# TOXIGENIC FUNGI IN BEANS (*PHASEOLUS VULGARIS* L.) CLASSES BLACK AND COLOR CULTIVATED IN THE STATE OF SANTA CATARINA, BRAZIL

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Submitted: April 16, 2001; Returned to authors for corrections: July 12, 2001; Approved: June 10, 2002

#### **ABSTRACT**

Toxigenic fungi were studied in beans (*Phaseolus vulgaris* L.) of Classes black and color, cultivated in different regions of the State of Santa Catarina, south region of Brazil. The mean counts of filamentous fungi were 2.8 x 103 and 6.7 x 103 CFU/g for beans Classes black and color, respectively. *Penicillium* spp., *Aspergillus* spp. and *Phoma* spp. were the most frequent genera isolated, followed by *Ryzopus* spp., *Alternaria* spp., *Helminthosporium* spp., *Cladosporium* spp., *Botrytis* spp., *Fusarium* spp., *Trichoderma* spp., *Curvularia* spp. and *Dreschelera* spp. Among beans Class black, 24.6% of the *Aspergillus* strains produced mycotoxins: 13.1% produced aflatoxins (AFs); 11.5% produced ochratoxin A (OTA) and 28.9% of *Penicillium* produced citrinin (CTR). On the other hand, 22.1% of *Aspergillus* strains isolated from beans Class color produced mycotoxins (16.7% produced AFs and 5.4% produced OTA), while *Penicillium* genera had 35.4% of CTR producing strains. The toxigenic species were *A. flavus*, *A. parasiticus*, *A. ochraceus* and *P. citrinum* Thom.

Key words: beans, Phaseolus, toxigenic fungi, aflatoxin, ochratoxin A, citrinin.

#### INTRODUCTION

Beans (*Phaseolus vulgaris* L.) originated from Latin America are grown and consumed mainly in Mexico, Central America, Peru, Equator, Bolivia and Brazil. They are cultivated in all Brazilian territory and there are three annual harvests. Brazil produces *ca.* 3.3 million tons of beans per year and most of the production comes from the South region, especially from the States of Paraná and Santa Catarina (3).

Beans cultivated in the State of Santa Catarina (SC) are mainly from the West, Mountain and South regions. However, the West region holds the highest production, with more than 120 thousand tones, which means *ca*. 38% of total SC production - 316 thousand tones in 1994/95 (3). It is important to emphasize that 60% of SC production is commercialized in other states of Brazil, especially, São Paulo.

Due to lack of appropriate management, agricultural products and their by-products, inclusive beans, may be exposed to high moisture and temperature during harvest and storage allowing fungi growth, leading to mycotoxin contamination (21).

The problem of food contamination with mycotoxins has led to an increasing concern of toxigenic fungi contamination, mainly *Aspergillus*, *Penicillium* and *Fusarium* genera. Widely spread in the Brazilian ecosystem, these fungi have been isolated from several food substrates, especially cereals, pulses and their by-products. Despite of that, there are only a few data on fungal contamination, their toxigenic potential and mycotoxins in pulses in Brazil, especially, on beans (4,5,7,22).

Considering that there is a lack of information on toxigenic fungi in the Brazilian varieties of beans, especially concerning to production region and storage conditions, this study was carried out to evaluate fungi contamination and their toxigenic potential for ochratoxin A (OTA), aflatoxins (AFs) and citrinin (CTR) in beans Classes *black* and *color*, grown in different regions of the Santa Catarina State (SC).

