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# Rapid Detection of Aflatoxin by Serological Methods

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## ABSTRACT

Antiserum was produced by using the following antigens: *Aspergillus flavus* (AFC), non aflatoxin producing, isolated in Thailand; *A. flavus* (AFB 5), high aflatoxin producing, isolated in Thailand; *A. parasiticus* (AP 827), high aflatoxin producing, isolated in U.S.A.; Aflatoxin B1 (AF). After collecting normalserum, antigens were prepared and injected into 5 rabbits. Antisera were collected at weekly intervals 10-20 times. Only low titers of antisera could be detected by precipitation reaction on slides. The antisera were not sufficient for use in ELISA. Preliminary tests using ALFA CHECK a product by UBE Industries Ltd, demonstrated clearly the high sensitivity of ELISA for Aflatoxin detection. AFB 1 was detected at concentrations as low as 4 ppb in 20 minutes.


## INTRODUCTION

It has been reported that aflatoxins can be detected by serological methods. The improvement of antiserum production and detection of aflatoxin by immunological methods has been investigated (Chu and Ueno, 1977). Kawamura et al. (1988) reported that monoclonal antibody has been produced against aflatoxin B1. Enzyme - linked immunosorbent assay (ELISA) combined with monoclonal antibody for estimation of aflatoxin B1 in peanut has been successful (Itoh & et al., 1987; Kawamura & et al., 1988).

So far these method have not been applied successfully in Thailand. A reliable and sensitive method is required for Thailands export quality control of maize. Currently only the time consuming and expensive method of thin-layer chromatography is used.

Our research aim is the development of ELISA technique for rapid aflatoxin detection.

## MATERIALS AND METHODS

### 1. Antigen production

The following antigens were selected:

1.1 *A. flavus*, non aflatoxin producing, isolated from maize in Thailand. (code = AFC)

1.2 *A. flavus*, high aflatoxin producing, isolatedfrom maize in Thailand. (code - AFB 5)

1.3 *A. parasiticus*, high aflatoxin producing isolated in U.S.A. (code = AP 827)

1.4 Aflatoxin B1 (AF).

The fungi were cultured on potato dextrose agar (PDA). Antigen of 10, 240 ppb aflatoxin was obtained in the laboratory, Department of Plant Pathology, Kasetsart University.

### 2. Antiserum production

Antigens AFC, AFB 5 and AP 827 were cultured on PDA, prepared by grinding 1 plate of each fungus in 1 ml phosphate buffered saline (PBS: 0.1 M neutral phosphate buffer + 0.15 NaCl). Aflatoxin antigen was diluted from 10,240 ppb to 204.8 ppb.

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Normalserum was bled from the ear of a rabbit before the injection of antigen. The first injections of antigens (Table 1) were prepared by mixing 1.0 ml. of antigen in 1.0 ml. of complete adjuvant. This was then injected intramuscularly into a rabbit. The following injections of antigen were prepared by mixing 1.0 ml. of antigen in 1.0 ml. incomplete adjuvant and injected intramuscularly again. The interval of injection was 3 days for a total of three times. Ten days after the last injection of antigen, antiserum was collected at weekly intervals for 10-20 times.

**3. Titer of antisera**

Antisera of AFC, AFB 5, AP 827, B1 (AF) and AP 827 + B1 (AF) were diluted in a two fold dilution step from 1:2 to 1:512. Antigens were diluted with PBS to 1:10 and 1:100. The reactions of antigen and specific antiserum were observed after an incubation period of 30 minutes in a precipitation on slide test.

Some antisera were selected and used to detect *A. flavus* with ELISA, as described by clark et al. (1974); Korpraditskul, (1979).

**4. Detection of aflatoxin**

Preliminary test for AFB 1 detection using Afla-Check products from UBE Industries, Ltd.

a) Kinds of samples
mathanol 55%
AFB 1 4 ppb (standard)
AFB 1 40 ppb (standard)
AFB 1 25 ppb
AFB 1 50 ppb
Corn sample No. 1
Corn sample No. 2
AFB 1 Benzene

b) The ELISA step instructions give by UBE industries, Ltd were followed (Anonymous, 1989).

