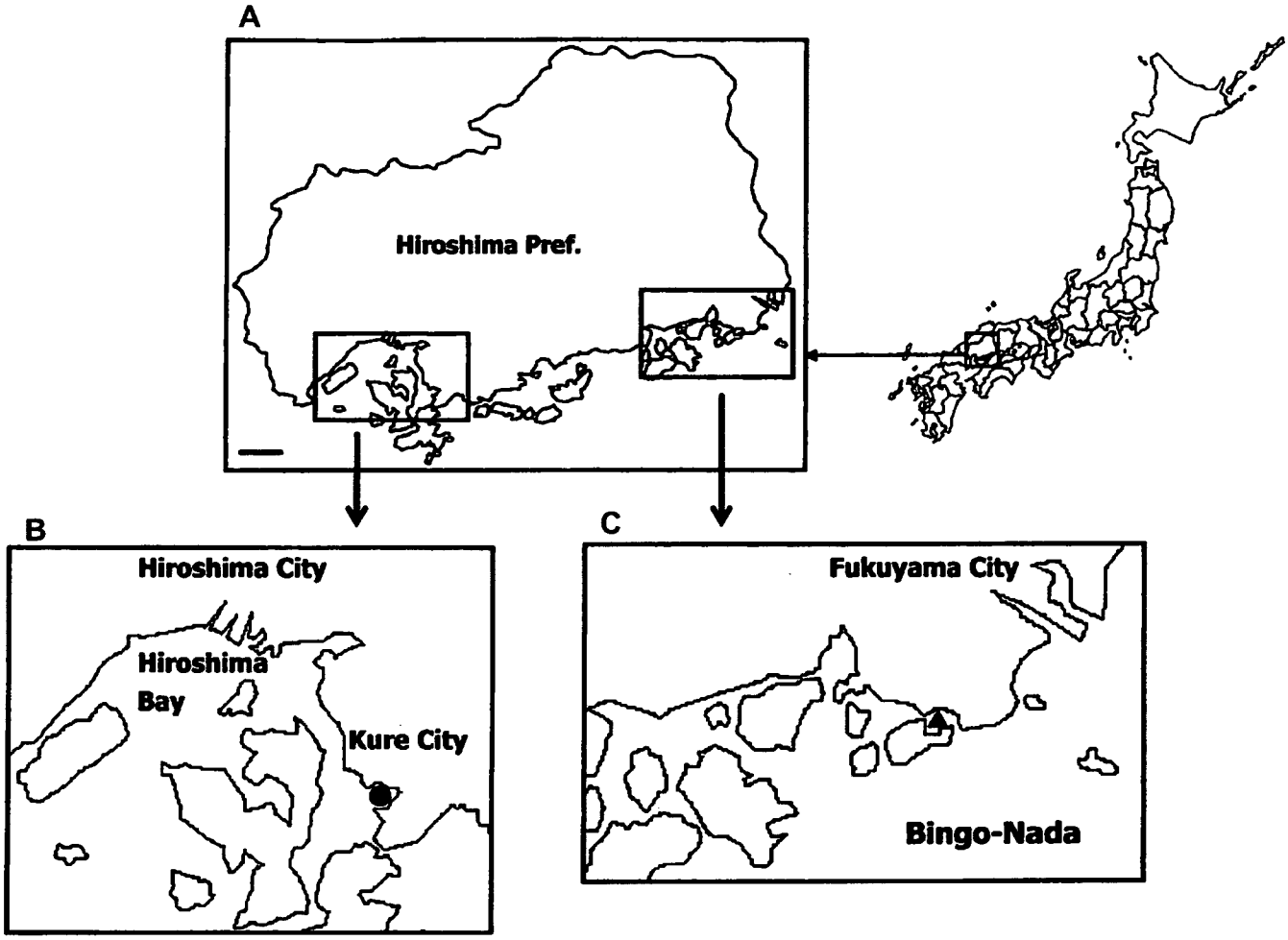


Fig. 1. Map showing sampling stations along with coast lines of Hiroshima Prefecture. (A) Hiroshima Prefecture, scale bar (–) means 10 km. (B) Western coastal sea; Kure Bay (●). (C) Eastern coastal sea; Uchiura Bay (▲).