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#### MATERIALS AND METHODS

- (a) Samples: 72 samples of beans (1kg), Classes *black* and *color* (variety carioquinha) cultivated in six regions of Santa Catarina State, Brazil: Florianópolis, North, West, Mountain, South and Itajai Valley (Table 1). Fig. 1 shows the percentage of samples collected per region. The sample collection was carried out during one year (from Aug/97 to Aug/98) by the Laboratory of Classification of Vegetable Products (*Companhia Integrada de Desenvolvimento Agrícola de Santa Catarina* CIDASC). Sample collection was done in farms and cooperatives, following the Ministry of Agriculture official method of sampling for grading (2).
- **(b) Mycotoxin standards:** aflatoxins  $B_1$ ,  $B_2$ ,  $G_1$  and  $G_2$  (AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub> and AFG<sub>2</sub>), ochratoxin A (OTA) and citrinin (CTR), from Sigma.
- **(c) Equipment:** universal moisture meter, Model CA 125 (Gehaka Co), thin layer chromatography (TLC) apparatus (Desaga) and UV cabinet, 245 and 365nm (Dist).
- (d) Mycoflora determination: sub-samples (25g) of beans were ground and homogenized with 225 mL of peptone water (0.1%), followed by decimal dilution preparation. Aliquots of 0.2 mL of each dilution were inoculated onto the surface of potato dextrose agar (PDA) acidified with 10% tartaric acid to reach pH 3.5 + 0.1 (2 groups of 5 Petri plates each). After incubation (5 days at 25°C) the colonies of filamentous fungi and yeast were counted, isolated and identified up to genera using Samson *et al.* (19) methodology. Toxigenic strains of *Aspergillus* and *Penicillium* were identified up to species according to the taxonomic criteria of Raper and Fennell (17), Pitt (15,16), Barnett and Hunter (1) and Klich and Pitt (13) for OTA, AFs and CTR production.
- (e) Evaluation of toxigenic potencial: all strains of the isolated filamentous fungi from beans Classes *black* e *color* were tested for their ability to produce AFs, OTA and CTR in coconut agar at pH  $5.0\pm0.1$  for CTR and  $7.0\pm0.1$  for AFs and OTA (10,13,15). After incubation (6 days at 25°C), the Petri dishes were exposed to ultraviolet light at 365 nm in a dark cabinet to check fluorescence. Next, an amount of the agar that surrounded the fungal colony (mainly areas were the fluorescence was more intense) was taken out and the fluorescent compound extracted with organic solvent (chloroform). The extract was submitted to TLC for identification and confirmation of the toxins using the solvent system:toluene:ethyl acetate:chloroform:90%formic acid (35:25:25:10 v/v) and standards of AFs (AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub> and AFG<sub>2</sub>), OTA and CTR (8.9).
- **(f) Statistical analysis:** the analysis of variance was applied and the results were considered significant when P<0.05. The

Tukey test was used for analyses of variance for the two Classes (bean black and color) studied for total counts of filamentous fungi, toxigenic fungi and moisture content. The software used was Stat Soft  $^{TM}$ .

## RESULTS AND DISCUSSION

#### Total counts of fungi and yeast

As expected, all samples of beans presented fungi and only four of them were contaminated with yeasts. Only a few samples of beans Class *black* presented yeast contamination and in very low amounts (max 8.7 x 10<sup>2</sup> UFC/g) and none were isolated from Class *color*. As shown in Table 1, the positive samples were from Dionísio Cerqueira (West region), Mafra, Canoinhas (North region) and São Ludgero (South region).

814 strains of fungi were isolated (Tables 2 and 3). The mean total counts of fungi in beans Classes *black* and *color* were rather different, being slightly lower ( $2.8 \times 10^3 \, \text{UFC/g}$ ) in the first class than in the second ( $6.7 \times 10^3 \, \text{UFC/g}$ ). In the first group (class *black*), the lowest count was  $1.90 \times 10^2$  (North region, city of Mafra) and the highest  $1.99 \times 10^4$  (Itajaí Valley region, city of Ituporanga). In the second group (Class *color*), the minimum was  $1.0 \times 10^2$  (Valley region, city of Itajai) and the maximum  $4.95 \times 10^4$  (South region, city of São Ludgero).

As far as the regions studied and the amount of fungi contamination are concerned, the beans Class *color* from South region of SC were the ones that presented the highest mean total count for filamentous fungi (max.  $4.95 \times 10^4$  UFC/g) followed by West region (max.  $2.43 \times 10^4$  UFC/g). The samples of the Mountain region had the lowest levels (max.  $2.9 \times 10^2$  UFC/g). On the other hand, the region that presented the highest contamination for beans Class *black* were Itajai Valley (Ituporanga city with  $1.99 \times 10^4$ ) followed by West (city of Campos Novos with  $7.0 \times 10^3$  UFC/g).

It is important to emphasize that 4% and 8.5% of the beans Class *black* and *color*, respectively, presented mean total count of filamentous fungi and yeast higher than 10<sup>4</sup> UFC/g, which it is the maximum level allowed by ICMSF (11).