## RESULT

**1. Antigen production**

The fungi were cultured on PDA for 10-20 days. Mixing suspension of fungi and complete ior incomplete adjuvant showed green-colloidial suspension.

**2. Antiserum production**

The injected rabbits looked healthy and produced useful volumes of serum. Antisera were bled from each infected rabbit and collected at weekly intervals 11-20 times.

**3. Titer of antisera**

Only very low titer of antibody against AFC, AFB 5, AP 827, B1 (AF) and AP 827 + B1(AF) could be detected by a precipitation on slide test. (Appendix 1, 2, 3, 4 and 5). In microprecipitin tests antisera showed homologous titiers of 1:6 for AP 1:32 for AP 827 + B1 (AF) and no titer for B1 (AF).

ELISA tests conducted with selected antisera produced in our laboratory showed a negative reaction.

**4. Detection of aflatoxin**

Result of Preliminary test for AFB 1 using Afla-Check products from UBE Industries, Ltd. are listed in Table 2

Result show that ELISA can be successfully used to detect AFB 1 at levels as low as 4 ppb.

## DISCUSSION

The very successful detection of aflatoxins with ELISA quich test kit using purchased serum showed the high potential of this method for applica-

**Table 1 Identification numbers of rabbiis and date of injecting.**

| Rabbet identification No. | Antigens injected | First injection date |
|---|---|---|
| 125 | AFC | 18 April, 1988 |
| 133 | AFB 5 | 18 April, 1988 |
| 161 | AP 827 | 18 April, 1988 |
| 153 | B1 (AF) | 28 June, 1988 |
| 151 | *AP 827+B1 (AF) | 28 June, 1988 |

* Antigen is prepared by mixing AP 827 1 ml. and BI (AF) 102.4 ppb. 1 ml.

**Table 2 Preliminary ELISA test for AFB 1 in Corn and standard sample**

| Kind of Sample | Absorbance |
|---|---|
| methanol 55% | 0.895 |
| AFB 1 4 ppb (standard) | 0.658 |
| AFB 1 40 ppb (standard) | 0.242 |
| AFB 1 25 ppb | 0.029 |
| AFB 1 50 ppb | 0.040 |
| Corn sample No.1 | 1.055 |
| Corn sample No.2 | 1.032 |
| AFB 1 Benzene | 0.341 |

**Appendix 1 Observation of antibody titers of AFC (rabbit No. 125) with precipitation method.**

| No. | Date | Titer slide method | vol. (ml) |
|---|---|---|---|
| Ns | 5. 7.87 | - | 13 |
| As 1 | 2. 5.88 | 1:0 | 15 |
| As 2 | 9. 5.88 | 1:0 | 17 |
| As 3 | 16. 5.88 | 1:0 | 16 |
| As 4 | 23. 5.88 | 1:0 | 11 |
| As 5 | 31. 5.88 | 1:4 | 13 |
| As 6 | 6. 6.88 | 1:0 | 16 |
| As 7 | 13. 6.88 | 1:0 | 11 |
| As 8 | 20. 6.88 | 1:4 | 16 |
| As 9 | 27. 6.88 | 1:16 | 14 |
| As 10 | 6. 7.88 | 1:32 | 17 |
| As 11 | 11. 7.88 | 1:16 | 11 |
| As 12 | 18. 7.88 | 1:0 | 15 |
| As 13 | 25. 7.88 | 1:32 | 16 |
| As 14 | 8. 8.88 | 1:16 | 13 |
| As 15 | 29. 8.88 | 1:4 | 12 |
| As 16 | 5. 9.88 | 1:8 | 15 |
| As 17 | 12. 9.88 | 1:8 | 15 |
| As 18 | 19. 9.88 | 1:8 | 15 |
| As 19 | 26. 9.88 | 1:0 | 15 |
| As 20 | 3.10.88 | 1:0 | 16 |
| As 21 | 10.10.88 | 1:8 | 15 |

