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Use of molds and their antifungal proteins for biocontrol of toxigenic molds on dry-ripened cheese and meats

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Traditional dry-ripened foods require the contribution of molds to achieve their particular flavor, but ripening conditions favor growth of spoilage and toxigenic molds. To control unwanted molds, physical and chemical methods are not appropriate for these foods. Lactic acid bacteria are not adequate for the low water activity at the food surface where molds grow. Yeasts are not very effective by themselves to prevent mycotoxins in dry-ripened foods. Molds producing antifungal proteins offer a quite promising means to combat unwanted molds. However, their performance on dry-ripening foods is yet to be tested. The microbial ecology of the ripened food has to be carefully considered, paying special attention to the effect on mycotoxin metabolism.

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Introduction

Mold-ripened foods can be found all over the world, being cheeses and meats the primary products in many countries. Traditional processing is characterized by long ripening times under natural environmental conditions, where molds are allowed to grow on the food's surface throughout the ripening process. Highly appreciated cheeses and ripened meats are commonly made of unpasteurized milk or raw meat, so that endogenous enzymes can play their key role in ripening. In addition, fungal enzymes are essential for proteolysis and flavor development in high quality products. The environmental conditions in cellars force the food to lose moisture throughout ripening, thus exerting a strong selective pressure on the microbiota. Temperature, pH, and, most importantly, water activity (a_w) changes may favor unwanted molds growth. Uncontrolled fungal populations lead to both spoilage and health hazards due to mycotoxins. Therefore, controlling fungal growth is a must for dry-ripened meats and cheese.

Among the means to combat unwanted molds, physical treatments can be instantly effective against molds. However, these methods destroy endogenous enzymes and microbiota, thus making them unsuitable for dry-ripened raw foods. Modified atmosphere storage or packaging greatly impairs fermentation, leading to off-flavor formation in mold-ripened foods. Chemical preservatives are efficient and cost-effective antifungal means, but they are against claims for 'natural' or 'traditional' products and raise concern about health risks. In addition, all these methods have the drawback of not being selective against toxigenic molds, but inhibiting desirable molds too. Their use at the end of processing would not be satisfactory for dry-ripened meats and cheese, due to the fact that preformed mycotoxins are not efficiently destroyed by physical or chemical treatments.

Microorganisms provide an additional tool against unwanted molds. Bacteria, yeasts, and molds are not as effective as physical or chemical antifungals, but controlled microbial populations respond to consumer health issues. Therefore, preventing growth of mycotoxigenic fungi in mold-ripened foods requires taking the advantages of an adequate combination of different strategies according to the stage and environmental parameters of the processed food. The present work offers an insight into the ecological strategy using microorganisms, focusing particularly on the use of the recently described antifungal proteins from molds.

Role of molds on dry-cured meats and cheeses

The ecology of dry-cured meat products and ripened cheese, particularly the low a_w at the surface, favor the development of spontaneous fungal population. Thus, molds commonly outgrow other microbial groups, dominating the microbial population for most of the ripening period. Molds are essential for different varieties of cheese, including the well-known white surface, blue-veined, and smear-ripened types. *Penicillium camemberti* and *Penicillium roqueforti* are frequently isolated, but also *Aspergillus*, *Cladosporium*, *Mucor*, *Geotrichum*, and *Fusarium* spp. can be found [1–3]. Molds grow on these cheeses and contribute to their flavor through lipolytic, proteolytic, and oxidative activities. *Penicillium* spp. are decisive for the appearance, texture, and flavor development of mold-ripened cheeses, leading to high production of ketones and alcohols with a significant impact on the sensory attributes [2,3].

In dry-cured meat products *Penicillium* is the prevailing group during most of ripening [4–7]. *Aspergillus* and *Eurotium* spp. become dominant in products with prolonged ripening process, especially as temperature increases and a_w decreases [4,5,7]. Other genera found less frequently are *Mucor*, *Cladosporium*, *Scopulariopsis*, *Geotrichum*, and *Alternaria* [4,7]. Fungi also play a central role in the development of the texture, flavor, and sensorial quality of meat products. Molds promote proteolysis and lipolysis increasing the concentration of peptides, free amino acids and free fatty acids in dry-cured meat products [8,9]. These compounds are substrates for further reactions leading to methyl branched oxy-compounds that contribute to the highly appreciated flavor of cured meats [10,11]. In addition, fungi decrease compounds derived from lipid oxidation, retarding rancidity during ripening [8,12].

