DOI:10.2478/10004-1254-63-2012-2206

Scientific paper

# CONTROL OF T-2 TOXIN IN *FUSARIUM LANGSETHIAE* AND *GEOTRICHUM CANDIDUM* CO-CULTURE*

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Received in January 2012
CrossChecked in August 2012
Accepted in September 2012

Due to contamination of barley grains by *Fusarium langsethiae,* T-2 toxin can be present in the brewing process. It has been observed that the presence of the yeast *Geotrichum candidum* during malting can reduce the final concentration of this mycotoxin in beer. In this work, a co-culture method was carried out for both microorganisms in order to evaluate the effect on T-2 mycotoxin concentration in comparison with the pure culture of *F. langsethiae* in the same conditions. The microbial growth of both microorganisms was assessed using three different methods: dry weight, DOPE-FISH, and DNA quantification. In co-culture, both microorganisms globally developed less than in pure cultures but *G. candidum* showed a better growth than *F. langsethiae.* The concentration of T-2 was reduced by 93 % compared to the pure culture. Hence, the interaction between *G. candidum* and *F. langsethiae* led to a drastic mycotoxin reduction despite the only partial inhibition of fungal growth.

**KEY WORDS:** *brewing*, *contamination, growth of microorganisms, mycotoxins*

## INTRODUCTION

Mycotoxins are toxic, non-protein, secondary metabolites produced by various mould species. The most frequent toxigenic fungi in Europe involved in the human food chain are *Aspergillus*, *Penicillium*, and *Fusarium* species (1, 2). There are more than 400 different types of toxins but the most common toxins found in foodstuffs are: aflatoxins, ochratoxins, zearalenone, fumonisins, and trichothecenes. Trichothecenes are subdivided into four groups (A, B, C, D), and the most important members belong to groups A and B. T-2 toxin, HT-2 toxin, neosolaniol, and diacetoxyscirpenol are the representative members of group A and deoxynivalenol, nivalenol, and fusarenon-X of group B. T-2 toxin is one of the most potent and toxic mycotoxins because it induces DNA fragmentation and inhibits protein synthesis (3-5).

Barley is one of the most commonly used cereals in beer production. The presence of *Fusarium langsethiae* was detected in kernels of barley and other cereals in several countries in Europe (6, 7). In fact, *F. langsethiae* has supplanted other species of *Fusarium* in the barley fields (8, 9). *F. langsethiae* has been implicated in the production of high levels of T-2 and HT-2 toxins in cereals in Norway and other

* The subject of this article has partly been presented at the International Symposium "Power of Fungi and Mycotoxins in Health and Disease" held in Primošten, Croatia, from 19 to 22 October 2011.

European countries (6, 10-12). Contaminated barley causes problems during beer production. In the malting process, the temperature and humidity conditions are suitable for fungal development, so the downstream process and the final beer quality are affected (13, 14).

*Geotrichum candidum* has been suggested to control T-2/HT-2 toxins levels (15). However information about the mechanism of action during the interaction between *F. langsethiae* and *G. candidum* is not available.

The aim of this work was to study the interaction of *G. candidum* and *F. langsethiae* in co-culture and to evaluate its effect on T-2 toxin concentration.

## MATERIALS AND METHODS

### *Strains and medium*

*Fusarium langsethiae* 033 was isolated from barley grains and was provided by the Institut Français des Boissons de la Brasserie et de la Malterie (IFBM, Nancy, France). *Geotrichum candidum* (Levure de malterie IFBM® *Geotrichum candidum* REF X16010211) was provided by CTS (Cargill France SAS). These microorganisms were stored at 4 °C on Potato Dextrose Agar (PDA) slants. Cultivation was performed using Ym medium with the following composition: glucose 5 g $L^{-1}$ (ACROS); yeast extract 1.5 g $L^{-1}$ (FISHER SCIENTIFIC), malt extract 1.5 g $L^{-1}$ (FISHER SCIENTIFIC); and Bacto tryptone, an enzymatic digest of casein, 2.5 g $L^{-1}$ (Becton, Dickinson and Company).

### *Microbial growth conditions*

For precultures of *F. langsethiae* 033, spores were first collected from Petri dishes after growth on PDA medium. These spores were then seeded at a final concentration of $1x10^6$ $mL^{-1}$ to 100 mL of Ym medium and incubated during three days at 22 °C and 200 rpm. In the case of *G. candidum*, after being collected from PDA in Petri dishes, arthrospores were seeded to 100 mL of Potato Dextrose Broth (PDB) medium and then incubated for 14 h to 16 h at 22 °C and 200 rpm.

