

Detection of *Fusarium* sp. contamination in corn by enzyme-linked immunosorbent assay

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(Received 7 February 2006; accepted 26 February 2006)

Abstract

Fusarium verticillioides is a primary corn pathogen and one of the main producers of fumonisins, a group of mycotoxins that cause several diseases in animals and probably also affect humans. An indirect competitive enzyme-linked immunosorbent assay (ic-ELISA) based on polyclonal antibodies was developed to detect the exoantigen of this fungus in corn and its correlation with traditional methods for mould detection was evaluated. Forty freshly harvested corn samples were analysed for *F. verticillioides* exoantigens, as well as for total mould count, ergosterol and fumonisin levels in order to evaluate the relationship between these parameters. In addition, *F. verticillioides* was grown in brain heart infusion (BHI) broth in order to evaluate the correlation between biomass and exoantigen concentration. There was no significant correlation between exoantigen concentration and total mould count, *Fusarium* sp. count and fumonisin levels. The correlation coefficient between exoantigens and ergosterol content was 0.52 and between biomass and *F. verticillioides* exoantigens in BHI broth was 0.84. These results suggest that this ic-ELISA has potential for *Fusarium* sp. detection in corn samples.

Keywords: *F. verticillioides*, exoantigens, fumonisins, enzyme-linked immunosorbent assay, corn.

Introduction

Fusarium verticillioides (Sacc.) Nirenberg (= *Fusarium moniliforme* Sheldon) is a primary corn pathogen and one of the main fumonisin producers. Twenty-eight fumonisin analogues have been characterized so far, however FB₁, FB₂ and FB₃ occur as natural contaminants at significant levels in corn and corn-based products and FB₁

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is the most toxic and abundant (Gelderblom et al. 1988, Musser & Plattner 1997, Rheeder et al. 2002).

Ingestion of corn and corn-based products contaminated with fumonisins is a matter of concern due to association with leukoencephalomalacia in equine, pulmonary oedema in swine, weight loss and immunosuppression in poultry (Gelderblom et al. 1991, Nagaraj et al. 1994, Mallmann et al. 1999). Epidemiologic studies suggest their association with human oesophageal and liver cancer (Gelderblom et al. 1992, Ueno et al. 1997).

High incidence of *F. verticillioides* and fumonisins in corn, associated with serious risks to human and animal health, require an effective control of the mould and mycotoxin contamination.

Fusarium species identification is complex due to the morphological variability in culture and mutations caused by successive subculturing. The traditional methods for mould identification and detection are based on culture in several media, microscopic examination and chemical analysis of chitin, ergosterol or secondary metabolites (Jarvis et al. 1983). Except for the identification of secondary metabolites (including mycotoxins), most of these methods have low specificity and reproducibility, and are time-consuming (Li et al. 2000). On the other hand, immunological methods have several advantages, such as high sensitivity, specificity and potential for use in rapid quality control. The development of the enzyme-linked immunosorbent assay (ELISA) (Engvall & Pearlmann 1972) could be a promising alternative to the traditional methods (Lin et al. 1986). Several ELISAs have been developed for detecting and identifying moulds with variable success (Notermans et al. 1986, Tsai & Yu 1997, 1999, Chen et al. 2000, Yong & Cousin 2001, Eibel et al. 2005, Yucel et al. 2005), however a limited number have been developed for the detection of *Fusarium* species in foods (Gan et al. 1997, Abramson et al. 1998, Iyer & Cousin 2003, Park et al. 2003).

The aims of this study were to develop an ELISA based on polyclonal antibodies for *Fusarium* sp. detection in corn and evaluate its correlation with traditional methods (total mould count and ergosterol assay) for mould detection and fumonisin levels.

Material and methods

Reagents and material

Microtitre plates (96 wells, flat bottom) were purchased from Corning Costar Co., USA, the anti-rabbit IgG-peroxidase conjugate, hydrogen peroxide, 3,3',5,5'-tetramethylbenzidine and fumonisin standards (FB₁ and FB₂) were from Sigma Co., USA. The solid-phase extraction cartridges (tC₁₈ Sep-Pak Vac, 3 ml column volume, 500 mg of sorbent, and Sep Pak accell plus QMA) were from Waters Co., USA. Methanol, acetonitrile and isopropanol (HPLC grade) were from J. T. Baker, USA. Ergosterol standard was purchased from Fluka, Germany. Other reagents were analytical grade.