Bay, Iwate Prefecture (Noguchi et al., 1981) and from a xanthid crab *Zosimus aeneus* from Kabira in Ishigaki Island, Okinawa Prefecture (Daigo et al., 1985). Due to the lack of standard toxins, the contents of N-sulfo carbamoyl derivatives of C1 (PX1 or epi-GTX8), C2 (PX2 or GTX8), C3 (PX3), C4 (PX4), GTX5 (B1) and GTX6 (B2) were estimated by comparing chromatograms before and after acid hydrolysis, assuming corresponding carbamate toxins of GTX2, GTX3, GTX1, GTX4, STX and neoSTX. Acid treatment was performed with 0.1 N hydrochloric acid for 15 min in boiling water. The HPLC results were expressed in relative amount of each toxin on molar basis (mol%).

## 3. Results and discussion

As Table 1 shows, six strains of the toxic dinoflagellate were isolated from the coastal water of the Seto Inland Sea in Hiroshima Prefecture. At first, four strains isolated from Uchiura Bay, which belong to the eastern part of coastal waters in Hiroshima Prefecture, Bingo-Nada, central region of the Seto Inland Sea, in 16 November 2004, 18 October 2005, 16 August 2003 and 14 June 2004, designated ATY041106, ATY051018, AC030816, and AC040614, were rather unexpectedly were identified as *A. tamiyavanichii* (formerly called *A. cohrticula*) and *A. catenella*. Seawater temperature at 0 m depth in Uchiura Bay, when the sea water sample was collected, was 20.0, 23.0, 26.0 and 19.0 °C, respectively. As for natural population densities of *A. tamiyavanichii* in 16 November 2004 and 18 October 2005, both were 40 cells/L, respectively.

The extracts of all strains isolated in the present study killed mice with typical symptoms associated with PSP toxins. Specific toxicities of these strains are summarized in Table 1. The specific toxicities of two *A. tamiyavanichii* strains were different from each other (ATY041106, $38.7 \times 10^{-6}$ MU/cell; ATY051018, $111.5 \times 10^{-6}$ MU/cell). These toxicity scores seemed to be several times or much higher than that of two *A. catenella* strains (AC030816, $4.5 \times 10^{-6}$ MU/cell; AC040614, $4.1 \times 10^{-6}$ MU/cell), isolated in the same area. However, paralytic toxicity from the bivalves such as mussels, clams and oysters in this and adjacent area was not detected by mouse bioassay at all. These phenomena were observed in the Gulf of

Table 1
Toxic dinoflagellate isolated from the coastal water of Hiroshima Prefecture, Japan

| Date/year | Isolation locale[a] (0 m depth) | Dinoflagellate | Strain no. | Toxicity ($\times 10^{-6}$ MU/cell) |
|---|---|---|---|---|
| November 16/2004 | Uchiura Bay (Eastern coast) | *Alexandrium tamiyavanichii* | ATY041106 | 38.7 ± 10.9 |
| October 18/2005 | | | ATY051018 | 111.5 |
| August 16/2003 | | *A. catenella* | AC030816 | 4.5 ± 2.8 |
| June 14/2004 | | | AC040614 | 4.1 ± 1.1 |
| April 20/2004 | Kure Bay (Western coast) | *A. tamarense* | AT040420 | 33.0 ± 12.5 |
| April 13/2005 | | | AT050413 | 3.7 ± 1.4 |

[a] Refer to Fig. 1.

Thailand. During monitoring *Alexandrium* spp. in the Gulf, four *Alexandrium* species, *A. fraterculus*, *A. leei* and *A. tamiyavanichii*, were found to occur, though no significant shellfish toxicity was observed (Fukuyo et al., 1989).