The moisture content (mc) of the beans for both Classes and the growth of filamentous fungi are shown in Table 1. A minimum of 13.8% and maximum of 20.9% of mc for samples Class *black* and minimum 14.0% and maximum of 22.4% for samples Class *color* were detected. The South region was the one that presented the highest levels of mc for both bean Classes, with averages of 17.5 and 21.0% for Class *black* and *color*, respectively. As expected, high fungi growth was observed in most of samples with high mc.

Only 20% of bean samples Class *black* and 24% Class *color* presented mc levels lower than the maximum limit allowed by the Brazilian regulation -15% (2). Therefore, *ca* 80% of the samples were above this limit, allowing fungi proliferation, which is of concern.

**Table 1.** Mean of total count of filamentous fungi, yeast and moisture content in beans (*Phaseolus vulgaris* L.) Classes *Black* and *Color* from the State of Santa Catarina, Brasil (1997 - 1998).

| C1    | Sampl                      | le collect         | Number _ | Fungi <sup>a</sup>  | Yeast <sup>a</sup> | mc d   |
|-------|----------------------------|--------------------|----------|---------------------|--------------------|--|
| Class | Region                     | City               | samples  | (CFU <sup>b</sup> / | $g \times 10^2)$   | (%)  |
| Black | West                       | Dionísio Cerqueira | 7        | 5.4                 | 3.2                | 13.8   |
|       |                            | Campos Novos       | 2        | 70.1                | $ND^{c}$           | 17.2   |
|       |                            | Aguas Frias        | 1        | 9.7                 | ND                 | 16.6   |
|       | North                      | Mafra              | 2        | 1.9                 | 8.7                | 14.8   |
|       |                            | Canoinhas          | 1        | 6.5                 | 1.2                | 19.1   |
|       | Mountain                   | S. José do Cerrito | 2        | 31.2                | ND                 | 17.4   |
|       | Itajaí Valey               | Ituporanga         | 1        | 199.0               | ND                 | 18.9   |
|       |                            | Blumenau           | 1        | 7.9                 | ND                 | 17.8   |
|       |                            | Itajaí             | 1        | 12.0                | ND                 | 17.3   |
|       |                            | Major Gercino      | 1        | 4.4                 | ND                 | 14.5   |
|       |                            | Nova Trento        | 1        | 3.2                 | ND                 | 16.5   |
|       | South                      | Araranguá          | 1        | 12.6                | ND                 | 16.5   |
|       |                            | São Martinho       | 1        | 24.8                | ND                 | 17.9   |
|       |                            | São Ludgero        | 2        | 26.8                | 1.1                | 20.9   |
|       |                            | Içara              | 1        | 5.4                 | ND                 | 13.8<br>17.2<br>16.6<br>14.8<br>19.1<br>17.4<br>18.9<br>17.8<br>17.3<br>14.5<br>16.5<br>16.5 |
|       | Total                      |                    | 25       |                     |                    |  |
| Color | West                       | Xanxerê            | 8        | 49.2                | ND                 | 14.8   |
|       |                            | Campos Novos       | 4        | 76.9                | ND                 | 15.9   |
|       |                            | Águas Frias        | 3        | 74.7                | ND                 | 18.4   |
|       |                            | Concórdia          | 2        | 29.4                | ND                 | 17.5   |
|       |                            | Lebom Régis        | 3        | 61.0                | ND                 | 22.1   |
|       |                            | Palmitos           | 2        | 1.6                 | ND                 | 17.5   |
|       |                            | São Lourenço       | 2        | 82.7                | ND                 | 17.0   |
|       |                            | Chapecó            | 2        | 8.0                 | ND                 | 14.3   |
|       |                            | Fraiburgo          | 2        | 2.8                 | ND                 | 16.4   |
|       |                            | Maravilha          | 2        | 7.1                 | ND                 | 15.0   |
|       |                            | Campo Êre          | 1        | 3.4                 | ND                 | 16.0   |
|       |                            | Caibi              | 1        | 35.8                | ND                 | 17.6   |
|       |                            | Cunha Porã         | 1        | 10.9                | ND                 | 14.5   |
|       |                            | Pinhalzinho        | 1        | 5.7                 | ND                 | 21.0   |
|       |                            | Tangará            | 1        | 243.0               | ND                 | 14.0   |
|       |                            | Joaçaba            | 1        | 129.0               | ND                 | 14.7   |
|       | Mountain                   | Curitibanos        | 1        | 1.5                 | ND                 | 17.0   |
|       |                            | Lages              | 1        | 2.9                 | ND                 | 15.5   |
|       | Itajaí Valley              | Pomerode           | 1        | 4.4                 | ND                 | 16.0   |
|       | -                          | Blumenau           | 2        | 34.8                | ND                 | 16.4   |
|       |                            | Itajaí             | 1        | 1.0                 | ND                 | 17.5   |
|       | Florianópolis <sup>e</sup> | São José           | 1        | 16.7                | ND                 |  |
|       | South                      | São Lugdero        | 2        | 495.0               | ND                 |  |
|       |                            | Içara              | 1        | 23.2                | ND                 |  |
|       |                            | Tubarão            | 1        | 272.0               | ND                 | 19.0   |
|       | Total                      |                    | 47       |                     |                    |  |