Fungal culture

F. verticillioides strain 97K was isolated from feed sample involved in animal intoxication outbreaks and provided by the Department of Food and Drug Technology (State University of Londrina, Paraná, Brazil).

Samples

A total of 40 freshly harvested corn (*Zea mays* L.) samples were collected directly from the trucks at the delivery post of a co-operative in the Northern region of the Paraná State, Brazil, during February and March 2004 crop. Each sampling was carried out by collecting 400–500 g of corn from 9–12 points of the truck bin to obtain a total of 4–5 kg, and the samples were classified following the Brazilian guideline protocol (Brasil 1976). A homogenized 1 kg portion of the sample, with initial moisture content between 14.3 and 19.0%, was sent to the laboratory and maintained at 4°C for no more than 7 days for microbiological analysis. For the determination of exoantigens, ergosterol and fumonisins, 200 g of corn kernels were ground to 50 mesh in a laboratory mill and maintained at –20°C until use.

Polyclonal antibody production

F. verticillioides 97K was used for the production of exoantigens and polyclonal antibodies as described by Biazon et al. (2006).

Briefly, aliquots of 1 ml spore suspensions (10^7 spores/ml) were inoculated into 1-l Erlenmeyer flasks containing 250 ml brain-heart infusion (BHI) broth. The cultures were incubated at 28°C in a rotary shaker (180 rpm) for 14 days and inactivated by 0.02% thimerosal at 4°C for 24 h. The cultures were then filtered through two layers of Whatman No. 1 filter paper and centrifuged at $4500 \times g$ at 4°C for 20 min. The supernatants containing the exoantigens were freeze-dried, stored at –20°C and later dissolved in 0.15M phosphate-buffered saline (PBS) and dialyzed for 24 h at 4°C against deionised water and PBS (two final changes). The dialyzates were used to immunize three young male New Zealand rabbits (Biazon et al. 2006). Pre-immunization blood samples from the rabbits were negative to *F. verticillioides* exoantigens in indirect ELISA. The exoantigen mixture was adjusted to give a protein concentration of 320 µg/ml and then mixed (v/v) with an equal amount of Freund's incomplete adjuvant. Each rabbit was immunized subcutaneously with three injections of exoantigens (0.5 ml containing 80 µg proteins) at one-week intervals. One week after the 3rd injection, the rabbits were bled and the sera were titred by indirect ELISA (Biazon et al. 2006).

Total mould count and exoantigen extraction from corn samples

Ground corn samples (10 g) were blended with 90 ml sterile 0.15M PBS and serial dilutions were carried out in tubes with 9.0 ml of the same diluent until 10^{-4} . One ml of each dilution was transferred into Petri dishes and pour-plated with 25 ml potato dextrose agar (PDA, pH 4.0) containing chloramphenicol (50 µg/ml) and incubated at 28°C for 7 days. After fungal counting, genera identification was carried out according to Nelson et al. (1983), Singh et al. (1991) and Samson et al. (1995).

The remaining corn suspension was filtered through Whatman No. 1 filter paper and stored at –20°C for further quantification of *F. verticillioides* exoantigens.

Exoantigen quantification

Exoantigen quantification in corn samples was carried out by indirect competitive ELISA (ic-ELISA).