By the way, isolation of *A. tamiyavanichii* from the Gulf of Thailand during a monitoring survey of toxic dinoflagellates and confirmation of its PSP production by culture experiments was reported, suggesting that this is a tropical species (Kodama et al., 1988). This species was originally found in the phytoplankton samples from the Bay of Mexico (Balech, 1967). Judging from all of these facts, *A. tamiyavanichii* has been considered to be a tropical or subtropical species so far. However, in 1988, this species was found in phytoplankton samples collected from Japanese coastal water, Aburatsubo in Sagami Bay, Japan, in November and its PSP production was confirmed (Ogata et al., 1990). Specific toxicity of these strains was in the range of 0.8–1.0 MU/$10^4$ cells. In addition, the first PSP-infestation of bivalves in the southeast coastal water of the Seto Inland Sea, Harima-Nada, Kagawa Prefecture, in early December 1999 and the isolation of A. *tamiyavanichii* with the specific toxicity as 7200 cells/MU, whose score is equivalent to $138.9 \times 10^{-6}$ MU/cell, from the Straits of Naruto was reported (Hashimoto et al., 2002; Yoshimatsu et al., 2001). This toxicity score is almost the same as that of the strain ATY051018 isolated in the present study. The Seto Inland Sea is surrounded by three major islands (Honshu, Shikoku and Kyushu) of Japan and is connected to the open ocean by only a few narrow straits such as Kitan, Naruto, Hoyo and Kanmon Straits. Though PSP compositions of this strain were not examined, its specific toxicity indicates the possibility of identity of our strain with the strain isolated from the Straits of Naruto. From a series of these studies including our present study, this species also seemed to be distributed in not only tropical or subtropical but also temperate water. In this connection, prior to this episode, PSP-infestation of green mussel *Perna viridis* due to the same species of dinoflagellate was confirmed in Okinawa Prefecture in early summer, 1996 (Koja et al., 2001).

Not only detection of PSP-producing phytoplankton *A. tamiyavanichii* in Bingo-Nada, the eastern part of coastal sea in Hiroshima Prefecture but also toxification of the bivalves in this area has not been reported so far. From the present results, there is a possibility of the contamination of bivalves during late fall to winter in this area with blooms of *A. tamiyavanichii* in near future. This is a huge threat to massive oyster culture area, Hiroshima Bay, belong to the western coastal waters of Hiroshima Prefecture, where cultured oysters are harvested mainly during late fall to winter.

On the other hand, two strains isolated at 0 m depth in Kure Bay, which is a part of Hiroshima Bay, in the eastern part of coastal waters in Hiroshima Prefecture, in April, 2004 and 2005, designated ATKR040420($33.0 \times 10^{-6}$ MU/cell) and ATKR050413($3.7 \times 10^{-6}$ MU/cell) were identified as *A. tamarense*, respectively (Table 1). At this time, seawater temperature at 0 m depth in Kure Bay was 15.7 and 12.0 °C, respectively. Cells density of this dinoflagellate on that time was 4.0 and 0.6 cells/mL. Specific toxicities of these two *A. tamarense* strains were much lower than those of two *A. tamiyavanichii* strains isolated in the present study, along with the data on other strains of *A. tamarense* reported so far (Asakawa et al., 1995,2005). In this connection, *A. tamiyavanichii* and *A. catenella* were not observed in this bay. In this area, *A. tamarense* has been mainly responsible for PSP (Asakawa et al., 1995,2005) since 1992.

Table 2 shows the PSP profiles of cultured cells of toxic dinoflagellate isolated in the coastal waters of the Seto Inland Sea in Hiroshima Prefecture. In *A. tamiyavanichii*, the amounts of C1 and 2 were only 20.6%. On the contrary, more than 70% of toxins were composed of C1 and 2 in *A. tamarense* and *A. catenella* regardless of the place of their collection. This may be the reason why specific toxicity of *A. tamiyavanichii* is higher than that of *A. tamarense* and *A. catenella*. The PSP composition of *A. tamiyavanichii* strain isolated in Harima-Nada, Kagawa Prefecture, in early December 1999 mentioned above show similar tendency. The total amounts of C1 and 2 were only 2.0% (Hashimoto et al., 2002).