<sup>&</sup>lt;sup>a</sup> mean of samples; <sup>b</sup> colony forms units/gram; <sup>c</sup> not deleted by the methodology used; <sup>d</sup> Moisture content; <sup>e</sup> region of Florianopolis.

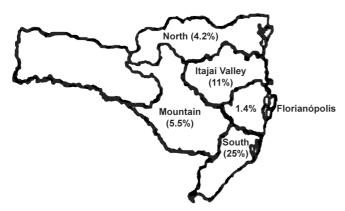
## Fungi genera versus bean Classes

The following genera were identified: Aspergillus spp., Penicillium spp., Phoma spp., Rizopus spp., Alternaria spp., Helminthosporium spp., Cladosporium spp., Botrytis spp.,

Fusarium spp., Trichoderma spp. Curvularia spp., and Drescheslera spp. (Fig. 2). As Aspergillus spp. and Penicillium spp. are the most important storage fungi, it is worth emphasizing that 246 and 313 strains of them were found in

| Sample collection |                     |            | Genera          |                  |            |                 |    |             |                 |                   |    |                 | Total |             |  |  |
|-------------------|---------------------|------------|-----------------|------------------|------------|-----------------|----|-------------|-----------------|-------------------|----|-----------------|-------|-------------|--|--|
| Samp              |                     |            | Aspergillus     |                  |            | Penicillium     |    |             | Others          |                   |    |                 |       |             |  |  |
| Region            | City                | of samples | N of<br>Strains | Toxi<br><b>N</b> | genic<br>% | N of<br>Strains |    | igenic<br>% | N of<br>Strains | Toxig<br><b>N</b> |    | N of<br>Strains |       | igenic<br>% |  |  |
| West              | Dionisio Cerqueira  | 7          | 11              | 3                | 3          | 32              | 3  | 9.4         | 21              | ND <sup>a</sup>   | ND | 64              | 6     | 9.4         |  |  |
|                   | Campos Novos        | 2          | 9               | 2                | 33.3       | 10              | ND | ND          | 8               | ND                | ND | 27              | 2     | 7.4         |  |  |
|                   | Águas Frias         | 1          | 4               | 2                | 50         | 5               | 2  | 40          | 1               | ND                | ND | 10              | 4     | 40          |  |  |
| North             | Mafra               | 2          | 3               | 2                | 67         | 10              | 5  | 50          | 2               | ND                | ND | 15              | 7     | 46.7        |  |  |
|                   | Canoinhas           | 1          | 5               | 1                | 20         | 5               | 5  | 100         | ND              | ND                | ND | 15              | 6     | 40          |  |  |
| Mountain          | São José do Cerrito | 2          | 8               | 2                | 25         | 9               | 2  | 22.2        | ND              | ND                | ND | 24              | 4     | 16.7        |  |  |
| Itajaí Valley     | Ituporanga          | 1          | ND              | ND               | ND         | 5               | 1  | 20          | 10              | ND                | ND | 15              | 1     | 6.7         |  |  |
|                   | Blumenau            | 1          | 5               | ND               | ND         | 5               | ND | ND          | 5               | ND                | ND | 15              | ND    | ND          |  |  |
|                   | Itajaí              | 1          | 5               | 1                | 20         | 5               | 1  | 20          | 5               | ND                | ND | 15              | 2     | 13.3        |  |  |
|                   | Major Gercino       | 1          | 2               | ND               | ND         | 5               | 1  | 20          | 3               | ND                | ND | 10              | 1     | 10          |  |  |
| South             | Araranguá           | 1          | 2               | 1                | 50         | 5               | 5  | 100         | 3               | ND                | ND | 10              | 6     | 60          |  |  |
|                   | São Martinho        | 1          | 2               | ND               | ND         | 5               | 4  | 80          | 3               | ND                | ND | 10              | 4     | 40          |  |  |
|                   | São Ludgero         | 2          | ND              | ND               | ND         | 10              | 4  | 40          | 15              | ND                | ND | 25              | 4     | 16          |  |  |
|                   | Nova Trento         | 1          | 5               | 1                | 20         | 5               | 2  | 40          | ND              | ND                | ND | 10              | 3     | 30          |  |  |
|                   | Içara               | 1          | ND              | ND               | ND         | 5               | ND | ND          | 5               | ND                | ND | 10              | ND    | ND          |  |  |
|                   | Total               | 25         | 61              | 15               | 24.6       | 121             | 35 | 28.9        | 93              | -                 | -  | 275             | 50    | 18.2        |  |  |

