Typical spoilage microorganisms in cereals

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INTRODUCTION

- **Cereals are one of the most important sources of food** (FAO, 2002), which have contributed to human nutrition for millennia. However, **cereals are exposed to numerous biotic and abiotic stress factors**, from cultivation and throughout their life cycle to processing.
- **Toxigenic fungi are a major problem in cereal crops** as they produce a multitude of toxic metabolites contaminating plants and food products.

ABOUT CEREAL

- **The major cereal crops** produced worldwide are **wheat** (*Triticum* spp.), **rice** (*Oryza* spp.), **maize** (*Zea mays* L.), and **barley** (*Hordeum vulgare* L.) (USDA, 2013).
- **Other cereals include millet**, **sorghum**, **rye**, **oat** and **triticale**.
- **Maize ranks first in quantity produced and cultivation area of cereals worldwide**, followed by wheat, rice and barley.
- Cereals are **important in human nutrition as a source of protein, dietary fiber**, and **carbohydrates**, as well as providing such as, **magnesium**, **zinc**, and **micronutrients E** and **B complex-vitamins** (McKevith, 2004).

ABOUT CEREAL

- Cereals are also used to produce **oils, starch, flour, sugar, syrup, malt, alcoholic beverages, gluten** and **renewable energy**.
- Indigenous microbiota in cereal grains consists of **virus, bacteria, filamentous fungi, yeast, slime moulds** and **protozoa**.
- Cereal grains are exposed to **contaminations** in the field from several sources (water, composted manure, soil, etc.), during **cultivation, harvest, storage,** and **transport.**

CEREAL SPOILAGE

- **Common phytogenic microorganisms** include **bacteria** (e.g. *Pseudomonadaceae*, *Micrococcaceae*, *Lactobacillaceae* and *Bacillaceae*), **yeasts** (e.g. *Candida*, *Cryptococcus*, *Pichia*, *Sporobolomyces*, *Rhodotorula*, *Trichosporon*) and **filamentous fungi** (e.g. *Alternaria*, *Aureobasidium*, *Cladosporium*, *Epicoccum*, *Fusarium*, *Helminthosporium, Claviceps*).

CEREAL SPOILAGE

- Additionally, potential secondary infections can occur **post-harvest**. **Grains can be contaminated during cleaning, milling, grading or packaging processes** (from residues in containers, equipment, screw-conveyors, etc).
- Common microorganisms infecting grains in storage include **xerophilic *Aspergillus glaucus*** group, and ***Penicillium*** spp., where the most important parameter for mould germination is the minimum a_w of 0.68 (14% moisture) (Lacaet al., 2006; Laitila, 2007; Noots et al., 1999).

CEREAL SPOILAGE

- After processing, **the main spoilage fungi affecting cereal products belong to the genera *Aspergillus*, *Penicillium*, and *Fusarium***.
- **Filamentous fungi** are a main safety concern due to the production of **mycotoxins** accumulated in grains pre- and post-harvest, which are associated with severe health problems.
- Mycotoxins can be **carcinogenic, mutagenic, genotoxic, teratogenic, neurotoxic, and oestrogenic**, including reproductive and developmental toxicity (Fung and Clark, 2004; Jestoi, 2008; Köppen et al., 2010).

CEREAL SPOILAGE

- High incidence of mycotoxin infections in cereals have been observed worldwide (Placinta et al., 1999), in different crops and regions (Manthey et al., 2004; Warzecha et al., 2011).
- **Mycotoxins**, such as ***Fusarium*** toxins, ***Alternaria*** toxins, and the **ergot alkaloid groups**, are common contaminants of cereal grains (Pleadin et al., 2012; Roscoe et al., 2008; Santos et al., 2012).

CEREAL SPOILAGE

- Table shows the most common mycotoxins detected in cereals and its health effects for humans and animals. Over the last two years, contaminations in cereals and bakery products by **aflatoxins** (48%) and **ochratoxin A** (OTA) (14%), by ***Aspergillus*** species, and **deoxynivalenol** (DON) (21%) and **fumonisins** (13%), by ***Fusarium*** species, were record (RASFF, 2012).