The microtitre plate wells were coated with 100 µl *F. verticillioides* 97K exoantigens (0.4 µg/well) in 0.2M carbonate/bicarbonate buffer pH 9.6, at 4°C for 16 h. After washing three times with PBS-0.05% Tween 20 (PBS-T), the wells were blocked with 150 µl 1% skim milk in PBS for 3 h at 25°C. After washing with PBS-T, 50 µl anti-*F. verticillioides* 97K immune serum and 50 µl corn sample extracts were incubated for 16 h at 4°C. After washing with PBS-T, 100 µl anti-rabbit IgG-peroxidase conjugate were added and incubated at 25°C for 1.5 h. After washing with PBS-T, 100 µl substrate solution (H₂O₂/3,3',5,5'-tetramethylbenzidine) were added and incubated for 30 minutes at 25°C. The reaction was stopped by adding 50 µl 1M H₂SO₄ and absorbance was determined at 450 nm (ELISA ELX 800 Reader – Bio Tek Instruments Inc.). Average absorbance was calculated from triplicate wells and results were expressed as the percentage of binding:

$$\text{Binding (\%)} = \frac{A^+}{A^-} \times 100$$

Where A⁺ is the mean absorbance in the presence of soluble exoantigens and A[−] is the mean absorbance in their absence.

The detection limit was estimated as the minimum concentration over 22% inhibition (3-fold SD of 0 ng exoantigens ml^{−1}) detected by ic-ELISA (Iijima et al. 1996).

Exoantigen recoveries were determined by spiking 10 g of ground corn in duplicate at three concentrations (10, 20 and 30 µg/g), in triplicate analysis.

F. verticillioides culture for biomass and exoantigen determination

Aliquots of 1 ml *F. verticillioides* 97K spore suspension (10⁷ spores/ml) were grown in BHI broth (50 ml), at 28°C in a rotary shaker (180 rpm) for different periods (1–7 days), in order to evaluate the correlation between biomass and exoantigen concentration. Uninoculated flasks of BHI were used as controls. Separate flasks of inoculated medium were analysed every 24 h for exoantigens (ic-ELISA) and biomass (mycelial dry weight).

Ergosterol analysis

Ergosterol content in corn samples was determined by high performance liquid chromatography (HPLC) according to Gessner and Schmitt (1996) with modification.

Ground corn samples (2 g) were mixed with 60 ml 0.14M KOH in pure methanol and refluxed for 3 h. After cooling, the final volume of the extract was adjusted to 30 ml with 0.14M KOH in methanol and 5.0 ml 0.75M HCl were added. The extract was then submitted to clean-up procedure using a solid phase extraction cartridge (tC₁₈ Sep Pak Vac.). After conditioning the cartridge with 2.5 ml methanol, followed by 2.5 ml 0.12M KOH in methanol:0.75M HCL (6:1, v/v), the sample was added to the cartridge at a flow rate of 1.0 ml/min. The cartridge was washed with 2.5 ml 0.4M KOH in 60% methanol and dried under a stream of air for 50 min. Ergosterol was eluted into tared HPLC vials with 1.4 ml 0.1M KOH in pure isopropanol and 0.3 ml 1M KOH in pure methanol was added immediately. Ergosterol was analysed by a reversed-phase isocratic HPLC system (Shimadzu LC-10AD pump, CTO-10A oven and SPD-10A UV/VIS detector), using a Nucleosil 100-5 C₁₈ (250 mm × 4.6 mm,

Macherey Nagel – Germany) and the absorbance was determined at 282 nm. The eluent was 100% methanol at a flow rate of 1.5 ml/min, at 38°C. The detection limit for ergosterol was 10 ng/g.

Ergosterol recovery was evaluated by spiking ground corn (2 g) in duplicate at two concentrations (10 and 50 µg/g), in triplicate analysis.