Pure culture method was carried out in 500 mL Erlenmeyer flasks containing 100 mL of Ym medium, at 22 °C and stirred at 200 rpm. Inoculation consisted in a 10 % preculture volume (biomass 0.5 g $L^{-1}$) for each strain. For co-cultures, the procedure was the same but 10 % of each preculture were mixed in Ym. After inoculation in Ym medium, pH in pure and co-cultures was set at 7.0 with sterile NaOH 5 mol $L^{-1}$ or sterile HCl 1 mol $L^{-1}$ in aseptic conditions.

### *Dry weight determination*

Microbial growth was measured by dry weight method. Five-to-ten-millilitre samples of culture (biomass and medium) were filtered through 0.45 µm pore-size membranes (cellulose nitrate, Sartorius). Membranes with cells were dried at 100 °C for 24 h. Biomass is expressed in grams of dry cell mass per litre of culture broth.

### *Determination and quantification of T-2 toxin by HPLC/FLD*

Standard T-2 toxin was purchased from Sigma Aldrich (Lyon, France). Stock solution was prepared by dissolving the toxin in pure acetonitrile HPLC grade (Fisher Scientific, France) to obtain a concentration of 1000 µg $mL^{-1}$. Working solutions were then diluted with pure acetonitrile HPLC grade and stored at 4 °C. Standards range was from 0.05 µg $mL^{-1}$ to 1 µg $mL^{-1}$. 4-dimethylaminopyridine (DMAP) and toluene (Chromasolv® Plus, HPLC grade, ≥99.9 %) were also purchased from Sigma Aldrich. 1-antroylnitrile (1-AN) was provided by Wako (SOBIODA SAS, France). DMAP and 1-AN were prepared in toluene as reported by Trebstein, Seefeldet (16). The HPLC-FLD methodology used in this study was adapted from Lippolis, Pascale (17), Pascale, Haidukowski (18), Trebstein, Seefeldet (16), Trebstein, Marschik (19), and Visconti, Lattanzio (20) to measure the T-2 toxin in liquid cultures. 4 mL aliquots (T-2 standards and assays) were dehydrated at 65 °C, 30 hPa for 20 h in a miVac DUO concentrator apparatus. Derivatisation procedure of the dried samples was performed as described by Trebstein, Seefeldet (16), with solvents evaporation using a speed vacuum machine miVac DUO. HPLC analysis was performed using a Kontron system consisting of a Kontron 525 binary pump, a Kontron 465 auto sampler, a Knauer degasser, a Kontron 582 column thermostat, a Spectra system FL 3000 fluorescence detector, and ChromGate version 3.3.1 software (Knauer, Berlin, Germany). Separation of samples was performed using a Luna Phenyl-Hexyl column (Phenomenex, France) following the methodology reported by Trebstein, Marschik (19). Quantification of the T-2 toxin was achieved by measuring the peak

area at the retention time of T-2 derivatives and comparing it with the calibration curve in the range of 0.05 μg $mL^{-1}$ to 1.0 μg $mL^{-1}$. For the calibration curve and linearity response of the T-2 derivative-peak area, equivalent aliquots of T-2 standard solutions from 0.05 μg $mL^{-1}$ to 1.0 μg $mL^{-1}$ were dried and derived with the same technique mentioned before.

*T-2 glucose concentration*

Glucose concentration in samples was determined during microbial growth time course by HPLC. Cell-free culture medium (800 μL) was treated with 100 μL of saturated $Ba(OH)_2$ and 100 μL of 5 % $Zn(SO_4)_2$ (w/w). After 5 min, samples were centrifuged at 13,000 rpm for 10 min at 4 ºC. HPLC analyses were performed using the Waters system consisting of a M45 Waters pump with a differential refractometer Waters 420 and a Waters C18 column for sugar separation. Refractometer detector temperature was set at 30 ºC. Mobile phase consisted of acetonitrile: $H_2O$ (70:30) with an isocratic flux of 1.2 mL $min^{-1}$. Quantification was done by a calibration curve in the range of 0.5 g $L^{-1}$ to 10 g $L^{-1}$.