CEREAL SPOILAGE

Mycotoxin	Fungi source	Cereal crops	Health effects in humans and animals	LD_{50} ($mg.kg^{-1}$)
Aflatoxins (B_1, B_2, G_1, G_2)	*Aspergillus* (*flavus, bombycis, nomius, ochraceoroseus, parasiticus, parvisclerotigenus, pseudotamarii, rambellii, toxicarius*); *Emericella* (*astellata, olivicola*)	Maize	Potent carcinogens, neurotoxins and immunosuppressants. Aflatoxicosis: death due to consumption of contaminated food; liver disease and cancer in humans and animals; hydroxylated aflatoxin metabolites (M1 and M2) found in milk.	**AFB_1** Mice: 9.0 [p.o.] Rabbit: 0.3 [p.o.] Dog: 0.5 – 1.0 [p.o.]
Alternaria alternata lycopersici (AALs)	*Alternaria* (*alternata, triticina, arborescens, cucumerina, dauci, kikuchiana, solani*)	Wheat, barley, oat	Carcinogenic; might be responsible for oesophageal cancer. Experiments with rodents indicate the following mycotoxin acute toxicity: altenuene (ALT) > (tenuazonic acid) TeA > (alternariol monomethyl ether) AME > (alternariol) AOH.	**AME, AOH** Mice: 400 [i.v.] **TeA** Mice: 162 – 115 [i.v.], 225 [p.o.] **ALT** Mice: 50 [i.v.]
Avenacein Y	*Fusarium* (*avenaceum, chlamydosporum, lateritium, tricinctum*)	Wheat	Significant antibiotic properties against phytopathogenic bacteria with low cell toxicity.	Not available
Butenolide	*Fusarium* (*avenaceum, crookwellense, culmorum, graminearum, poae, sambucinum, sporotrichioides, tricinctum, venenatum*)	Broad	Associated with cattle diseases, synergistic effects with enniatins B.	Mice: 44 [i.p.], 275 [p.o.]
Citreoviridin	*Aspergillus terreus*; *Eupenicillium cinnamopurpureum*; *Penicillium* (*citreonigrum, manginii, miczynskii, smithii*)	Rice	Possibly involved in acute cardiac beriberi, sporadically associated with yellow rice disease.	Mice: 7.5 [i.p.], 20 – 29 [p.o.], 11 [s.c.]
Citrinin (CTN)	*Aspergillus* (*terreus chemotype II, carneus, niveus*); *Blennoria sp.*; *Clavariopsis aquatic*; *Monascus ruber*; *Penicillium* (*manginii, chrzaszii, citrinum, expansum, odoratum, radicicola, verrucosum, westlingii*)	Broad	Potent nephrotoxin.	Mice: 35 – 58 [i.p.], 110 [p.o.] Rat: 50 [p.o.] Rabbit: 19 [i.p.]
Culmorin and derivates	*Fusarium* (*crookwellense, culmorum, graminearum, langsethiae, poae, sporotrichioides*)	Broad	Synergistic effect with DON towards caterpillars.	Low toxicity in *in vitro* assays
Cyclochlorotine	*Penicillium islandicum*	Rice	Chlorine containing cyclic peptides associated with yellowed rice toxicosis.	Mice: 0.3 [i.p., i.v.], 6.5 [p.o.], 0.48 [s.c.] Rat: 50 [i.p.], 5 [p.o.], 0.4 [s.c.]
Cyclopiazonic acid	*Aspergillus* (*flavus, lentulus, oryzae, parvisclerotigenus, pseudotamarii, tamarii*); *Penicillium* (*camemberti, commune, dipodomyicola, griseofulvum, palitans*)	Broad	Potent organ damaging calcium chelating mycotoxin; produces focal necrosis in most vertebrate inner organs.	Rat: 2.3 [i.p.], 36 – 63 [p.o.]

