Medicinal Food and Marine Toxins Analysis

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Highlight

1. Authentication of Chinese medicinal food, using Cordyceps sinensis as an example
2. Briefing on the control of common marine toxins in seafood
Introduction of GL
Administration

Food and Health Bureau

- Food and Environmental Hygiene Department
- Department of Health
- Agricultural, Fisheries and Conservation Department
- Government Laboratory

No. of staff: ~500, Founded in 1913
HQ & Satellite Labs

1. Headquarters, Homantin
2. Meteorological Station
3. Public Works Central Laboratory Building
4. LCK Govt. Offices
5. Public Health Laboratory
6. Food Safety Laboratory
7. Science & Technol. Parks
   (Hong Kong Chinese Materia Medica Standards)
Analytical Services

Foods
Chinese medicines
DNA
Dangerous chemicals
Pharmaceuticals
Commodities
Consumer products
Legal measures
Environment
Forensic Services

Scene of Crime Investigation

Trace Contact Evidence

DNA

Questioned Documents

Physical Investigation

Controlled Drugs

Forensic Toxicology
Our Role

To provide advisory and scientific testing services to various government departments in Hong Kong to uphold:

- Law and Order
- Public Health and Food Safety
- Environmental Protection
- Consumer Protection
- Government Revenue
1. Chinese Medicinal Food

- What is Cordyceps??
- Endoparasitic fungus

**HOST**

**WHOLE FUNGUS**

**CARCASS**

**stroma**

**Development of mycelia**

**Infection**

**Growth**
Cordyceps

- Over 400 species, distributed worldwide. Mostly abundant in tropical forests

  Cordyceps cicadea (cicada)
  Cordyceps unilateralis (ant)
  Cordyceps formosana (beetle)
  Cordyceps amazonica (grasshopper)
Cordyceps Sinensis

- Winter worm
- Summer grass or caterpillar fungus

**SPRING**
- Germination

**DEVELOPMENT OF MYCELIA**

**SUMMER**
- Infected by spores

**WINTER**
- EGG
  - GHOST MOTH
- LARVA
  - 5-year
- PUPA

- Winter worm summer grass or caterpillar fungus
Cordyceps Sinensis

- Well known Chinese medicinal herb, used as food or dietary supplement to improve immune system & body health today
- Having an appearance of a plant and an worm
- Only found in Tibet Highland
Cordyceps Sinensis

- Ghost moths live in Tibet area at 4,500 to 6,000 m above sea level.
Cordyceps Sinensis

- Has been used for more than 1,500 years as an important Chinese and Tibetan medicine
- Became popular when two Chinese female broke the world records in 1500, 3000 & 10000 meters running in 1993
- Negative doping, but admitted taking cordyceps sinensis
- Since then, the price has sky-rocketed. It increased > 1000% within 10 years from 2003. Retail price is ~ USD 35,000 per kg
Cordyceps Sinensis

- Hunting for fungus in spring to summer time
# Fake Items

## Fungus and Insect Host

<table>
<thead>
<tr>
<th>Fungus and Insect Host</th>
<th><img src="https://example.com/fake_items_fungus_insect_host.jpg" alt="Image" /></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cordyceps militaris + Lepidoptera pupa</td>
<td><img src="https://example.com/fake_items_cordyceps_militaris.jpg" alt="Image" /></td>
</tr>
<tr>
<td>Cordyceps liangshanensis + Lepidoptera larva</td>
<td><img src="https://example.com/fake_items_cordyceps_liangshanensis.jpg" alt="Image" /></td>
</tr>
<tr>
<td>Cordyceps ophioglossoides</td>
<td><img src="https://example.com/fake_items_cordyceps_ophioglossoides.jpg" alt="Image" /></td>
</tr>
<tr>
<td>Cordyceps hawkesii + Lepidoptera larva</td>
<td><img src="https://example.com/fake_items_cordyceps_hawkesii.jpg" alt="Image" /></td>
</tr>
<tr>
<td>Cordyceps barnesii + Lepidoptera larva</td>
<td><img src="https://example.com/fake_items_cordyceps_barnesii.jpg" alt="Image" /></td>
</tr>
<tr>
<td>Cordyceps ramosa + Lepidoptera larva</td>
<td><img src="https://example.com/fake_items_cordyceps_ramosa.jpg" alt="Image" /></td>
</tr>
</tbody>
</table>

