Effect of commercial starter cultures and native yeasts on Ochratoxin A production by *Penicillium nordicum* in meat products

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Introduction:

Ochratoxin A (OTA) is a secondary metabolite produced by *Penicillium* and *Aspergillus* genera and is considered one of the most important mycotoxins occurring in animal and human food chains. Oxidative stress, inhibition of protein synthesis, disruption of calcium homeostasis, inhibition of mitochondrial respiration and DNA damage are some of OTA´s mechanisms of action causing teratogenicity, immunotoxicity, neurotoxicity and mostly nephrotoxicity.

In dry-cured and fermented meat products, OTA is strongly associated with *Penicillium nordicum.* Fungal growth and OTA production in meat products can be influenced by environmental conditions, physico-chemical characteristics of the matrix, and its endogenous flora. OTA is highly stable, so its destruction during normal food processing is very difficult to achieve. Bacteria and fungi have been tested as biocontrol agents against fungal development and OTA production, with variable results.

Aims:

This work aimed to i) evaluate the role of yeasts, previously isolated from meat products, and one commercial starter culture, on the growth of *P. nordicum* as well as on OTA production, by using meat-based culture media as model systems and to ii) understand the mechanisms underlying the observed effect.

Methods:

1. Yeast, starter and fungus preparation:

Yeasts (*Candida zeylanoides* (*Cz*) and *Rhodotorula mucilaginosa* (*Rh*) were grown in PDB at 28 °C on a rotary shaker for 24h, Starter culture (St) in MRS broth at 37 °C for 24h, and *P. nordicum* (Pn) in MEA for 7 days at 25 ºC

2. Meat-based Media preparation and inoculation:

Ham (Ham, 3% NaCl/0.98 a_w), traditional dry-sausage (Trad, 3% NaCl/0.95 a_w) and industrial dry-sausage (Chour, 3% NaCl/0.95 a_w) media were inoculated by incorporation with 10^5 cells/mL of Cz, Rh, Mix (Cz+Rh) and St. Fungal spores ($2x10^4$) were co-inoculated by three-point inoculation. Petri dishes were incubated at 20 °C for 15 days.

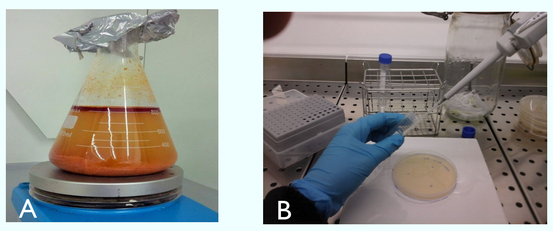

Figure 1: **A:** cooking the meat for the medium. **B:** Spore inoculation on medium with incorporated cells

3. Co-inoculation and mechanisms of action:

Pn was co-inoculated with each Cz/ St in different ways: **A)** Effect of incorporated live cells **B)** Effect of cell-free culture filtrate (BC), **C)** Effect of incorporated dead cells (DC), **D)** Effect of diffusible compounds, **E)** Effect of volatile compounds

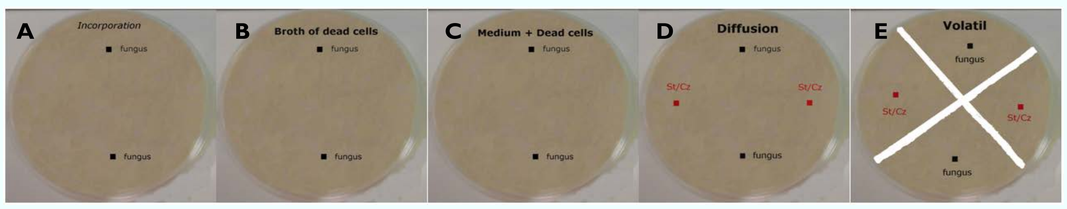

Figure 2: Co-inoculation of microorganisms in different methods

4. OTA evaluation:

OTA was extracted from agar plugs with methanol and quantified by HPLC-FLD (λex 330 nm and λem 463 nm) with a RP-C18 column (100 x 4.6 mm), with isocratic elution in water: acetonitrile: acetic acid (29.5:70:0.5) at 0.8 mL/min.