CEREAL SPOILAGE

Deoxynivalenol (DON) and derivatives	*Fusarium* (*culmorum, graminearum, pseudograminearum*)	Broad	Nausea, vomiting and stomach pains; chronic and fatal toxic effects. At the cellular level, the main toxic effect is the inhibition of protein synthesis via binding to ribosome.	Mice: 49 – 70 [i.p.], 46 – 78 [p.o.] Duckling: 27 [s.c.] Chicks: 140 [p.o.]
Diacetoxyscirpenol (DAS)	*Fusarium* (*venenatum, poae, equiseti, sporotrichioides, langsethiae, sambucinum*)	Broad	Effects in immune system, inhibits initiation of protein synthesis, killing rapidly proliferating cells.	Mice: 23 [i.p.] Rabbit: 1.0 [i.v.] Swine: 0.37 [i.v.]
Enniatins (ENNs) (A, A1, B, B1) and cyclic peptides	*Fusarium* (*acuminatum, avenaceum, langsethiae, lateritium, poae, sambucinum, sporotrichioides*); *Halosarpeia sp.*; *Verticillium hemipterigenum*	Broad	Antibiotic and ionophoric activity. Induction of apoptosis. Enniatin B often occurs together with enniatin B1 and A.	Mice: 10 – 40 [i.p.] (death within 2 – 5 days)
Ergot alkaloids (ergolines)	*Claviceps* (*fusiformis, paspali, purpurea*)	Rye	Ergotism in human and animals, ergot alkaloids cause vasoconstriction and neurotoxicity including hallucinations.	**Ergometrine** Mice: 160 [i.v.], 448 [p.o.] Rabbit: 3.2 [i.v.] **Ergotamine** Mice: 265 [i.v.] Rabbit: 3 [i.v.], 550 [p.o.]
Fumonisins (B_1, B_2, B_3)	*Fusarium* (*anthophilum, dlamini, napiforme, nygamai, proliferatum, thapsinum, verticillioides*)	Maize, millet, sorghum, rice	Interfere with some steps that contribute to cell growth. Weak link with increased risk of throat cancer. Affect nervous system of horses.	***F. verticillioides* extract** Mice: 45.4 – 51.7 [i.p.], > 1000 [p.o.] Chicks: 81 – 88 [i.p.]
Fusaproliferin (FUS)	*Fusarium* (*globosum, guttiform, proliferatum, pseudocircinatum, pseudonygamai, subglutinans, verticillioides*)	Maize	Recent mycotoxin which shows teratogenic and pathological effects in cell assays. Toxic in *in vitro* trials to brine shrimp and mammalian cells.	Not available
Fusarenon-X (FUS-X)	*Fusarium* (*culmorum, graminearum, cookwellense, poae, nivale, equiseti, tricinctum*)	Rice, wheat	It is toxic to murine thymocytes, lymphocytes and gastric epithelial cells and to human hepatoblastoma cells, acute toxic effects on gastric epithelial cells in animals such as vomiting.	Mice: 4.5 [p.o.] Rat: 4.4 [p.o.]
Moniliformin (MON)	*Fusarium* (*avenaceum, napiforme, nygamai, oxysporum, proliferatum, subglutinans, tricinctum, thapsinum, verticillioides*)	Corn, sorghum, millet, rice	Cytotoxic, inhibits protein synthesis and enzymes, chromosome damages, induce heart failure in mammals and poultry.	Mice: 21 – 29 [i.p.] Rat: 42 – 50 [i.p.] Chicks: 5.4 [p.o.]
Nivalenol (NIV)	*Fusarium* (*graminearum, poae, culmorum, venenatum, equiseti, crookwellense*)	Broad	Hormone (oestrogen) mimic, limited evidence of genotoxicity. Estrogenic toxin affects reproduction. Inhibition of protein synthesis.	Mice: 4.1 [i.p.]
Ochratoxin A (OTA)	*Aspergillus* (*carbonarius, cretensis, flocculosus, lacticoffeatus, niger, ochraceus, pseudoelegans, roseoglobulosum, sclerotioniger, sclerotiorum, steynii, sulphureus, westerdijkiae*); *Neopetromyces muricatus*; *Penicillium* (*nordicum, verrucosum*); *Petromyces* (*albertensis, alliaceus*)	Rice, wheat	Toxic to the kidneys (nephrotoxic) and the immune system, it is classified as a probable human carcinogen. Neurotoxins and immunosuppressants.	Mice: 22 – 40 [i.p.], 26 – 34 [i.v.], 46 – 58 [p.o.] Rat: 12.6 [i.p.], 20 – 30 [p.o.] Chicken/swine: 2.1 – 4.7 [p.o.]
Patulin	*Aspergillus* (*clavatonanica, clavatus, giganteus, longivesica, terreus*); *Byssochlamys nivea*; *Penicillium* (*carneum, clavigerum, concentricum, coprobium,*	Rye, rice	Very toxic with various toxic effects; can harm the immune system and gastrointestinal tract.	Rat: 5 – 15 [i.p.], 15 – 25 [i.v.], 25 – 46 [p.o.] Mice: 7.6 [i.p.]