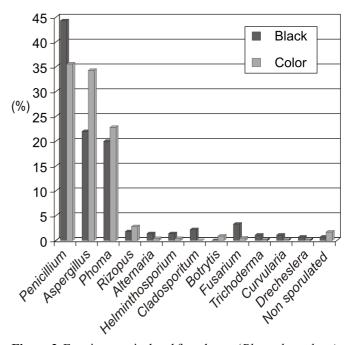
<sup>&</sup>lt;sup>a</sup> colony forms units/gram; <sup>b</sup> not detected by the methodology used.



**Figure 1.** State of Santa Catarina, Brazil, divided in regions for bean (*Phaseolus vulgaris* L.) samples collection.

the beans studied, respectively (Table 4). Beans Class *color* presented genera *Aspergillus* and *Penicillium* almost in the same proportion (35.6 and 34.3%, respectively). For beans Class *black*, the genera *Penicillium* presented higher values with 44.3% of the total of fungi isolated compared to only 22% for *Aspergillus* (Fig. 2).

These data are similar to those reported by Ruiz *et al* (18) in Argentine; the highest incidence was of *Aspergillus* spp. and *Penicillium* spp, with 40 and 15%, respectively, for freshly harvested beans. Scussel *et al.* (22) studied bean samples Classes *black* and *color*, from different Brazilian states (Bahia,



**Figure 2.** Fungi genera isolated from beans (*Phaseolus vulgaris* L.) Classes *black* and *color* collected from different regions of the State of Santa Catarina, Brazil (1997-1998).

Ceará, Minas Gerais, Mato Grosso, Mato Grosso do Sul, Paraná, and Rio Grande do Sul) and also reported the prevalence of these two genera (29.7 and 19.3%, respectively).

Apart from Aspergillus and Penicillium, other fungal genera were isolated from beans grown in Santa Catarina. A high proportion of Phoma spp. (20%) was isolated from beans Class black, followed by smaller amounts of Fusarium spp., Cladoporium spp. and Rizopus spp. (3.3, 2.2 and 1.8%). For all other genera (Alternaria spp., Helminthosporium spp., Trichoderma spp., Curvularia spp., and Drecheslera spp.) the percentage was lower than 1.4%. In the samples of bean Class color the following genera were also isolated: a significantly high percentage of Phoma spp. (22.8%), followed by small amounts of Rizopus spp., and Botrytis spp. (2.8 and 0.9% respectively). Genera Fusarium spp., Alternaria spp., Helminthosporium spp., Trichoderma spp., Curvularia spp. and Drecheslera spp. were less than 0.5% of the total genera isolated (Fig. 2).

Genera Fusarium was isolated in higher proportion in beans Class black (3.2%) than color (0.5%). On the other hand, in the South region, the presence of Fusarium spp. was higher in beans Class black and reached 9.1% of the total of fungi isolated.