The relative abundance of β-epimers of 11-hydroxysulfate toxins (C2, GTX3, GTX4) always exceeded those of α-epimers(C1, GTX2, GTX1). In many *Alexandrium* species, this phenomenon has been observed in common (Hall et al., 1990; Oshima et al., 1990; Asakawa et al., 1995,2005). As for *A. tamiyavanichii* (ATY041106), the PSP toxins exist almost exclusively as β-epimers (C2, GTX3, GTX4), which accounted for 72.7 mol%. The toxin profiles of this strain are featured by the presence of a large amount of GTX3 (59.1 mol%) in comparison with the PSP compositions of

Table 2
Toxin compositions of PSP-producing dinoflagellates isolated from the coastal water of Hiroshima Prefecture. Japan

| Area | Uchiura Bay[a] | | | Kure Bay[a] | |
|---|---|---|---|---|---|
| Dinoflagellate | *Alexandrium tamiyavanichii* | *A. catenella* | | *A. tamarense* | |
| PSP Components | ATY041106 | AC030816 | AC040614 | AT040420 | AT050413 |
| C1 | 8.1 | 16.2 | 32.6 | 21.8 | 4.0 |
| C2 | 12.5 | 80.5 | 46.3 | 61.2 | 88.6 |
| GTX1 | 9.3 | 0.8 | 1.3 | 5.5 | 0.6 |
| GTX2 | 9.9 | 1.0 | 9.2 | 5.1 | 0.5 |
| GTX3 | 59.1 | Trace | 0.6 | 2.3 | 3 |
| GTX4 | 1.1 | 1.3 | 9.6 | 1.5 | 0.2 |
| neoSTX | 0 | 0.2 | 0.4 | 2.6 | 3.1 |

All results are shown in mol%. Trace: less than 0.1%.
[a] Refer to Fig. 1.

*A. catenella* and *A. tamarense*. In addition, it revealed that the toxin profiles of two *A. catenella* strains (AC030816, AC040614) exist almost exclusively as β-epimers (C2, GTX3, GTX4), which accounted for 81.8 and 56.6 mol%, as the same manner. The toxin profiles of these strains are featured by the presence of a large amount of C2 (80.5, 46.3 mol%) in comparison with the PSP compositions of *A. tamiyavanichii*.

Until the 1980s, in Japanese coastal waters, contamination of bivalves with PSP by toxic dinoflagellates had been restricted to some areas such as the Hokkaido and Tohoku regions. But since the 1990s, toxic dinoflagellates have bloomed at new areas in western and eastern Japan. In Hiroshima Bay, PSP outbreaks have occurred almost every year (Asakawa et al., 1993, 2005). The causative organisms of PSP in Japan are mostly *A. tamarense*, *A. catenella* and *G. catenatum* as shown in many reports on the occurrence of these dinoflagellates. (Asakawa et al., 2005; Fukuyo, 1985; Noguchi et al., 1990; Takatani et al., 1998a,b). As for *A. tamiyavanichii*, there was no report on the occurrence of toxic dinoflagellate in eastern coastal waters of the Seto Inland Sea, Hiroshima Prefecture. In early December 1999, a bloom due to *A. tamiyavanichii*, which has not been reported so far as causative plankton for PSP infestation of bivalves, occurred around the southeast coast of the Seto Inland Sea in Kagawa Prefecture, resulting in PSP toxification of mussels *Mytilus edulis* and ark shell *Anadara broughtonii* (Hashimoto et al., 2002). These data including our results shows that it is also distributed in temperate waters. In addition to this, the present paper is the first record of harmful influence of *A. tamiyavanichii* in Hiroshima Prefecture. The present study adds *A. tamiyavanichii* to the list of possible causative organisms of PSP in Hiroshima Prefecture. Actually, in Hiroshima Bay, the western part of coastal water, this species has not been detected until now.