## Others

<table>
<thead>
<tr>
<th>Others</th>
<th><img src="https://example.com/fake_items_others.jpg" alt="Image" /></th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant Radix Stachys geobombycis &amp; Radix Stachys sieboldii</td>
<td><img src="https://example.com/fake_items_plant_radix_stachys.jpg" alt="Image" /></td>
</tr>
<tr>
<td>Flour, plaster and pigments</td>
<td><img src="https://example.com/fake_items_flour_plaster_pigments.jpg" alt="Image" /></td>
</tr>
</tbody>
</table>
# Genuine vs Fake

<table>
<thead>
<tr>
<th></th>
<th>Genuine</th>
<th>Fake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>2 pairs of feet close to the head and tail are flat, middle 4 pairs are more obvious</td>
<td>Feet are neatly arranged; or no foot</td>
</tr>
<tr>
<td>Colour</td>
<td>Natural brown</td>
<td>Turbid, pale yellow</td>
</tr>
<tr>
<td>Odour</td>
<td>Mushroom flavour</td>
<td>Wood-like smell</td>
</tr>
<tr>
<td>Immerse in water</td>
<td>Colour remains</td>
<td>Lose pigment, sticky</td>
</tr>
</tbody>
</table>
How about these?

- Commercial products: Powder in capsules and many claim to contain *cordyceps sinensis*. Really?
- Relied on a DNA-based method
Workflow of DNA Sequence Analysis for Cordyceps Authentication

Raw materials or powder → DNA Extraction → PCR Amplification of DNA markers → Sequence Analysis

*Cordyceps sinensis*
- Partial ribosomal RNA gene (ITS1) ~350bp
- Partial RNA polymerase II largest subunit gene (RPB1) ~700bp
<table>
<thead>
<tr>
<th>No.</th>
<th>Products</th>
<th>Sample nature</th>
<th>Claims</th>
<th>DNA Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Raw material</td>
<td></td>
<td>Cordyceps sinensis</td>
<td>Fungus from the genus <em>Metarhizium</em></td>
</tr>
<tr>
<td>2</td>
<td>Powder</td>
<td></td>
<td>Cordyceps sinensis</td>
<td><em>Cordyceps sinensis</em></td>
</tr>
<tr>
<td>3</td>
<td>Powder</td>
<td></td>
<td>100% Cordyceps</td>
<td><em>Tolypocladium inflatum</em></td>
</tr>
<tr>
<td>4</td>
<td>Powder</td>
<td></td>
<td>Cordyceps Mycelia</td>
<td><em>Paecilomyes genus</em></td>
</tr>
<tr>
<td>5</td>
<td>Powder</td>
<td></td>
<td>100% Cordyceps Mycelia</td>
<td>Fungus from the family <em>Trichocomaceae</em></td>
</tr>
</tbody>
</table>
About 100 catties (~ 60.5 kg) of suspected fake dried abalone slices from 31 dried seafood retail shops

The items carrying trade descriptions of:

- High Quality Abalone slices
- Australian abalone slices
- Japanese abalone slices
- American abalone slices
- South African abalone slices

All slices were found to be of CONCH origin.

A total of 33 retailers and 5 wholesalers were convicted and fined a total of HKD446,000 (£36,000). Some convicted parties were sentenced to 4 months and 1 month suspended imprisonment.
(ii) Dried Deer Tendon

Fifty one pieces of suspected fake dried deer tendons (~£65 each) from several dried food retailers

Deer (2/51)

Cattle (41/51)

Water buffalo (2/51)

Deer + Cattle (6/51)

The retailers shops were fined a total sum of HKD 338,500 (£27,000).
Chinese Medicinal Foods

DNA tests assist authentication

- Fish maws
- Shark fins
- Sea cucumber
- Edible Bird’s nest
- Ginseng
- Crocodile meat
2. Marine Toxins

- Most marine toxins are originated from toxic planktonic algae, some are from bacteria. Among the 5,000 species of marine planktonic algae, some 300 species can cause red tides, while only ~80 species have the capacity to produce potent toxins.