CEREAL SPOILAGE

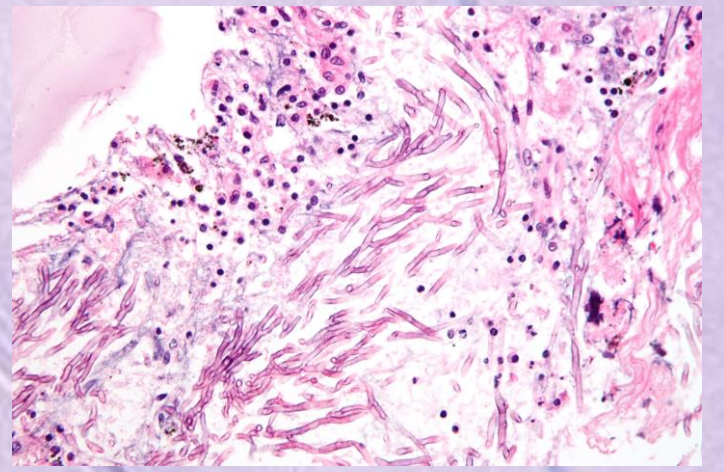
	dipodomyicola, expansum, formosanum, gladioli, glandicola, griseofulvum, marinum, paneum, sclerotigenum, vulpinum)			
Penitrem A	*Penicillium* (*clavigerum, crustosum, glandicola, janczewskii, melanoconidium, tulipae*)	Broad	Mycotoxic indol-terpene with tremorgenic properties, implicated with mycotoxicoses of animals, suspected to be implicated in tremors in humans.	Mice: 1 [i.p.]
T-2 toxin and HT-2 toxin	*Fusarium* (*sporotrichioides, langsethiae, poae, sambucinum*)	Broad	It is the most toxic of the *Fusarium* trichothecenes. Interferes with protein synthesis and DNA/RNA synthesis (HT-2 toxin derivate is less toxic).	Mice: 5.2 [i.p.], 5.2 – 10.5 [p.o.] Rat: 5.2 [p.o.] Swine: 1.2 [i.v.]
Trichodermin	*Trichoderma viride*	Wheat, maize	Potent inhibitor of plant growth with several phytotoxic effects. It inhibits wheat coleoptile growth. Inhibits protein synthesis by binding to ribosomes, proposed as antifungal and antineoplastic, used as tool in cellular biochemistry.	Mice: 500 [s.c.]
Zearalenone (ZEA)	*Fusarium* (*graminearum, culmorum, equiseti, crookwellense*)	Broad	Estrogenic activity in farm animals and it is implicated in hyperestrogenic syndromes in humans.	Mice: > 500 [i.p., p.o.]
Xanthomegnin	*Aspergillus* (*auricomus, bridgeri, elegans, flocculosus, insulicola, melleus, neobridgeri, ochraceus, ostianus, persii, petrakii, roseoglobulosus, sclerotiorum, steynii, sulphureus, westerdijkiae*); *Microsporon cookie; Neopetromyces muricatus*; *Penicillium* (*cyclopium, freii, janthinellum, mariaecrucis, melanoconidium, tricolor, viridicatum*); *Trichophyton* (*megninii, mentagrophytes, rubum, violaceum*)	Broad	Mycotoxicosis in animals, toxic to liver and kidneys in mammals.	Mice: 450 [p.o.]

[i.p.] intraperitoneal administration;
[i.v.] intravenous administration;
[p.o.] oral administration;
[s.c.] subcutaneous administration.