Bivalves are suddenly infested with PSP, causing a serious damage to fisheries and related industries. In late autumn of 1988, the scallop *P. yessoensis* was suddenly and unexpectedly infested with PSP in Funka Bay, Hokkaido, where is one of the representative scallop culture areas in Japan, causing a serious damage to fisheries and its related industries. The responsible dinoflagellate was identified as *A. catenella*, whose lethal potency was estimated to be $2.5 \times 10^4$ cells/MU. Scallops became toxic with the highest toxicity score exceeding 400 MU/g digestive gland. Before 1988, scallops have extensively been infested with *A. tamarense* in late spring to early summer in almost every year, but never in autumn or winter. So is the case with Hiroshima Bay. In this connection, from the results of feeding the cultured *A. tamiyavanichii* cells to green mussel to examine toxin accumulation, this dinoflagellate is harmful enough to make bivalves inedible in a short time, when they are exposed to the bloom (Wisessang et al., 1991). Judging from the history of other areas where PSP toxification or blooms of toxic dinoflagellate has occurred, these episodes tend to occur repeatedly every year. Therefore, attention should be paid to this species as well as the other causative dinoflagellates throughout the coastlines in Hiroshima Prefecture. It would allow farmers to harvest seafood products before they can become contaminated with dinoflagellate toxins, relocate aquaculture stocks to non-affected areas, and to adjust marketing strategies.

Finally, it is very important to monitor *A. tamiyavanichii* strictly in addition to *A. tamarense* and *A. catenella*,, especially in fall to early winter, because a large number of culture oysters are harvested in Hiroshima Bay.

## References

Asakawa, M., Miyazawa, K., Noguchi, T., 1993. Studies on paralytic shellfish poison (PSP) toxification of bivalves in association with appearance of *Alexandrium tamarense*, in Hiroshima Bay, Hiroshima Prefecture. J. Food Hrg. Soc. Jpn. 34, 50–54.

Asakawa, M., Miyazawa, K., Takayama, H., Noguchi, T., 1995. Dinoflagellate *Alexandrium tamarense* as the source of paralytic shellfish poison (PSP) contained in bivalves from Hiroshima Bay, Hiroshima Prefecture, Japan. Toxicon 33, 691–697.

Asakawa, M., Takayama, H., Beppu, R., Miyazawa, M., 2005. Occurrence of paralytic shellfish poison (PSP)-producing Dinoflagellates *Alexandrium tamarense* in Hiroshima Bay, Hiroshima Prefecture, Japan, during 1993–2004 and its PSP profiles. J. Food Hyg. Soc. Jpn. 46, 246–250.

Balech, E., 1967. Dinoflagellate nuevos o intersantes del Golfo de Mexico. Revist. Mus. Argent. Cienc. Nat. Ber. Riv. Hidrobiol. 2, 77–129.

Daigo, K., Uzu, A., Arakawa, O., Noguchi, T., Seto, H., Hashimoto, K., 1985. Isolation and some properties of neosaxitoxin from a xanthid crab *Zosimus aeneous*. Nippon Suis. Gakk. 51, 309–313.

Fukuyo, Y., 1985. Morphology of *Protogonuaulax tamarensis* (Lebour) Taylor and *Protogonyaulax catenella* (Whedon and Kofoid) Taylor from Japanese coastal waters. Bull. Mar. Sci. 37, 529–537.

Fukuyo, Y., Yoshida, K., Ogata, T., Ishimaru, T., Kodama, M., Pholpunthin, P., Wisessang, S., Phanichyakarn, V., Piyakarnchana, T., 1989. Suspected causative dinoflagellates of paralytic shellfish poisoning in the Gulf of Thailand. In: Okaichi, T., Anderson, D.M., Nemoto, T. (Eds.), Red Tides:Biology, Environmental Science, and Toxicology. Elsevier, New York, pp. 403–406.

Hall, S., Strichartz, G., Moczydloski, E., Ravindran, A., Reichardt, P.B., 1990. The saxitoxin: sources, chemistry, and pharmacology. The saxitoxins: sources, chemistry and pharmacology. In: Hall, S., Strichartz, G. (Eds.), Marine Toxins – Origin, Structure and Molecular Pharmacology. American Chemical Society, Washington, DC, pp. 29–65.

Harada, T., Oshima, Y., Kamiya, H., Yasumoto, T., 1982. Confirmation of paralytic shellfish toxins in the dinoflagellate *Pyrodinium bahamense* var *compressa*. Nippon Suisann gakkaishi 48, 821–825.

Hashimoto, K., Noguchi, T., 1989. Recent studies on paralytic shellfish poison in Japan. Pure. Appl. Chem. 61, 7–18.