Fumonisin analysis

Fumonisin B₁ and B₂ were analysed according to Shephard et al. (1990) with modification (Ueno et al. 1993). Ground corn samples (10 g) were mixed with 30 ml methanol:water (3:1, v/v). After standing for 10 min at room temperature, the suspension was shaken at 150 rpm for 30 min and centrifuged at 4500 × g for 10 min. The crude extract (1.0 ml) was applied to a preconditioned clean-up cartridge. After washing the cartridge with methanol:water (3:1, v/v, 6 ml) followed by methanol (3 ml), the fumonisins were eluted with 10 ml methanol containing 0.5% acetic acid. The eluate was evaporated to dryness under a stream of nitrogen at 45°C, the residue was dissolved in methanol:water (3:1, 800 µl) and a 200 µl aliquot dried under nitrogen. After derivatization with 200 µl O-phthalaldehyde reagent (40 mg OPA, 1 ml methanol, 5 ml 0.1M sodium borate and 50 µl 2-mercaptoethanol), HPLC injections were made within 1 min. Fumonisin were analysed by a reversed-phase isocratic HPLC system (Shimadzu 10AT pump and RF 10A fluorescence detector), using a Shim-pack CLC-ODS (M) column (4.6 × 250 mm, Shimadzu). Excitation and emission wavelengths were 335 nm and 450 nm, respectively. The eluent was CH₃OH:0.1 M NaH₂PO₄ (80:20, v/v) adjusted to pH 3.3 with ortho-phosphoric acid. The flow rate was 1 ml/min. The detection limits for FB₁ and FB₂ were 50ng/g and 80 ng/g, respectively.

Statistical analysis

The correlation between exoantigen concentration and *Fusarium* sp. count, total mould count, biomass, fumonisin and ergosterol levels was analysed by Pearson's correlation test. Statistical analysis was performed by STATISTICA software version 5.1 (Statsoft Inc., Tulsa, USA).

Results and discussion

Polyclonal antibodies raised against *F. verticillioides* exoantigens were used to develop an ic-ELISA for detecting *Fusarium* sp. in naturally contaminated corn samples. The detection limit of this assay was 1.6 µg/g. Cross-reactivities of polyclonal antibodies against exoantigens from 14 fungi species from other genera (*Aspergillus*, *Penicillium*, *Cladosporium*, *Mucor*, *Alternaria*, *Cercospora* and *Colletotrichum*) were low (4.5–14.7%). High cross-reactivities were observed for exoantigens from *F. graminearum*, *F. subglutinans* and *F. sporotrichioides* (51–76%) (Biazon et al. 2006).

Total mould and *Fusarium* sp. count, ergosterol, exoantigen and fumonisin levels in 40 freshly harvested corn samples from the Northern region of Paraná State, Brazil, are summarized in Table I. Total mould count ranged from 1.5×10^4 to 9.9×10^5 CFU/g (Table I), and 75% of the samples (Table II) showed high contamination (10^5 to $<10^6$ CFU/g). *Fusarium* sp. prevailed (100%) with mean contamination of 2.1×10^5 CFU/g and contamination in a range of 10^5 to $<10^6$ CFU/g was detected in

Table I. Total mould count, *Fusarium* sp. count, ergosterol, exoantigen and fumonisin levels in 40 freshly harvested corn samples from the Northern region of Paraná State, Brazil (February and March/2004 crop).

Parameter	Range	Mean
Total count (CFU/g)	1.5×10^4 – 9.9×10^5	2.8×10^5
<i>Fusarium</i> sp. count (CFU/g)	6.5×10^3 – 7.5×10^5	2.1×10^5
Ergosterol (µg/g)	0.8–31.7	7.2
Exoantigens (µg/g)	8.9–956.0	217.3
Fumonisin (µg/g)		
FB ₁ *	0.03–2.4	0.64
FB ₂ **	0.02–0.71	0.28
Total	0.03–2.4	0.8

*Fumonisin B₁; **Fumonisin B₂.

58% of the samples (Table II). *Penicillium* sp. and *Aspergillus* sp. were detected in 93% and 37% of the samples, respectively.

These results were similar to the data obtained by Ono et al. (1999) that showed higher contamination with *Fusarium* sp. (98.7–100%) and *Penicillium* sp. (93–100%) than with *Aspergillus* sp. (non-detected to 27.7%) in 150 freshly harvested corn samples from three regions of Paraná State, Brazil. Total mould count in a range from 10^5 to $<10^6$ CFU/g was detected in 55.5–66.3% of the samples (Ono et al. 1999). Almeida et al. (2000) analysed natural contamination in 66 corn samples from different regions of São Paulo State (Brazil), and *Fusarium* sp. were detected in 55.5–80% of the samples, with predominance of *F. verticillioides* (60.6%), while *Penicillium* sp. and *Aspergillus* sp. were detected in 40–50% and from 22.2–23.3% of the samples, respectively.