TYPICAL SPOILAGE MICROORGANISMS

Aspergillus

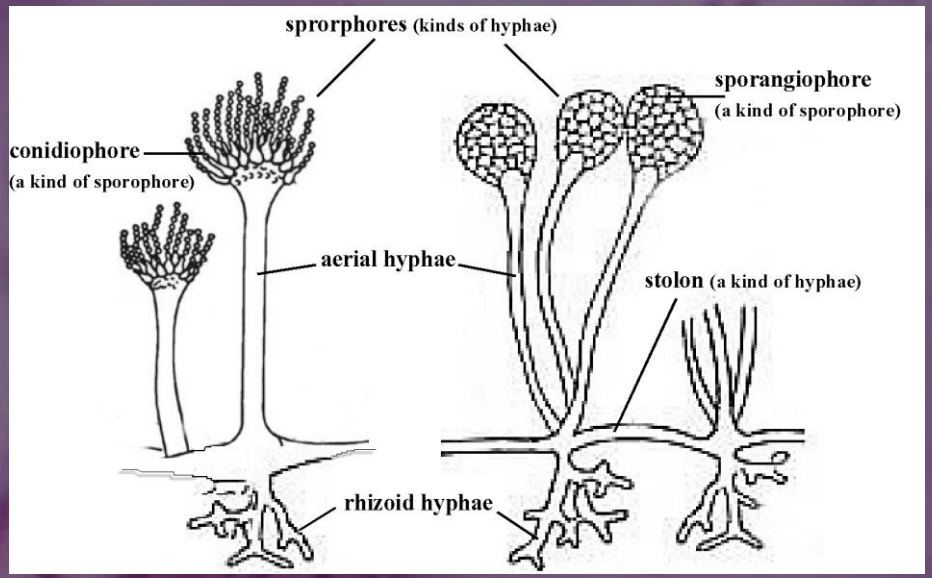
- Can live in high **osmotic concentration** (high sugar, salt, etc.) environment
- Like highly **aerobic** condition
- Use **carbon-rich substrates**(monosaccharides-glucose, polysaccharide-amylose)
- Live in **starchy foods, plants and trees**
- *Aspergillus* spp. cause disease on many grain crops, especially maize, and some variants synthesize mycotoxins, including **aflatoxin**.
- Aspergillosis (fever, cough, chest pain, or breathlessness-Pulmonary aspergillosis)

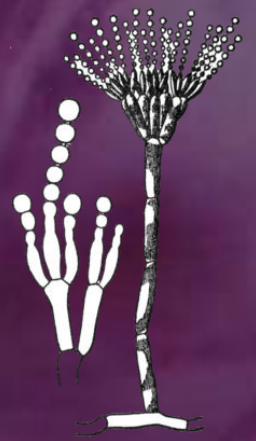


TYPICAL SPOILAGE MICROORGANISMS

Penicillium

- **Thallus** consists of a **highly branched network** of multinucleate, septate, usually colorless hyphae
- Many-branched **conidiophores** sprout on the mycelia, bearing individually constricted conidiospores.
- The **conidiospores** are the main dispersal route of the fungi, and often are green in color.
- *Penicillium* **growth** can still occur indoors even if the relative **humidity is low.**





TYPICAL SPOILAGE MICROORGANISMS

Fusarium

- widely distributed in **soil** and associated with **plants**
- Some **species produce mycotoxins in cereal crops** that can affect human and animal health if they enter the food chain.
- The main toxins produced by these *Fusarium* species are **fumonisins** and **trichothecenes**.