Hashimoto, T., Matsuoka, S., Yoshimastsu, S., Miki, K., Nishibori, N., Nishio, S., Noguchi, T., 2002. First paralytic shellfish poison (PSP) infestation of bivalves due to toxic dinoflagellate *Alexandrium tamiyabanichii*, in the southeast coasts of the Seto, Inland Sea, Japan. J. Food Hyg. Soc. Jpn. 43, 1–5.

Ikeda, T., Matsuno, S., Sato, S., Ogata, T., Kodama, M., Fukuyo, Y., Takayama, H., 1989. First report on paralytic shellfish poisoning caused by *Gtmnodinium catenatum* Graham (Dinophyceae) in Japan. In: Okaichi, T., Anderson, D.M., Nemoto, T. (Eds.), Red Tides: Biology, Environmental Science, and Toxicology. Elsevier, New York, pp. 411–414.

Ito, K., Asakawa, M., Beppu, R., Takayama, H., Miyazawa, K., 2004. PSP-toxification of the carnivorous gastropod Rapana venosa inhabiting the estuary of Nikoh River, Hiroshima Bay, Hiroshima Prefecture, Japan. Mar. Pollut. Bull. 48, 1116–1121.

Kawabata, T., 1978. Assay method for paralytic shellfish poison. Food Hygiene Examination Manual. In: Environmental Health Bureau, Ministry of Health and Welfare, vol. 2. Japan Food Hygiene Association, Tokyo, pp. 240–244.

Kodama, M., Ogata, T., Fukuyo, Y., Ishimaru, T., Wisessang, S., Saitanu, K., Panichyakarn, V., Piyakarnchana, T., 1988. *Protogonyaulax cohorticula*, a toxic dinoflagellate found in the Gulf of Thailand. Toxicon 26, 707–712.

Koja, A., Tamanaha, K., Abe, Y., Oshiro, N., Teruya, N., 2001. Studies on paralytic shellfish poisons in Okinawa Prefecture II. Okinawaken Eisei Kankyo Kenkyujyo Shoho 35, 59–61.

Noguchi, T., Kohno, M., Ueda, Y., Hashimto, K., 1981. Isolation of gonyautoxin-2, a main component of paralytic shellfish poison from toxic scallop and its properties. J. Chem. Soc. Jpn. 5, 652–658.

Noguchi, T., Asakawa, M., Arakawa, O., Fukuyo, Y., Nishio, S., Tanno, K., Hashimoto, K., 1990. First occurrence of Alexandrium catenella in Funka Bay, Hokkaido, along with its unique toxin composition. In: Graneli, E., Sundstrom, B., Edler, L., Anderson, D.M. (Eds.), Toxic Marine Phytoplankton. Elsevier, New York, pp. 493–498.

Ogata, T., Pholpunthin, P., Fukuyo, Y., Kodama, M., 1990. Occurrence of *Alexandrium cohoticula* in Japanese coastal water. J. Appl. Phycol. 2, 351–356.

Oshima, Y., Hasegawa, H., Yasumoto, T., Hallegraeff, G.S., Blackburn, S., 1987. Dinoflagellate *Gymnodinium catenatum* as the source of paralytic shellfish toxins in Tasmanian shellfish. Toxicon 25, 1105–1111.

Oshima, Y., Sugino, K., Itakura, H., Hirota, M., Yasumoto, T., 1990. Comparative studies on paralytic shellfish toxin profile of dinoflagellates and bivalves. In: Graneli, E., Sundstrom, B., Edler, L., Anderson, D.M. (Eds.), Toxic Marine Phytoplankton. Elsevier Science Publishing, New York, pp. 391–396.

Takatani, T., Morita, T., Anami, A., Akaeda, H., Kamijyo, Y., Tsutsumi, K., Noguchi, T., 1998a. Appearance of *Gymnodinium catenatum* in association with the toxification of bivalves in Kamae, Oita Prefecture. Jpn. J. Food Hyg. Soc. Jpn. 39, 275–280.

Takatani, T., Akaeda, H., Kaku, T., Miyamoto, M., Mukai, H., Noguchi, T., 1998b. Paralytic shellfish poison infestation to oyster Crassostrea gigas due to dinoflagellate *Gymnodinium catenatum* in the Amakusa Islands, Kumamoto Prefecture. Jpn. J. Food Hyg. Soc. Jpn. 39, 292–295.