Fumonisin were detected in 100% (FB₁) and 52.5% (FB₂) of the samples, with levels ranging from 0.03–2.4 µg/g for FB₁ and 0.02–0.71 µg/g for FB₂ (Table I). These results showed lower fumonisin levels than previously obtained by Ono et al. (1999) and Camargos et al. (2000). Ono et al. (1999) detected FB₁ in 96.3–100% (range from 0.07–13.46 µg/g) and FB₂ in 63–100% of the samples (range from 0.08–6.92 µg/g). Camargos et al. (2000) evaluated the fumonisin occurrence in 23 samples cultivated at experimental stations in different regions of São Paulo State (Brazil) and these mycotoxins were detected in all samples at levels ranging from 1.63–25.79 µg/g (FB₁) and from 0.38–8.60 µg/g (FB₂).

Ergosterol is the main fungal sterol and plays an important role in cell membranes. This sterol was found to be a useful method to evaluate the fungal contamination in

Table II. Levels of mould contamination in 40 freshly harvested corn samples from the Northern region of Paraná State, Brazil (February and March/2004 crop).

CFU/g	10^2 – $<10^3$		10^3 – $<10^4$		10^4 – $<10^5$		10^5 – $<10^6$	
	AF*	RF** (%)	AF	RF (%)	AF	RF (%)	AF	RF (%)
Total count	–	–	–	–	10	25	30	75
<i>Fusarium</i> sp.	–	–	3	7	14	35	23	58
<i>Penicillium</i> sp.	1	2	10	25	17	43	9	23
<i>Aspergillus</i> sp.	9	22	5	13	1	2	–	–

*Absolute frequency; **Relative frequency.

Table III. Exoantigen recoveries from corn samples spiked at three levels.

Concentration ($\mu\text{g/g}$)	Recoveries (%) [*]
10	95.8 $\pm$ 4.2
20	80.7 $\pm$ 4.8
30	106.4 $\pm$ 13.4

^{*} Recoveries based on duplicate spiking and triplicate analysis by ic-ELISA.

grains, which can quantify total fungal biomass, whereas the plating technique detects only viable fungi (Gourama & Bullerman 1995).

Ergosterol content in 40 corn samples ranged from 0.8–31.7 $\mu\text{g/g}$, with mean value of 7.2 $\mu\text{g/g}$ (Table I). Abramson et al. (1998) reported a mean value of 16.87 $\mu\text{g/g}$ ergosterol in hard wheat and 14.25 $\mu\text{g/g}$ in soft wheat, both artificially contaminated. Mean recoveries of ergosterol from corn spiked at two concentrations were 89.6 $\pm$ 4.4% at 10 $\mu\text{g/g}$ and 96 $\pm$ 12.7% at 50 $\mu\text{g/g}$, based on duplicate spiking and triplicate analysis. These data were similar to Gessner and Schmitt (1996), who reported recoveries from 85.1–98.1% in samples of different plant tissues spiked with 114 μg ergosterol in 50 mg of sample. Moreover, recoveries from canola samples spiked at 10 $\mu\text{g/g}$ and 20 $\mu\text{g/g}$ were 103% and 90%, respectively (Abramson et al. 2005).

F. verticillioides exoantigen concentrations in corn samples ranged from 8.9–956.0 $\mu\text{g/g}$, with mean value of 217.3 $\mu\text{g/g}$ (Table I). Exoantigen recoveries from corn spiked at three concentrations, based on duplicate spiking and triplicate analysis, were 95.8% at 10 $\mu\text{g/g}$, 80.7% at 20 $\mu\text{g/g}$ and 106.4% at 30 $\mu\text{g/g}$ (Table III). Exoantigen concentration did not correlate with *Fusarium* sp. count ($r=0.11$; $p < 0.05$) nor with total mould count ($r=0.15$, $p < 0.05$) (Table IV). These results were similar to Yong and Cousin (2001) who developed a double antibody sandwich ELISA for detection of aflatoxin producer *Aspergillus* species in naturally contaminated corn and no correlation between exoantigens and total mould count was observed. This was probably due to differences in substrate composition and storage condition of the corn samples, since they were obtained from different sources (Yong & Cousin 2001). Furthermore, total mould count is not a precise method for total biomass estimation, taking into account that only the viable propagules can be

Table IV. Correlation matrices between total mould count, *Fusarium* sp. count, fumonisin levels and exoantigens in 40 freshly harvested corn samples from the Northern region of Paraná State, Brazil (February and March/2004 crop).