*Double-labeling of Oligonucleotide Probes for Fluorescent in situ Hybridisation (DOPE-FISH)*

*F. langsethiae* 033 has natural yellow auto fluorescence when observed at 470 nm. For this reason, DOPE-FISH was only used in *G. candidum* to differentiate these microorganisms in the co-culture; natural yellow for F. *langsethiae* 033 and green for *G. candidum*. DOPE-FISH was used to increase fluorescent signal. Specific fluorescent oligonucleotide probe for *G. candidum* pB-01541 (Table 1) was purchased from GeneCust - (Custom Services for Research, Luxembourg) and DyLight dye 488 from Thermo FISHER, Fr. and labelling of the probe was carried out both at 5'and 3' ends for DOPE-FISH study. DOPE-FISH of *G. candidum* in pure and co-culture was performed on glass slides every 24 h during microbial growth time course. One mL aliquot of each culture was centrifuged for 10 min at 4 °C and 13,000 rpm to eliminate culture media. Pellets were washed three times adding 1 mL of buffer solution A ($NaH_2PO_4$ 0.05 mol $L^{-1}$; NaCl 0.15 mol $L^{-1}$; pH 7.2). After cell washing, 1 mL of solution B (buffer solution A plus 3 % paraformaldehyde) was used to carry out the fixing reaction during 60 min at room temperature. After centrifugation and elimination of supernatant, cell washing was repeated three more times. Finally, cells were suspended in 1 mL of buffer solution A and 10 μL were dropped on a glass slide and left to dry at 50 °C for 5 min on an electric plaque. Yeast wall digestion was performed using 20 μL of Lysing Enzyme from *Trichoderma harzianum*, 15 μg $μL^{-1}$ of which was dissolved in buffer solution A. Glass slides were incubated at 37 °C for 2 h. Thereafter, the slides were washed three times with 20 μL of FISH-NaCl buffer (Tris HCl 20 mol $L^{-1}$; SDS 0.1 %; EDTA 5 mmol $L^{-1}$; and NaCl 0.9 mol $L^{-1}$; pH 7.2) and dehydrated with 20 μL of ethanol 50 %, 70 %, and 99.9 %. Samples were then dried at 50 °C for 30 min. Hybridisation reaction was achieved by placing 20 μL of 15 ng $μL^{-1}$ of *G. candidum* oligonucleotide probe dissolved in FISH-NaCl buffer containing 20 % formamide (v/v) on glass slides and heating them at 50 °C for 2 h. After hybridisation, 50 μL of FISH buffer (Tris HCl 20 mmol $L^{-1}$; SDS 0.1 %; EDTA 5 mmol $L^{-1}$) were added and slides were incubated at 50 °C for 30 min. Three washing cycles followed post-hybridisation reaction before slides were observed with a microscope at 470 nm.

**Table 1** *Characteristics of* G. candidum *probe (www.microbial-ecology.net).*

| Accession number | pB-01541 |
|---|---|
| Specificity | *Geotrichum* sp. |
| Target molecule | 26S rRNA |
| Sequence | 5'-TTA CGG GGC TGT CAC CCT-3' |
| Length / nt | 18 |
| G+C content / % | 61.1 |
| Tm / °C | 53 |
| MW / g $mol^{-1}$ | 5463 |
| Formamide / % | 20 |

*DNA extraction and quantification*

Genomic DNA of *F. langsethiae* 033 and *G. candidum* in pure and co-cultures were extracted from 0.5 g $L^{-1}$ to 5 g $L^{-1}$ of freeze-dried biomass using a Fast DNA Kit (Q-BIOgene). DNA extraction was quantified by measuring the absorbance at 260 nm for nucleic acids and 280 nm for proteins with 1-cm path length cuvettes. DNA purity was evaluated by comparing the absorbance ratios A260/A280. An optical density unit measured at 260 nm is equivalent to 50 μg $mL^{-1}$ of DNA. Genomic DNA samples were diluted to obtain a concentration of 20 μg $mL^{-1}$. Volumes of 50 μL were then sent to the IFBM (Nancy, France) for DNA quantification by q-PCR. The results were expressed as the number of genomes for each strain.

## RESULTS

### *Interaction between* F. langsethiae *033 and* G. candidum *in co-culture*

*F. langsethiae* 033 and *G. candidum* growth kinetics of pure cultures and co-culture were studied by dry weight measurement. The results are shown in Figure 1. Maximal growth was achieved after 24 h of cultivation of both microorganisms in pure cultures and in the co-culture. In the latter case, *F. langsethiae* and *G. candidum* biomass could not be separated by the dry weight method. In the chosen culture conditions, after 24 h, *F. langsethiae* growth in pure culture (4.5 g $L^{-1}$) was by 50 % higher than the growth of both microorganisms in the co-culture (3 g $L^{-1}$) and by 125 % higher than the growth of *G. candidum* in the pure culture (2 g $L^{-1}$).