- Marine toxins can accumulate in seafood through the food chain.

- **Shellfish Toxins**
  - Ciguatoxins
  - Tetrodotoxin
Shellfish Toxins

Five main types of shellfish toxins

- Paralytic Shellfish Poisoning Toxins, PSP
- Amnestic Shellfish Poisoning Toxins, ASP
- Neurotoxic Shellfish Poisoning Toxins, NSP
- Diarrhetic Shellfish Poisoning Toxins, DSP
- Azaspiracid Shellfish Poisoning Toxins, AZP
Ciguatoxins

- Ciguatoxins that cause ciguatera poisoning are produced by dinoflagellates.

- Particularly high concentrations in some large predatory tropical coral fish.

- Grouper, Sea Bass, Snapper and Barracuda are commonly associated with ciguatoxin poisoning.
Three different classes of Ciguatoxins have been identified.

- Pacific Ciguatoxins (neurological symptoms predominate)
- Caribbean Ciguatoxins (gastrointestinal symptoms are a dominant feature)
- Indian Ciguatoxins (cause a cluster of symptoms reminiscent of hallucinatory poisoning)
Tetrodotoxin

- Tetrodotoxin (TTX) has been isolated from different animal species, including newts, toads, blue-ringed octopuses, several sea stars and certain angelfish. Also common in puffer fish, a delicacy in Asia.
  - TTX is roughly 100 times more poisonous than potassium cyanide (LD$_{50}$: 334 µg/kg)
  - TTX block the sodium ion channel, causes paralysis of voluntary muscles and loss of sensation.
Control of Marine Toxins in HK

FAO Report (2011):
Consumption: 505,553 tonnes seafood > 70 kg per person per year

Ranking per capita:
2\textsuperscript{nd} in Asia
7\textsuperscript{th} in the world
Control of Marine Toxins in HK

- **Routine Surveillance Programme**
  - Shellfish Toxins

- **Ad-hoc Projects**
  - In response to overseas food alert to conduct risk assessment ad-hoc project on other marine toxins

- **Urgent Testing Services**
  - In response to local food incident to provide urgent testing services to prevent further spreading of the questionable seafood to the market and consumers
  - Testing scope: Shellfish toxins, Tetrodotoxin and Ciguatoxins (in 2014)
Ciguatera in HK

280 Cases, 800+ persons were affected
Ciguatoxins Analysis

- Ciguatoxin Standards
  - Ciguatoxin standards cannot be directly obtained from algae.
  - It should only be extracted from fish containing ciguatoxin.
  - The production cost of ciguatoxin is extremely high.
  - US$1 per 1 ng (i.e. 10ug ⇒ US$10,000)

- Pacific Ciguatoxin standards obtained from a toxin research team at the University of Queensland, Australia

- Conventional quantification method (using calibration curve) is not suitable for Ciguatoxin analysis
Screening was done by comparing the responses of the MRM peaks of P-CTX1, P-CTX2 and P-CTX3 in sample to the corresponding peak responses (control points) set out by the positive control samples.

The control point was a cut-off between a “negative” and a “positive” result, is set at 0.06 μg/kg.

Any positive sample will be quantified by using standard addition method.
MRM Chromatogram

MRM Spectrum of Squaretail Grouper (西星斑) - Spike at Control Point (0.06 ppb in sample)
Previous Case: Tetrodotoxin in Grilled Grouper Snack

- In a routine DNA sequencing testing of snack, a sample of dried fish meat was found not to match its claim. It contained pufferfish (*Lagocephalus lunaris*) instead of grouper.

- Suspect food item might also contain tetrodotoxin.

- LC-MS/MS showed that the sample contained tetrodotoxin ranging from 0.31 µg to 1.7 µg/kg.

- Centre for Food Safety immediately recalled and warned the public to stop consuming the product.
THANK YOU