**Appendix 2 Observation of antibody titers of AFB 5 (rabbit No. 133) with precipitation method.**

| No. | Date | Titer slide method | vol. (ml) |
|---|---|---|---|
| Ns | 5. 7.87 | - | 10 |
| As 1 | 2. 5.88 | 1:0 | 15 |
| As 2 | 9. 5.88 | 1:0 | 14 |
| As 3 | 16. 5.88 | 1:0 | 14 |
| As 4 | 23. 5.88 | 1:0 | 19 |
| As 5 | 31. 5.88 | 1:8 | 18 |
| As 6 | 6. 6.88 | 1:4 | 19 |
| As 7 | 13. 6.88 | 1:8 | 15 |
| As 8 | 20. 6.88 | 1:0 | 15 |
| As 9 | 27. 6.88 | 1:8 | 16 |
| As 10 | 11. 7.88 | 1:16 | 10 |
| As 11 | 18. 7.88 | 1:32 | 16 |
| As 12 | 25. 7.88 | 1:16 | 17 |
| As 13 | 1. 8.88 | 1:16 | 15 |
| As 14 | 8. 8.88 | 1:0 | 13 |
| As 15 | 29. 8.88 | 1:0 | 14 |
| As 16 | 5. 9.88 | 1:4 | 15 |
| As 17 | 12. 9.88 | 1:0 | 16 |
| As 18 | 19. 9.88 | 1:4 | 17 |
| As 19 | 27. 9.88 | 1:4 | 16 |
| As 20 | 3.10.88 | 1:0 | 17 |
| As 21 | 10.10.88 | 1:8 | 18 |
| As 22 | 17.10.88 | 1:4 | 18 |
| As 23 | 24.10.88 | 1:0 | 8 |
| As 24 | 31.10.88 | 1:0 | 13 |

**Appendix 3 Observation of antibody titers of AP 827 (rabbit No. 161) with precipitation method.**

| No. | Date | Titer slide method | vol. (ml) |
|---|---|---|---|
| Ns | 15. 7.87 | - | 16 |
| As 1 | 2. 5.88 | 1:0 | 12 |
| As 2 | 9. 5.88 | 1:0 | 18 |
| As 3 | 16. 5.88 | 1:0 | 13 |
| As 4 | 23. 5.88 | 1:0 | 17 |
| As 5 | 31. 5.88 | 1:16 | 15 |
| As 6 | 6. 6.88 | 1:16 | 17 |
| As 7 | 13. 6.88 | 1:8 | 18 |
| As 8 | 20. 6.88 | 1:0 | 17 |
| As 9 | 27. 6.88 | 1:8 | 17 |
| As 10 | 6. 7.88 | 1:16 | 15 |
| As 11 | 11. 7.88 | 1:4 | 19 |
| As 12 | 18. 7.88 | 1:8 | 18 |
| As 13 | 25. 7.88 | 1:0 | 15 |
| As 14 | 1. 8.88 | - | 16 |
| As 15 | 8. 8.88 | 1:4 | 15 |
| As 16 | 29. 8.88 | 1:4 | 17 |
| As 17 | 5. 9.88 | - | 16 |
| As 18 | 12. 9.88 | 1:8 | 13 |
| As 19 | 19. 9.88 | 1:8 | 15 |
| As 20 | 27. 9.88 | - | 15 |
| As 21 | 4.10.88 | - | 15 |
| As 22 | 12.10.88 | 1:4 | 15 |
| As 23 | 19.10.88 | - | 17 |
| As 24 | 26.10.88 | - | 16 |