This finding is of concern, as *Fusarium* is considered an important field contaminant in food, that could lead to fumonisins production. In addition, the high incidence of *Phoma* (the third highest percentage) found in the beans studied represents a risk to the consumer as some *Phoma* species can produce mycotoxins – the cytochalasins (10).

The Santa Catarina regions that presented the highest incidence of fungi were (a) North, with 50% of *Penicillium* and 26.6% of *Aspergillus*, (b) West, with 46.5% of *Penicillium* and 23.7% of *Aspergillus* and (c) South, with 45.4% of *Penicillium* and only 7.2% of *Aspergillus*. The South region presented a high incidence of *Phoma* and *Fusarium*.

**Toxigenic potential of isolated strains and fungi species:** the fungi that presented toxigenic potential were from genera *Aspergillus* spp. and *Penicilium* spp. (Tables 2, 3 and 4).

From the total of *Aspergillus* strains isolated (61) from beans Class *black*, 24.6% were aflatoxigenic and ochratoxigenic. From the *Penicillium* strains (121), 28.9% were citrinogenic (Table 4).

**Table 3.** Toxigenic fungi in beans (*Phaseolus vulgaris* L.) Class *Color* from different regions of the State of Santa Catarina, Brazil.

| Sample collection |              | Number of | Genera Aspergillus Penicilliu |    |       |         |     |        | Ot      |                 | Total |         |       |       |
|-------------------|--------------|-----------|-------------------------------|----|-------|---------|-----|--------|---------|-----------------|-------|---------|-------|-------|
| Region            | City         | samples   | N of                          |    | genic | N of    | Tox | igenic | N of    | Toxig           | genic | N of    | Toxig | genic |
|                   | City         |           | Strains                       | N  | %     | Strains | N   | %      | Strains | N               | %     | Strains | N     | %     |
| West              | Xanxerê      | 8         | 28                            | 6  | 21.4  | 28      | 8   | 28.6   | 16      | ND <sup>a</sup> | ND    | 72      | 14    | 19.4  |
|                   | Campos Novos | 4         | 32                            | 9  | 28.1  | 11      | 3   | 27.3   | 6       | ND              | ND    | 49      | 12    | 24.5  |
|                   | Águas Frias  | 3         | 7                             | ND | ND    | 15      | 6   | 40.0   | 12      | ND              | ND    | 34      | 6     | 17.6  |
|                   | Concórdia    | 2         | 2                             | ND | ND    | 6       | 2   | 33.3   | 7       | ND              | ND    | 15      | 2     | 13.3  |
|                   | Lebom Régis  | 3         | 16                            | 5  | 31.3  | 11      | 4   | 36.4   | 8       | ND              | ND    | 35      | 9     | 25.7  |
|                   | Palmitos     | 2         | 7                             | ND | ND    | 10      | ND  | ND     | 3       | ND              | ND    | 25      | ND    | ND    |
|                   | São Lourenço | 2         | 7                             | 2  | 28.6  | 9       | 7   | 77.8   | 9       | ND              | ND    | 25      | 9     | 36.0  |
|                   | Chapecó      | 2         | 7                             | 2  | 28.6  | 6       | ND  | ND     | 12      | ND              | ND    | 25      | 2     | 8.0   |
|                   | Fraiburgo    | 2         | 5                             | 1  | 20.0  | 10      | 4   | 40.0   | 5       | ND              | ND    | 20      | 5     | 25.0  |
|                   | Maravilha    | 2         | 6                             | 2  | 33.3  | 9       | 2   | 22.3   | 10      | ND              | ND    | 26      | 4     | 15.3  |
|                   | Campo Erê    | 1         | 3                             | ND | ND    | 5       | 2   | 40.0   | 5       | ND              | ND    | 10      | 2     | 20.0  |
|                   | Caibi        | 1         | 3                             | ND | ND    | 5       | 5   | 100    | 7       | ND              | ND    | 15      | 5     | 33.3  |
|                   | Cunha Porã   | 1         | 3                             | ND | ND    | 10      | 6   | 60.0   | 2       | ND              | ND    | 15      | 6     | 40.0  |
|                   | Pinhalzinho  | 1         | 8                             | 3  | 37.5  | 2       | 1   | 50.0   | ND      | ND              | ND    | 10      | 4     | 40.0  |
|                   | Tangará      | 1         | 3                             | ND | ND    | 5       | ND  | ND     | 7       | ND              | ND    | 15      | ND    | ND    |
|                   | Joaçaba      | 1         | 5                             | ND | ND    | 5       | ND  | ND     | 10      | ND              | ND    | 20      | ND    | ND    |
| Mountain          | Curitibanos  | 1         | 10                            | 3  | 30.0  | ND      | ND  | ND     | ND      | ND              | ND    | 10      | 3     | 30.0  |
|                   | Lages        | 1         | 2                             | ND | ND    | 5       | ND  | ND     | 3       | ND              | ND    | 10      | ND    | ND    |
| Itajaí Valley     | Pomerode     | 1         | 5                             | 2  | 40.0  | 3       | 1   | 33.4   | 2       | ND              | ND    | 10      | 3     | 30.0  |
|                   | Blumenau     | 2         | 5                             | 1  | 20.0  | 14      | 6   | 42.9   | 4       | ND              | ND    | 23      | 7     | 30.4  |
|                   | Itajaí       | 1         | 4                             | ND | ND    | 5       | 2   | 40.0   | 2       | ND              | ND    | 11      | 2     | 18.9  |
| Florianópolis     | São José     | 1         | 2                             | ND | ND    | 5       | 5   | 100    | 3       | ND              | ND    | 10      | 5     | 70.0  |
| South             | São Ludgero  | 2         | 8                             | 2  | 25.0  | 10      | 4   | 40.0   | 7       | ND              | ND    | 25      | 6     | 24.0  |
|                   | Içara        | 1         | 5                             | 3  | 60.0  | 3       | ND  | ND     | 7       | ND              | ND    | 15      | 3     | 20.0  |
|                   | Tubarão      | 1         | 5                             | ND | ND    | ND      | ND  | ND     | 15      | ND              | ND    | 20      | ND    | ND    |
|                   | Total        | 47        | 185                           | 41 | 22.1  | 192     | 68  | 35.4   | 162     | -               | -     | 539     | 109   | 20.2  |