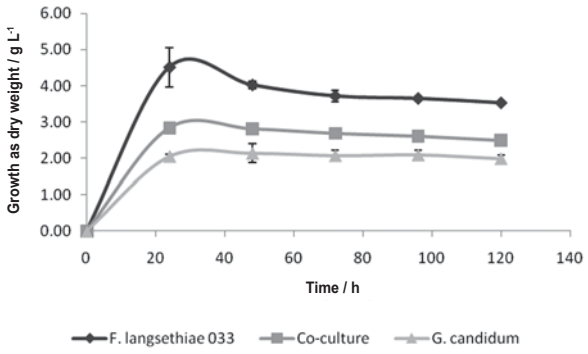


**Figure 1** F. langsethiae *033 and* G. candidum *growth as dry weight in pure culture and co-culture.*

T-2 toxin concentration was measured by HPLC-FLD in the pure culture of *F. langsethiae* 033 and in the co-culture (Figure 2). The pure culture of *F. langsethiae* 033 produced up to 0.63 μg $mL^{-1}$ ± 0.27 μg $mL^{-1}$ after 72 h whereas in co-culture its concentration was only 0.04 μg $mL^{-1}$ ± 0.01 μg $mL^{-1}$ after 94 h. Thus the concentration of T-2 toxin was reduced by more than 93 % in the presence of *G. candidum*.

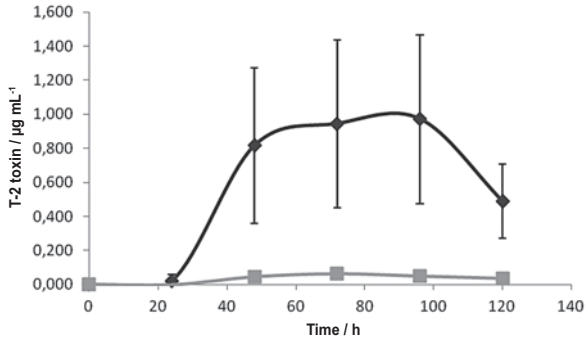


**Figure 2** *T-2 toxin concentration in* F. langsethiae *033 pure culture and co-culture of* F. langsethiae *and* G. candidum *(-◆- pure culture and -■- co-culture).*

## DOPE-FISH

The growth of *F. langsethiae* 033 and *G. candidum* in pure cultures and co-culture was also assessed by fluorescence microscope observation by DOPE-FISH. *G. candidum* appeared green, and *F. langsethiae* naturally fluoresced in yellow. The results are shown in Figure 3. Samples from each culture were taken every 24 h to follow the interaction in the co-culture in comparison with pure cultures. Figures 3a to 3f display the development of *F. langsethiae* 033 during its growth in the pure culture. Figures 3g to 3l show *G. candidum* growth in the pure culture. In figures 3m to 3r, yellow and green colorations, respectively, show *F. langsethiae* 033 and *G. candidum* development. We can observe that in the co-culture, the green coloration was slightly predominant over the yellow coloration. This suggests that *G. candidum* growth could be more important than the *F. langsethiae* 033 one in the co-culture.

### *DNA quantification*

The results of DNA quantification by Q-PCR of *F. langsethiae* 033 and *G. candidum* in pure cultures and co-culture are shown in Figure 4, Figure 5, and Figure 6, respectively, in comparison with dry weight biomass. The number of genomes detected in *F. langsethiae* 033 pure culture has the tendency to increase in time (Figure 4) although there is a stationary phase after 48 h of time course detected by dry weight measurement. For *G. candidum*, the values of DNA quantification showed an increase from 2670 to 216 000 genomes after 48 h and then this figure decreased to 12 300 during the stationary phase (Figure 5). The decay phase is not so marked by dry weight measurement. DNA quantification of *G. candidum* and *F. langsethiae* in the co-culture showed that both microorganisms were able to grow (Figure 6). Maximal DNA quantification was obtained after 48 h, reaching 90000 and 30000 genomes for *G. candidum* and *F. langsethiae* respectively. Then, DNA quantification for both microorganisms decreased until 96 h after incubation. However, the behaviour observed in the pure culture of *F. langsethiae* 033 was quite different since the quantification of DNA increased during the first 96 h.