**Appendix 4 Obervation of antibody titers of B1 (AF) (rabbit No. 153) with precipitation method.**

| No. | Date | Titer slide method | vol. (ml) |
|---|---|---|---|
| Ns | 10. 3.88 | - | 15 |
| As 1 | 11. 7.88 | 1:0 | 13 |
| As 2 | 18. 7.88 | 1:0 | 16 |
| As 3 | 25. 7.88 | 1:0 | 16 |
| As 4 | 1. 8.88 | 1:0 | 15 |
| As 5 | 8. 8.88 | - | 16 |
| As 6 | 29. 8.88 | - | 16 |
| As 7 | 5. 9.88 | - | 15 |
| As 8 | 12. 9.88 | 4 | 15 |
| As 9 | 19. 9.88 | 1:0 | 15 |
| As 10 | 27. 9.88 | 1:0 | 15 |
| As 11 | 4.10.88 | 1:0 | 17 |
| As 12 | 12.10.88 | - | 18 |
| As 13 | 19.10.88 | - | 16 |
| As 14 | 26.10.88 | 1:0 | 16 |
| As 15 | 2.11.88 | - | 15 |
| As 16 | 9.11.88 | 1:4 | 15 |
| As 17 | 16.11.88 | - | 14 |
| As 18 | 23.11.88 | - | 12 |
| As 19 | 30.11.88 | - | 8 |
| As 20 | 7.12.88 | - | 8 |

**Appendix 5 Observation of antibody titers of AP827 + B1(AF) (rabbit No. 151) with precipitation method.**

| No. | Date | Titer slide method | vol. (ml) |
|---|---|---|---|
| Ns | 15. 3.88 | - | 16 |
| As 1 | 17. 7.88 | 1:0 | 15 |
| As 2 | 18. 7.88 | 1:0 | 15 |
| As 3 | 25. 7.88 | - | 15 |
| As 4 | 1. 8.88 | 1:4 | 13 |
| As 5 | 8. 8.88 | - | 13 |
| As 6 | 29. 8.88 | - | 15 |
| As 7 | 5. 9.88 | - | 14 |
| As 8 | 12. 9.88 | 1:4 | 15 |
| As 9 | 19. 9.88 | 1:4 | 16 |
| As 10 | 26. 9.88 | 1:16 | 16 |
| As 11 | 4.10.88 | 1:16/32 | 16 |
| As 12 | 12.10.88 | 1:32 | 18 |
| As 13 | 19.10.88 | 1:16/32 | 17 |
| As 14 | 26.10.88 | 1:0 | 12 |
| As 15 | 2.11.88 | - | 16 |
| As 16 | 9.11.88 | 1:4 | 10 |
| As 17 | 16.11.88 | - | 11 |
| As 18 | 23.11.88 | 1:0 | 15 |
| As 19 | 30.11.88 | 1:0 | 8 |
| As 20 | 7.12.88 | - | 2 |

tion in quality control. The local production of antiserum is the only limiting factor and requires further research especially the production of monoclonal antibody anainst AFB 1.

## LITERATURE CITED

Anonymous. 1989. Afla-Check Aflatoxin B1 Quick Test Kit. UBE Industries, Ltd. Japan. 18 p.

Chu, F.S. and E. Ueno, 1977. Production of antibody againt aflatoxin B1. Applied and Environmental Microbiology. 33 (5) : 1125-1128.

Clark, M.F., A.N. Adams and D.J. Barbara. 1976. The detection of plant viruses by enzyme-linked immunosorbent assay (ELISA). Acta Hort. 67 : 43-49.

Itoh, Y., E. Hifumi, K. Sudoh, T. Uda, K. Ohtani, O. Kawamura, S. Nagayama, S. Satoh and Y. Ueno. 1987. Detection of aflatoxin B1 by direct ELISA. Proc. Jpn. Assoc. Mycotoxicol. No. 26 : 31-35.

Korpraditskul, P. 1979. Untersuchungen Zur differenzierung von isolaten des cymbidium virus mit serologischen, elektronenmikroskopischen und biologischen Verfahren unter besonderer Berucksichtigung des enzyme-linked immunosorbent assay (ELISA). Dissertation Zur Erlangung des Doktorgrades der Landwirtschaftlichen Fakultat der George-August Universitat der Gottingen. 128 p.

Kawamura, O., S. Nagayama, S. Sato, K. Ohtani, I. Ueno and Y. Ueno. 1988. A monoclonal antibody-based enzyme-linked immunosorbent assay of aflatoxin B1 in peanut products. Mycotoxin Research 4 : 75-88.