	Total count (CFU/g)	<i>Fusarium</i> sp. (CFU/g)	Fumonisin [*] ($\mu\text{g/g}$)	Exoantigens ($\mu\text{g/g}$)
Exoantigens ($\mu\text{g/g}$)	$\rho=0.15$ $p=0.349$	$\rho=0.11$ $p=0.516$	$\rho=0.05$ $p=0.760$	$\rho=1.00$ $p=—$
Total count (CFU/g)	$\rho=1.00$ $p=—$	$\rho=0.95$ $p=0.000$	$\rho=-0.02$ $p=0.928$	$\rho=0.15$ $p=0.349$
<i>Fusarium</i> sp. (CFU/g)	$\rho=0.95$ $p=0.000$	$\rho=1.00$ $p=—$	$\rho=0.07$ $p=0.663$	$\rho=0.11$ $p=0.516$
Fumonisin [*] ($\mu\text{g/g}$)	$\rho=-0.02$ $p=0.928$	$\rho=0.07$ $p=0.663$	$\rho=1.00$ $p=—$	$\rho=0.05$ $p=0.760$

^{*}Fumonisin: FB₁+FB₂; ρ =correlation; p =probability (correlations are significant at $p < 0.05$).

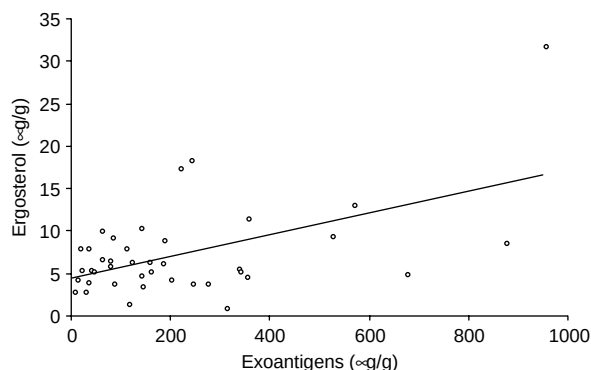


Figure 1. Correlation between ergosterol and exoantigen concentration in naturally contaminated corn samples ($n=40$). Linear regression equation ($y=4.4792+0.01243 \cdot x$) was obtained with correlation coefficient of 0.52. x and y represent exoantigen and ergosterol concentrations determined by ic-ELISA and HPLC, respectively.

detected and the degree of spore production affects the results of plate counts (Gourama & Bullerman 1995). On the other hand, there was a positive correlation between ergosterol content, viable plate count and exoantigens when corn and peanut samples were inoculated artificially with *Aspergillus parasiticus* (Yong & Cousin 2001).

The correlation coefficient between ergosterol and exoantigen concentration was 0.52 ($p < 0.05$) (Figure 1), but there was no correlation between fumonisin and exoantigen concentration ($r=0.05$, $p < 0.05$) (Table IV, Figure 2). Abramson et al. (1998) analysing *F. sporotrichioides* exoantigens by ic-ELISA, in hard and soft wheat samples artificially inoculated with *F. graminearum*, reported a correlation coefficient of 0.66 (hard wheat) and 0.81 (soft wheat) between exoantigens and ergosterol, and a correlation coefficient of 0.8 and 0.76 between deoxynivalenol and exoantigen concentrations.