<sup>&</sup>lt;sup>a</sup> colony forms units/gram; <sup>b</sup> not detected by the methodology used; <sup>c</sup> variety carioquinha.

| C           | N                 |                            | Toxigenic potential |    |                  |    |                  |     |      |
|-------------|-------------------|----------------------------|---------------------|----|------------------|----|------------------|-----|------|
| Genera      | Number of strains | $\overline{\mathrm{AF^a}}$ |                     | (  | CTR <sup>b</sup> | (  | )TA <sup>c</sup> |     |      |
|             | Class black       | N                          | (%)                 | N  | (%)              | N  | (%)              | N   | (%)  |
| Aspergillus | 61                | 8                          | 13.1                | -  | -                | 7  | 11.1             | 15  | 24.6 |
| Penicillium | 121               | -                          |                     | 35 | 28.9             | -  |                  | 35  | 28.9 |
| Other       | 93                | -                          | -                   | -  | -                | -  | -                | -   |      |
| Total       | 275               | 8                          | 2.9                 | 35 | 12.8             | 7  | 2.5              | 50  | 18.2 |
|             | Class color d     |                            |                     |    |                  |    |                  |     |      |
| Aspergillus | 185               | 31                         | 16.7                | -  | -                | 10 | 5.4              | 41  | 22.1 |
| Penicillium | 192               | -                          | -                   | 68 | 35.4             | -  | -                | 68  | 35.4 |
| Other       | 162               | -                          | -                   | -  | -                | -  | -                | -   | -    |
| Total       | 539               | 31                         | 5.8                 | 68 | 12.6             | 10 | 1.0              | 109 | 20.2 |

**Table 4.** Toxigenic potential of strains isolated from beans (*Phaseolus vulgaris* L.) Classes *black* and *color* from State of Santa Catarina, Brazil.

Beans Class *color* had 22.1% of the *Aspergillus* strains that produced AF and OTA. With a higher percentage (35.4%) of citrinogenic *Penicillium* than the Class *black* (Table 3). Cyclopiazonic acid and fumonisin production was not evaluated. As far as toxigenic potential of the strains and the sample collection regions are concerned, no difference among them could be observed.