Therefore, molds are used as starter cultures in both cheese and meat products. These cultures may also play a role as biocontrol agents even though they had not been selected by their ability to inhibit unwanted microorganisms.

Detrimental effects of molds on dry-cured meats and cheeses

Despite the beneficial effects of molds, their uncontrolled growth can be responsible for food spoilage, allergies and mycotoxin contamination. Molds growth on dry-ripened foods has been associated with off-flavors and unpleasant appearance. For example, *Cladosporium oxysporum* is responsible for black spot formation in sausages [13], and *Scopulariopsis brevicaulis* or *Mucor* spp. lead to ammoniacal odor and taste in cheeses [14].

I the same way, molds growing on ripened foods spread into the air, which may cause respiratory diseases or urticaria to workers [15,16]. Ingestion of moldy cheese rind or dry-fermented sausages may cause allergic reactions [17,18]. In addition, some strains of *Penicillium nalgiovense* or *Penicillium olsonii* isolated from dry-cured foods are able to produce penicillin [19,20]. If penicillin was produced on foods, it would pose a threat to allergic individuals.

Likewise, growth of mycotoxigenic molds on foods is a serious potential hazard for consumers. Various species isolated from dry-ripened foods are mycotoxin producers, including *Aspergillus flavus*, *Aspergillus ochraceus*, *Aspergillus parasiticus*, *P. camemberti*, *Penicillium commune*, *Penicillium expansum*, *Penicillium griseofulvum*, *Penicillium nordicum*, *Penicillium polonicum*, *P. roqueforti* and *Penicillium verrucosum*. Although the environmental conditions needed for mycotoxin synthesis are usually more restrictive than those necessary for mold growth [21], ochratoxin A (OTA), aflatoxins, patulin, citrinin, and cyclopiazonic acid production on dry-cured meats have been reported [6,22–24]. OTA produced by mold on surface of meat product diffuses at least 5 mm into the inner tissues in few days [24]. Some of mycotoxins, such as patulin, rapidly decrease after direct contamination of dry-cured ham, but cyclopiazonic acid, citrinin and OTA are more stable [22].

Various mycotoxins can be produced by molds during cheese-making, such as roquefortine C, PR toxin, penicillic acid, patulin, mycophenolic acid, and penicillic acid by *P. roqueforti*; cyclopiazonic acid by *P. camemberti*; or sterigmatocystin and ochratoxin by other *Penicillium* or *Aspergillus* spp. [1,25].

Given that high mold counts are found in the air inside food producing facilities and in raw materials, fungal contamination of dry-ripened foods cannot be prevented, but only minimized. Therefore, strategies to control growth of toxigenic molds and mycotoxin production on dry-ripened foods should be adopted. The use of mold starter cultures has proved to be efficient for some fermented sausages and white-surface mold cheeses, but more complex strategies are required for foods with long dry-ripening periods.

Biological control

Biological control using microorganisms is an emergent alternative to efficiently control fungi and mycotoxin production. For this purpose, lactic acid bacteria (LAB), yeasts, and molds found naturally in dairy and meat products could be used (Table 1).

The wide antimicrobial spectrum of LAB has been attributed to the synergistic actions of several low-molecular-weight compounds, including organic and carboxylic acids, fatty acids, reuterin, hydrogen peroxide, phenolic compounds, lactones, cyclic dipeptides, diacetyl, and proteinaceous compounds [26,27]. The antifungal mechanism of action of most of these compounds remains unknown. Reuterin causes oxidative stress to fungi [26], whereas the supernatant from a culture of *Lactobacillus plantarum* K35 containing lactic acid, indene, fatty acids and other organic acids caused severe damage to cell wall and cytoplasmic membrane of *A. flavus* and *A. parasiticus* [27]. LAB also produce proteinaceous compounds, such as defensin-like proteins, peptides with homology to lacticin, or peptides derived from the proteolytic activity of LAB against caseins [26]. Furthermore, some LAB are able to remove mycotoxins, including aflatoxins, OTA, patulin and fumonisins, from different matrices by physical adsorption to the cell wall [28,29]. *Lactobacillus* spp. and *Lactococcus lactis* have been proposed as bioprotective

Table 1

Antifungal biocontrol agents and their mode of action.