Wisessang, S., Ogata, T., Kodama, M., Fukuyo, Y., Ishimaru, T., Saitanu, K., Yongvanichi, T., Piyakarnchana, T., 1991. Accumulation of paralytic shellfish toxins by green mussel *Perna viridis* by feeding on cultured cells of *Alexandrium cohorticula* isolated from the Gulf of Thailand. Nippon Suisann Gakkaishi 57, 127–131.

Yoshimatsu, S., Ochi, Y., Ueda, T., Yamanishi, S., Miki, K., 2001. An annual report at 1999 edited by Kagawa Akashiwo Research Institute. Kagawaprefecture, 13–15.

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研究ノート

# 西表島産イワスナギンチャク *Palythoa tuberculosa* の毒性について

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## Toxicity of *Palythoa Tuberculosa* Collected on the Reef of Iriomote Island

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## 緒　言

イワスナギンチャク *Palythoa tuberculosa* は，軟質サンゴの一種である腔腸動物門花虫網スナギンチャク目のイワスナギンチャクの一種で，本州中部沿岸以南，トカラ列島，ミクロネシア，ベトナム沿岸，インド洋，紅海に分布し，低潮線付近より数m深の，サンゴ礁の浅海の岩に着生している。各ポリプの大きさは不同で，高さ10－20㎜，直径6－10㎜であり，やや紅褐色や黄色のものがある[1]。*P. tuberculosa* は，我々がしばしば目にする動物でもなく，一般にはあまりなじみがない生物であるが，1960年代に本種より極めて致死活性の高い毒であるパリトキシン(PTX)が発見されたことにより，世界の天然物化学者からの注目を集めた[2]。

PTXは強心作用を有する毒で，その分子量は2680であり，糖やアミノ酸，核酸の反復構造を含まない生体高分子としては最大の部類に入る[3]。その毒性は，フグ毒テトロドトキシンの約20倍で，静脈注射による50%致死量は25 ng/kg（ウサギ）～450 ng/kg（マウス）である。

現在では，PTXおよびその関連毒はオウギガニ科のヒロハオウギガニ *Lophozozymus pictor*，ウロコオウギガニ *Demania scaberrima* などの毒ガニ，紅藻ハナヤナギ *Chondria armata*，カワハギ科のソウシハギ *Aluterus scriptus*，また，ミクロネシアでシガテラ魚とされているモンガラカワハギ科のクロモンガラ *Melichthys vidua* などにおいて存在が確認されている[2]。

*P. tuberculosa* の毒性に関する報告は，国内では沖縄県石垣島や奄美大島に生息するもののみであり，その他の地域の本種の毒性に関しては報告例が無いため，本研究では西表島に生息する本種に含有されるPTX成分について調べた。

## 方　法

### 材料

2006年6月に沖縄県西表島（図1）で採取したイワスナギンチャク150 gを試料とした。試料は採取後，直ちに凍結し，四国大学短期大学部に送付し，実験に供するまで－30℃で冷凍保管した。なお，供試する際には流水中で急速解凍した。

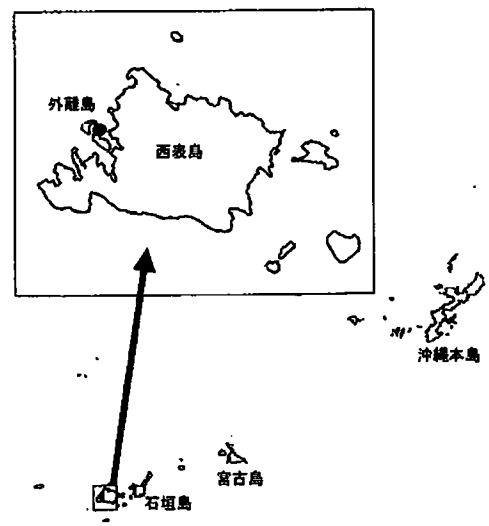


図1　イワスナギンチャクの採取場所（●）

試験液の調整

試験液の調製は，Taniyamaら[4]の方法に準拠した。

試料に3倍量の酢酸酸性75%エタノール（pH 3.5）加えて10分間ホモジナイズし，遠心分離（10,000 g，20分間，室温）して上清を得た。残渣については同様の作業を2回繰り返して上清を合一した。次いで上清（粗抽出液：150 ml）に等量のジエチルエーテルを加え，脱脂して水画分（50 ml）とジエチルエーテル画分（100 ml）に分画後，前者を水：1-ブタノール（1：1）で分配し，1-ブタノール画分（50 ml）と水溶性画分（100 ml）とし，粗抽出液ならびに各画分を試験液とした（図2）。