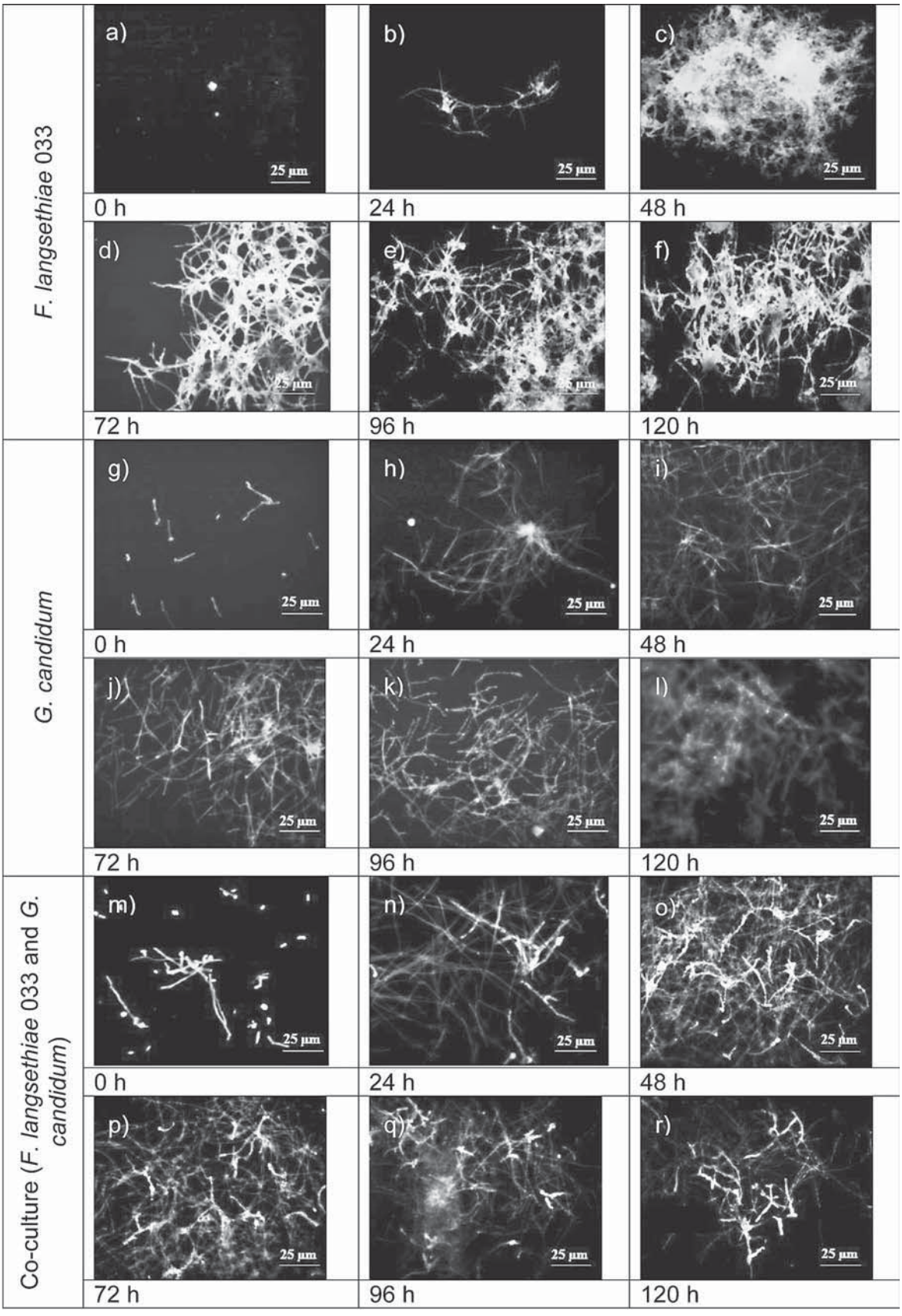


**Figure 3** *Differentiation of* F. langsethiae *033 and* G. candidum *by DOPE-FISH in pure culture and co-culture. Pictures from a) to f),* F. langsethiae *033 growth development; g) to l),* G. candidum *growth development in pure culture; m) to r),* F. langsethiae *033 and* G. candidum *growth development in co-culture.*