The correlation coefficient in this study was lower than that obtained by Abramson et al. (1998), probably because naturally contaminated corn samples (different hybrids) collected from different areas were analysed instead of artificially inoculated samples. Fungal growth and mycotoxin production depend on crop susceptibility, mould toxigenicity and environmental factors, such as rainfall, temperature, relative humidity during plant development and crop harvest (Pestka & Casale 1990, Ono

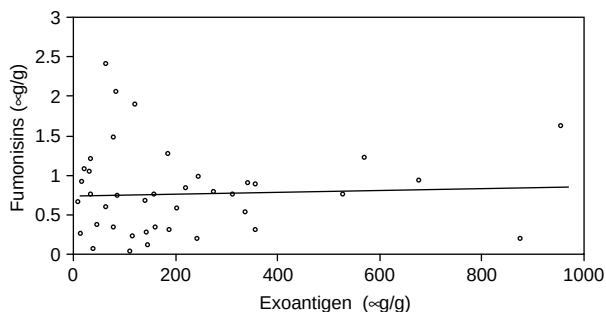


Figure 2. Relationship between fumonisin (FB_1+FB_2) levels, determined by HPLC, and exoantigen concentration, determined by ic-ELISA, in naturally contaminated corn samples ($n=40$).

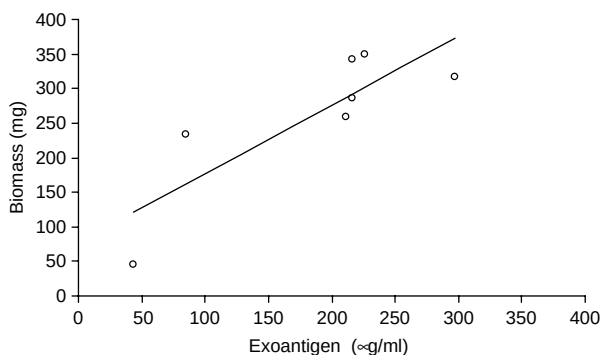


Figure 3. Correlation between biomass and exoantigen concentration for *F. verticillioides* 97K grown in BHI broth. Linear regression equation ($y = 78.197 + 0.99006 \cdot x$) was obtained with correlation coefficient of 0.84. x and y represent exoantigen concentration and biomass determined by ic-ELISA and mycelial dry weight, respectively. Circles represent the mean value from duplicate analysis.

et al. 1999). Studies with artificially contaminated samples minimize many of these factors; however they do not correspond to all the variability observed in naturally contaminated samples. In addition, ergosterol quantification indicates total mould contamination of samples, and the differentiation among genera or species is not possible. Moreover, different fungal species produce different levels of ergosterol, and the synthesis of this metabolite can be influenced by substrate composition, mycelial growth phase and storage conditions (Tothill et al. 1992). As shown in Table II, *Penicillium* sp. and *Aspergillus* sp. were detected in corn samples and the ELISA developed in this study detects only *Fusarium* species, showing low cross-reactivity (4.54–14.67%) with other fungal genera (*Aspergillus*, *Penicillium*, *Cladosporium*, *Mucor*, *Alternaria*, *Cercospora* and *Colletotrichum*) (Biazon et al. 2006).

F. verticillioides 97K was cultured in BHI broth in order to evaluate the relationship between biomass (dry mycelium weight) and exoantigen production, in different periods. The correlation coefficient between these parameters was 0.84 ($p < 0.05$) (Figure 3). Notermans et al. (1986) also demonstrated a correlation between dry mycelium weight and exoantigens analysed by an ELISA for detection of *Penicillium* species, *Mucor racemosus* and *Geotrichum candidum* inoculated in several fruit juices.

Research carried out so far had demonstrated that exoantigen immunoassays can be useful for the detection of several fungi in food and feed stuff. In this study, there was a positive correlation between *F. verticillioides* exoantigen and ergosterol content analysed in naturally contaminated corn samples, as well as between exoantigen concentration and biomass in BHI broth. Therefore, this ic-ELISA is promising as a screening method to detect *Fusarium* sp. contamination in corn samples.

Acknowledgements

The authors thank the CNPq (the Brazilian Government Organization for grant aid and fellowship to Brazilian researchers), the Araucária Foundation, Paraná Fund/SETI, FINEP and CAPES (Coordination for Formation of High Level Professionals) for the financial support.

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