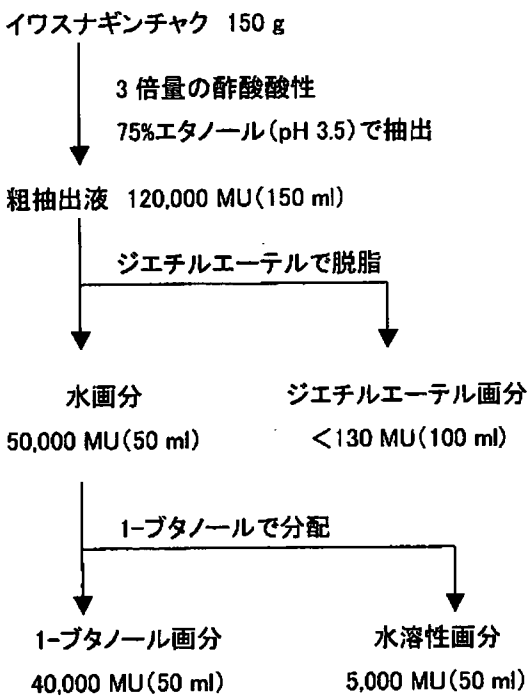


図2　イワスナギンチャクからの活性画分の精製

マウス毒性試験

マウス毒性試験はTaniyamaらの方法[5),6)]に準じて行った。各試験液をddY系雄マウスに1 ml腹腔内投与して48時間観察し，生死を確認した。本研究において，1マウス単位(mouse unit：MU)は供試マウス1尾を約48時間で死亡させる毒量と定義した[6)]。

試験液の前処理（固相抽出）

各試験液2 mlにつき，有毒画分をメタノールと蒸留水で平衡化した2種のOASIS MAX 3 ccまたはOASIS MAX 6 cc(Waters)ミニカラムに吸着させ，2%アンモニア水と100%メタノールで洗浄後，1%酢酸-80%メタノールで溶出させた[7)]。次いで，溶出液をメタノールと蒸留水で平衡化したSep-Pak C18(Waters)ミニカラムに付し，蒸留水，20%メタノール，50%メタノールおよび80%メタノールで順次洗浄し，100%メタノールで有毒成分を溶出させ，HPLC分析に供した。

HPLC分析

カラムにPurospher STAR RP-8e(φ2 mm×250 mm, Merck)を使用した。移動相Aに0.1%ギ酸-20%アセトニトリル，移動相Bに0.1%ギ酸-80%アセトニトリルを用い，移動相Aから移動相Bに60分間かけて切替えるリニアグラジエント法を用い，流速を0.2 ml/minとした。有毒成分の検出にはPTX標品特有の紫外部極大吸収の263 nm[8)]を使用した。PTX標準品は和光純薬工業株式会社製を使用した。

## 結果および考察

マウス毒性試験

イワスナギンチャク150 g相当の粗抽出液の毒力は，120,000 MU，水画分は50,000 MU，ジエチルエーテル画分は130 MU未満，1-ブタノール画分は40,000 MU，水溶性画分は5,000 MUであった（いずれもPTX換算）（図2）。

固相抽出

まず，OASIS MAX 3 ccミニカラムを用いて粗抽出液（1 g試料相当量/ml）を固相抽出に供したところ，非吸着画分で80 MU，2%アンモニア水洗浄画分で20 MU，100%メタノール洗浄画分で200 MU未満，1%酢酸-80%メタノール溶出画分で100 MUの毒力を示し，回収率はそれぞれ5%，1%，13%，6%であった。水画分（3 g試料相当量/ml）につ