The fungi species identified from the bean samples contaminated with *Aspergillus* and *Penicillium* were *A. flavus*, *A. parasiticus*, *A. ochraceus* and *P. citrinum* Thom.

The *Aspergillus* species isolated from beans Class black and the types of toxins produced were as follows: 11.5% of *A. ochraceus* produced OTA; 6.5% of *A. flavus* produced only AFB<sub>1</sub> and 3.3% produced AFB<sub>1</sub> and AFB<sub>2</sub>. In addition, 3.3% produced the four aflatoxins (AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub> and AFG<sub>2</sub>). The OTA production was higher for Class black (11.5%) than for color (5.4%). The incidence of *P. citrinum* was also lower in this Class. Apart from OTA producers, 8.6% of the *A. flavus* strains isolated from beans Class color produced AFB<sub>1</sub> and AFB<sub>2</sub>; and 4.8% produced only AFB<sub>1</sub>. *A. parasiticus* (3.3%) produced AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub> and AFG<sub>2</sub>. It was observed that 3.5% of *P. citrinum* produced citrinin.

The analysis of variance of the two Classes of bean showed that there was not a significative difference among them, either for fungi total counts, toxigenic fungi potential and mc of the samples.

It is important to emphasize that the detection of toxigenic fungi in food does not mean presence of mycotoxin, especially if the fungus is not exposed to conditions that allow its growth. However, presence of toxigenic fungi indicates that there are potential risks of mycotoxin contamination. When the food is a good substrate for fungi growth and mycotoxin production. When the food is exposed to physical factors such as high moisture content and temperature, the risk of contamination increases, especially during long term storage (12,21).

The toxigenic tests do not give all the information due to differences on fungi behavior in different medium and/or different substrates (23). Therefore, the data obtained have to be considered only as an indicative.

The high incidence of ocratoxinogenic and citrinogenic strains in both Classes of beans is of concern, as the target organs for both toxins are the kidneys. Considering that beans are the staple food of the Brazilian population, extensive to some other Latin American countries, it is important to take into perspective that this exposure can lead to nephropathies with unknown cause. A parallel study in bean samples demonstrated that OTA contamination was much higher than AFB<sub>1</sub> (6).

## **ACKNOWLEDGEMENTS**

The authors acknowledge the *Companhia Integrada de Desenvolvimento Agricola* - CIDASC - through Mr. O. Volpato and staff of the Laboratory of Classification of Vegetable. Products of Santa Catarina State for the samples collection.

#### **RESUMO**

## Fungos toxigênicos em feijão (*Phaseolus vulgaris* L.) classes preto e cores cultivado no Estado de Santa Catarina, Brasil

Foram estudados fungos toxigênicos em feijão (*Phaseolus vulgaris* L.), classes preto e cores, cultivados em diferentes regiões do Estado de Santa Catarina, região Sul do Brasil. A média total de fungos filamentosos foi de 2,8x10<sup>3</sup> e 6,7x10<sup>3</sup> UFC/g para feijão classe preto e cores, respectivamente. *Penicillium* spp., *Aspergillus* spp. e *Phoma* spp. foram os gêneros mais frequentes isolados, seguidos por *Ryzopus* spp., *Alternaria* spp., *Helminthosporium* spp., *Cladosporium* spp., *Botrytis* spp., *Fusarium* spp., *Trichoderma* spp., *Curvularia* spp. e

<sup>&</sup>lt;sup>a</sup> aflatoxins; <sup>b</sup> citrinin; <sup>c</sup> ochratoxin A; <sup>d</sup> variety *carioquinha*.

Dreschelera spp. No feijão classe preto, 24,6% das cepas de Aspergillus isolados eram toxigenicas: 13.1% eram produtoras de aflatoxinas (AFs) e 11,5% de ocratoxina A (OTA); e 28,9% de Penicillium produziram citrinina (CTR). Por outro lado, 22,1% de cepas de Aspergillus isolados do feijão classe cores, produziram micotoxinas (16,7% produziram AF e 5,4% produziram OTA), já do gênero Penicillium, 35,4% das cepas produziram CTR. As espécies toxigênicas isoladas foram A. flavus, A. parasiticus, A. ochraceus e P. citrinum Thom.

Palavras-chave: feijão, *Phaseolus*, fungos toxigênicos, aflatoxina, ocratoxina A, citrinina.

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