TYPICAL SPOILAGE MICROORGANISMS

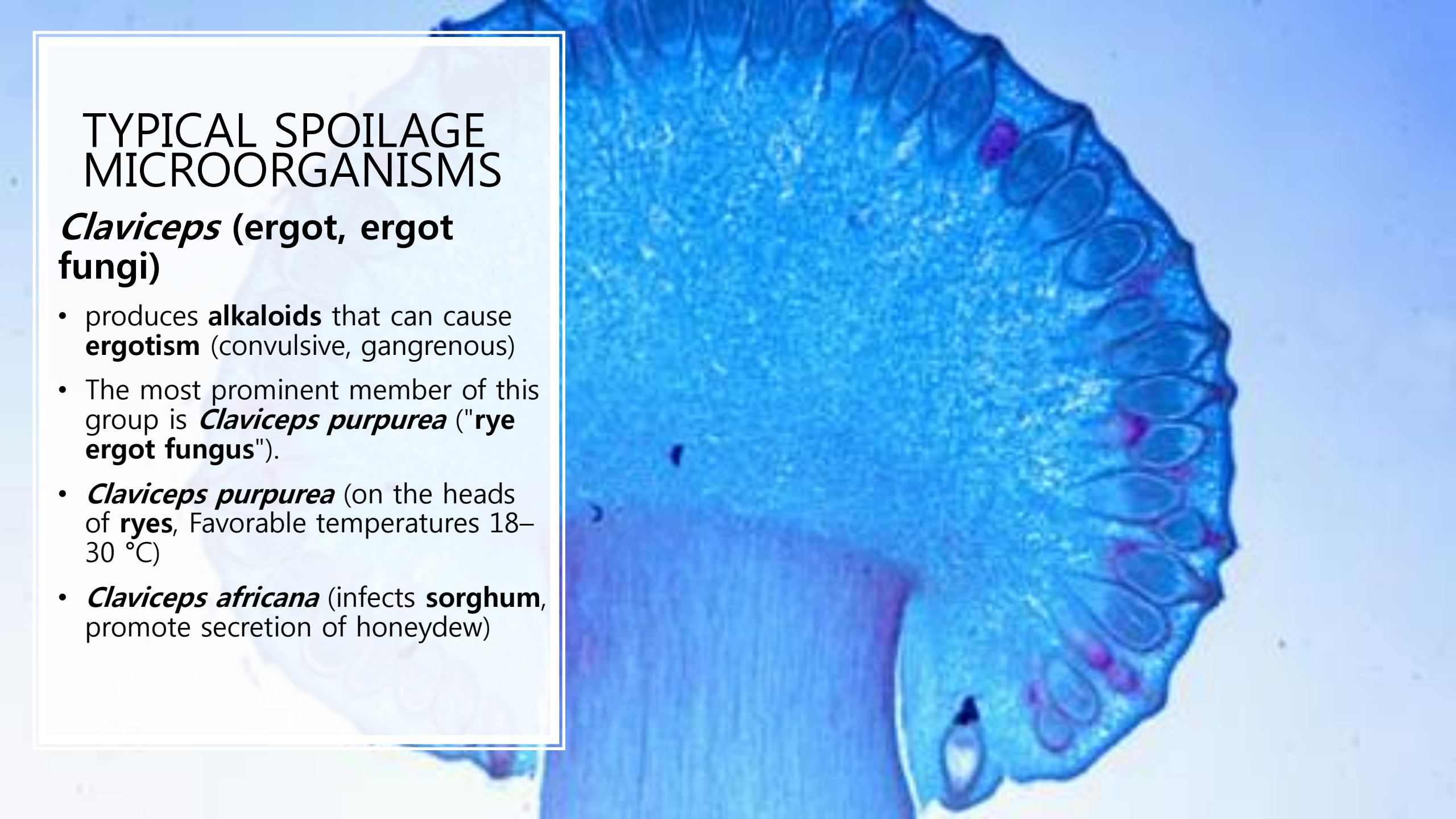
Alternaria

- The club-shaped spores are **single or form long chains**.
- Can grow thick colonies which are usually **green, black, or gray**
- Grow on **skin** and **mucous membranes**, including on the **eyeballs** and within the **respiratory tract**
- **Allergies** are common, but serious infections are rare.
- **Alternariosis**

TYPICAL SPOILAGE MICROORGANISMS

Claviceps (ergot, ergot fungi)

- produces **alkaloids** that can cause **ergotism** (convulsive, gangrenous)
- The most prominent member of this group is ***Claviceps purpurea*** ("**rye ergot fungus**").
- ***Claviceps purpurea*** (on the heads of **ryes**, Favorable temperatures 18–30 °C)
- ***Claviceps africana*** (infects **sorghum**, promote secretion of honeydew)



SUMMARY

- **The major cereal crops**: **wheat** (*Triticum* spp.), rice(*Oryza* spp.), **maize** (*Zea mays* L.), and **barley** (*Hordeum vulgare* L.)
- **Typical spoilage microorganisms**: ***Aspergillus, Penicillium, Fusarium, Alternaria, Claviceps (genus)***
- Filamentous fungi produces **mycotoxins**.
- Mycotoxins can be **carcinogenic, mutagenic, genotoxic, teratogenic, neurotoxic, and oestrogenic.**

CONCLUTION / FURTHER STUDY

- Fungal infections cause quality and quantity losses in the food cereal chain.
- Cereal crop diseases lack effective prevention strategies and cost € billions.
- Antifungal lactic acid bacteria is a suitable hurdle antifungal technology.
- LAB bioprotection can be applied effectively pre-/post-harvest and post-processing.
- Antifungal LAB provides product safety, nutritional, and organoleptic advantages.

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THANK YOU