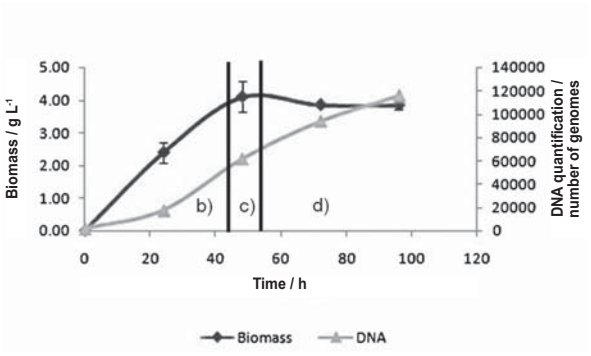


**Figure 4** *DNA quantification of* F. langsethiae *033 in pure culture. Growth exponential phase, b); transition phase, c); and stationary phase d).*

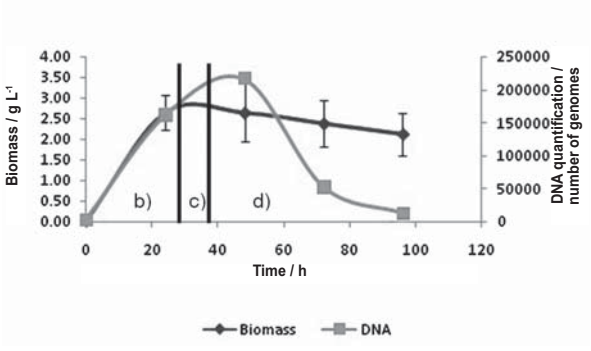


**Figure 5** *DNA quantification of* G. candidum *in pure culture. Growth exponential phase, b); transition phase, c); stationary/decay phase, d).*

## DISCUSSION

*F. langsethiae* 033 and *G. candidum* developed well in pure cultures in Ym liquid medium (22 °C, 200 rpm, pH 7.0). Growth morphology of *F. langsethiae* 033 in liquid culture displayed developed filaments and formed biomass conglomerations of different sizes probably due to the orbital agitation. These biomass conglomerations affected sampling, and as a result, some of the dry cell mass values have significant standard deviations. The measurement of glucose consumption revealed that it was entirely depleted after 48 h of pure culture cultivation (Table 2). *F. langsethiae* 033 growth was kept constant during the 120 h of cultivation and this suggests that *F. langsethiae* 033 used a different carbon/energy source. This source may have been provided by the components of the tryptone present in the medium Ym. *G. candidum* growth morphology was also filamentous but did not form biomass conglomerations like *F. langsethiae* 033 did in the liquid culture. After reaching the stationary phase, spores began to form. Glucose consumption was also measured and results showed that after 96 h of pure culture (Table 2), a glucose residual concentration of 1.5 g $L^{-1}$ ± 0.3 g $L^{-1}$ was detected. Adour, Couriol (21) studied the amino acids consumption and catabolism of *G. candidum* and *Penicillium camembertii* in two media, one based only on peptones and the other on the combination with lactic acid. They found that *G. candidum* metabolizes more amino acids than *P. camembertii*, 79.9 % and 64.4 %, respectively. This is explained by *G. candidum* using amino acids as a source of carbon and energy. They also found that peptones are preferably consumed by *G. candidum* over other energy sources; 64.8 % of the peptones were consumed by *G. candidum* in the presence of lactic acid and 44.9 % by *P. camembertii*. Literature concerning *F. langsethiae* 033 nutrients consumption is very scarce. Blesa, Meca (22) studied the effect of glucose concentration in the T-2 toxin concentration. They found that the production of this mycotoxin is directly correlated with the concentration of glucose present in the medium. In their study with a 20 % glucose concentration, a maximal T-2 toxin concentration of 1.45 mg $kg^{-1}$ was produced by *F. sporotrichioides* on PDA plates. They did not measure glucose consumption through time; T-2 toxin quantification was made after 11 days of cultivation at 30 °C on PDA plates. *F. langsethiae* 033 produced a maximal concentration of T-2 toxin of 0.63 µg $mL^{-1}$ ± 0.27 µg $mL^{-1}$ in Ym liquid culture containing 5 g $L^{-1}$ of glucose after three days of cultivation. The amount of mycotoxins produced by certain strains depends on different factors