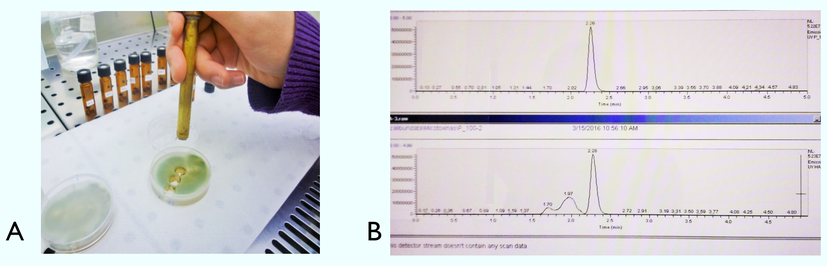

Figure 3: A: OTA extraction by the three agar plug method. **B:** Chromatogram of OTA (Top: OTA standard; bottom: OTA produced in ham-based medium)

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Results:

1. Co-inoculation with *Penicillium nordicum*:

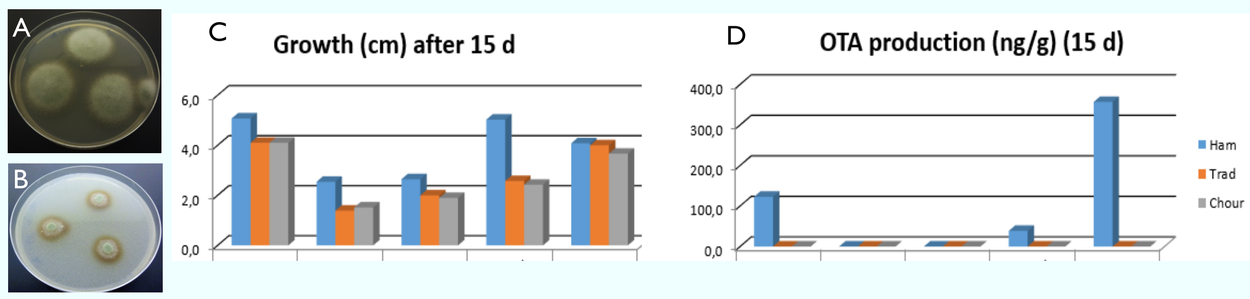

Figure 4: Growth and OTA production by Pn under different conditions after 15 d incubation. **A:** Pn on ham (control); **B:** Pn with Cz on ham; **C:** Pn growth (colony diameter, in cm); **D:** OTA production (ng/g agar)

- All co-inoculants seem to inhibit Pn growth except St
- OTA production by Pn was only detected in ham media.
- In ham medium, Starter significantly stimulated OTA production
- **NOTE:** Rh showed limited growth, thus limited effect, in ham medium

2. Mechanisms of action of Cz/ St:

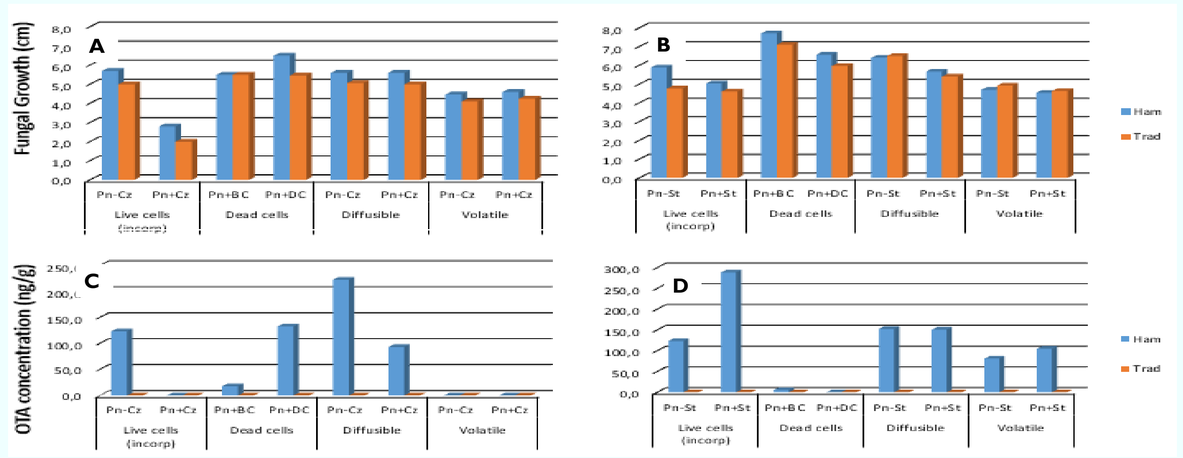

Figure 5: A: Pn growth under different methods of co-culture with Cz; **B:** Pn growth under different conditions of co-culture with St; **C:** OTA production under different methods of co-culture with Cz; **D:** OTA production under different methods of co-culture with Cz

- Cz seems to inhibit Pn growth and OTA production, when incorporated as live cells (direct contact to the fungus) on both cuture media tested
- St has no significant effect on Pn growth, while when in direct contact with the fungus it increases OTA production .
- **NOTE:** Pn showed the inability to produce OTA in Trad medium under all conditions

Conclusions:

P. nordicum did not respond to microorganisms in the same way. The use of biocontrol agents with the intent of reducing fungal growth and mycotoxin production can have unexpected effects, thus leading to unforeseen safety problems. Further experiments are recommended to properly understand the reasons behind the different effects of microorganisms, to ensure their safe use as biocontrol agents.