**Table 2** *Glucose consumption of* F. langsethiae *033 and* G. candidum *in pure culture.*

| Time / h | *F. langsethiae* 033 / g $L^{-1}$ | *G. candidum* / g $L^{-1}$ |
|---|---|---|
| 0 | 4.6 ± 0.3 | 4.6 ± 0.4 |
| 24 | 1.3 ± 1.1 | 2.8 ± 0.2 |
| 48 | 1.1 ± 0.1 | 2.4 ± 0.1 |
| 72 | 0 | 2.0 ± 0.0 |
| 96 | 0 | 1.5 ± 0.3 |

including species, origin, pH, temperature, nutrient availability, etc (23). In this case, *F. langsethiae* 033 culture conditions were different from the culture conditions of *F. sporotrichioides*, liquid culture and agar plates used, respectively. For this reason, the effect of initial glucose concentration is not comparable. We observed that glucose concentration was almost fully depleted by *F. langsethiae* 033 after 48 h of growth in the liquid culture. *F. langsethiae* 033 continued to develop probably using a different carbon/energy source as *P. camembertii* did. On the other hand, our finding concerning glucose consumption of *G. candidum* is in agreement with the result obtained by Adour, Couriol (21).

For the co-culture, the method used (dry cell measurement) to estimate biomass did not permit to separate the biomass of *F. langsethiae* 033 from the one of *G. candidum*. Total biomass obtained was 3 g $L^{-1}$, which was less than the sum of the biomass obtained for each microorganism in pure cultures, 4.5 g $L^{-1}$ and 2 g $L^{-1}$ respectively. However, *F. langsethiae* 033 was able to produce a maximal concentration of 0.63 μg $mL^{-1}$ ± 0.27 μg $mL^{-1}$ of T-2 toxin at 72 h in the pure culture, yet the T-2 toxin concentration detected in the co-culture was below 0.05 μg $mL^{-1}$. This result indicates that the presence of *G. candidum* in the co-culture with *F. langsethiae* 033 led to a reduction of the T-2 toxin concentration of more than 90 %.

The filamentous morphology of *F. langsethiae* 033 and *G. candidum* prevented the differentiation and separate quantification of *F. langsethiae* and *G. candidum* in the co-culture. The dry weight method was impractical in the co-culture for providing information about the effect produced by the interaction between these microorganisms. The hybridisation method DOPE-FISH was used to differentiate the development of *F. langsethiae* 033 and *G. candidum* in the co-culture. *F. langsethiae* 033 presented auto-fluorescence (yellow) at 470 nm and for this reason *G. candidum* was only hybridised to be green. This yellow auto-fluorescence may possibly be explained by the presence of a pigment or other secondary metabolite produced during growth. Figure 3 shows in yellow the development of *F. langsethiae* 033 in the pure culture (a to f), in green the pure culture of *G. candidum* (g to l), and yellow/green the co-culture of these microorganisms (m to r). From these series of pictures it is possible to observe a prevalence of the green colour that corresponds to *G. candidum* development. We can therefore assume that in the co-culture this microorganism developed better than the other one. We used DNA quantification which can be specific for each microorganism to make quantification. Microbial growth is divided in 4 phases: 1) lag phase, in which DNA helicoidal tension is recovered and gene expression increases; 2) exponential phase in which cellular division occurs thanks to nutrient availability; 3) transition phase takes place when nutrients decrease, the slope changes, the proteins and DNA synthesis reduce in a non-synchronic way causing an increment in the DNA/protein ratio, and cellular division continues at the same velocity as in the exponential phase; and 4) stationary phase, which is characterised by depleted nutriment, unchanged biomass concentration, and reduced synthesis of DNA, RNA, and proteins (24). DNA quantification of *G. candidum* in the pure culture (Figure 5) showed that the number of genomes increased during the exponential phase of *G. candidum* reaching the value of 150 000 for 2.5 g $L^{-1}$ dry weight. An increase in the values of the number of genomes observed at 48 h could not be correlated with the transition phase where, typically, the DNA/protein ratio increases once the nutriment depletion starts. Afterwards, genomic values decreased when the culture entered the stationary phase. This phenomenon corresponds to the behaviour described by Ramírez Santos (24) where growth was related to DNA quantification. *F. langsethiae* 033 genomic quantification yielded different results: the number of genomes increased in the course of culture growth (Figure 4). The maximal biomass obtained at the end of the growth phase generated 4.5 g $L^{-1}$ of dry weight corresponding to 110 000 genomes. In the co-culture, the profile of genomic values of *F. langsethiae* 033 was completely different and decreased after 48 h (Figure 6). In the co-culture, the number of genomes

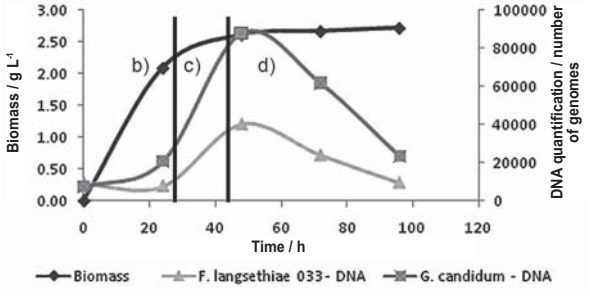


**Figure 6** *DNA quantification of* F. langsethiae *033 and* G. candidum *in co-culture. a); exponential phase, b); stationary phase, c); and decay phase, d).*

of both microorganisms presented a period where DNA helicoidal tension was recovered, followed by an increase due to growth. Then, a maximal value that may be associated with the transition phase was achieved, and finally ensued a decrease in genetic expression related to the stationary phase as mentioned by Ramírez Santos (24). We can therefore estimate, by approximation based on the results observed in pure cultures, that 90 000 genomes measured for *G. candidum* corresponded to 1.6 g $L^{-1}$ and 35 000 genomes measured for *F. langsethiae* corresponded to 1.3 g $L^{-1}$ of dry weight in the co-culture. Therefore, both microorganisms were able to grow in the co-culture, although *F. langsethiae* 033 slightly less, as it was partially inhibited by the direct contact of *G. candidum*. Its growth was reduced by about two thirds. Contrary to this, the T-2 toxin concentration was drastically affected and its concentration was reduced by more than 90 % in the co-culture.

## CONCLUSIONS

The T-2 toxin concentration may be reduced by 90 % by the presence of *G. candidum* in a co-culture with *F. langsethiae.* DOPE-FISH is a method that allowed us to differentiate these filamentous microorganisms in the co-culture. DNA quantification permitted to better understand the interaction behaviour of these microorganisms in the co-culture. In this study we found that *G. candidum* partially inhibited *F. langsethiae* 033 growth but its effect was greater on the T-2 toxin concentration. Two mechanisms could be proposed: either toxin degradation after its production or production inhibition by the fungi.

*Acknowledgements*

We would like to acknowledge the financial support of the project ANR-BARSAFE. Elida Gastélum-Martinez also gratefully thanks for the financial support for her doctoral studies from The Consejo Nacional de Ciencia y Tecnología Mexicano (CONACYT) and the Federation of European Microbiological Societies (FEMS) for the conferred FEMS Young Scientist Meeting Grant to participate in the Meeting 2011-17: “Power of Fungi and Mycotoxins in Health and Disease”, Primošten, Croatia. 19-22 October 2011.

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**Sažetak**

KONTROLA TOKSINA T-2 U KOKULTURI *FUSARIUM LANGSETHIAE* I *GEOTRICHUM CANDIDUM*

Zbog kontaminacije zrna ječma kulturom *Fusarium langsethiae* u postupku proizvodnje piva može doći do pojave T-2-toksina. Uočeno je da prisutnost plijesni *Geotrichum candidum* tijekom faze pretvaranja ječma u slad može sniziti krajnju koncentraciju ovog mikotoksina u pivu. U ovom je radu izvršena kokultura obaju mikroorganizama kako bi se procijenio učinak na koncentraciju T-2-mikotoksina u odnosu na čistu kulturu *F. langsethiae* u istim uvjetima. Mikrobni rast obaju mikroorganizama procijenjen je s pomoću triju metoda: mjerenjem suhe mase, fluorescencijskom *in situ* hibridizacijom s dvostruko obilježenim oligonukleotidnim sondama (*Double-labeling of Oligonucleotide Probes for Fluorescent* in situ *Hybridisation* – DOPE-FISH) i kvantifikacijom DNA. Ukupan razvoj obaju mikroorganizama u kokulturi bio je manji nego u pojedinačnim kulturama, ali je *G. candidum* pokazao nešto veći rast od *F. langsethiae.* Koncentracija T-2 snizila se za 93 % u usporedbi s čistom kulturom. Dakle, interakcija između *G. candidum* i *F. langsethiae* dovela je do drastičnog smanjenja koncentracije mikotoksina unatoč samo djelomičnoj inhibiciji fungalnog rasta.

**KEY WORDS:** *kontaminacija, mikotoksini, proizvodnja piva, rast mikroorganizama*

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