Biocontrol agent	Activity/active compounds	Effect on toxigenic molds/mycotoxins	Reference
Lactic-acid bacteria	Reuterin	Oxidative stress	[26]
	Low-molecular-weight compounds (lactic acid, indene, fatty acids and other organic acids)	Damage to the cell wall and cytoplasmic membrane, massive loss of cytoplasmic content, formation of membrane-bound vesicles, and destruction of organelles	[27]
	Cell wall compounds	Mycotoxin adsorption	[28,29]
Yeasts	Competition for space	Suppression the adherence of molds to food surface	[37]
	Competition for nutrients	Consumption of limiting nutrients for toxigenic molds	[38]
	Volatile compounds	Change gene expression, protein profile and the activity of enzymes	[41]
	Killer proteins	Disruption of cytoplasmic membrane, block of DNA synthesis, inhibition cell wall synthesis, morphological changes	[43,44,58]
	Lytic enzymes	Degradation of hyphae, loss of cellular integrity, inhibition hyphal growth, leakage of cytoplasmic components	[42,44]
	Cell wall compounds	Mycotoxin adsorption	[45,46•]
	Yeast metabolism	Biodegradation of mycotoxins	[46•]
		Reduction in the expression of toxin-biosynthetic genes	[45]
Molds	Competition for space and nutrients	Reduction of growth	[1,47]
	Antifungal proteins	Membrane changes, reduction of metabolic activity, oxidative stress, and apoptosis	[52•]

cultures for fresh cheese, raw meat, and raw smoked sausages to delay and decrease fungal growth [30,31•,32]. However, their limited resistance to intermediate a_w values makes them unsuitable for foods subjected to a long-ripening period, such as cheeses and dry-cured meats.

Yeasts have been found throughout the ripening of dry-ripened foods, and are involved in the generation of volatile compounds contributing to the flavor of dry-ripened foods [9]. In addition, some yeasts may retard mold growth in dairy and meat products, due to competition for space and nutrients, production of killer toxins, cell wall hydrolytic enzymes, and volatile compounds [33–36]. Upon colonizing a food surface, fast-growing yeasts may suppress the adherence of filamentous fungi to the surface [37]. Additionally, yeasts could rapidly consume limiting nutrients for toxigenic molds, thus retarding fungal growth [38]. Yeasts also produce some volatile compounds, such as alcohols, acids, and esters, which show antifungal properties [39,40•]. The antifungal effect of such volatile compounds has been related to changes in gene expression, protein profile and enzymes activity in sensitive fungi [41]. Certain killer proteins are effective against filamentous fungi [42], interacting with components of the cell wall and causing disruption of cytoplasmic membrane [43], or as lytic enzymes that degrade fungal hyphae and spores [44]. Furthermore, some yeasts can reduce the mycotoxin concentration, which may be due to adsorption to cell wall, degradation of mycotoxins, or reduction in the expression of toxin-biosynthetic genes [45,46•]. Several yeasts, such as *Debaryomyces hansenii*, *Candida famata*, *Endomyces fibuliger*, and *Candida zeylanoides* have been proposed as protective cultures, because they are able to reduce growth of ochratoxigenic molds and OTA accumulation in dry-cured meat products [5,33,35].

Non-toxigenic molds could be used as potential protective cultures in dairy and meat products to prevent growth of toxigenic molds and mycotoxin production, while keeping the beneficial contribution of fungi to the ripening of dry-cured meat products. *Penicillium chrysogenum* and *P. nalgiovense* are able to limit growth of mycotoxigenic molds in fermented sausages and dry-cured meats [34,47]. However, the processing conditions and the evolution of the protective mold starter culture should be efficiently controlled [6]. In cheese, *P. camemberti*, *P. roqueforti*, and *Geotrichum candidum* inoculated as ripening cultures efficiently compete against toxigenic contaminants [1,14,25].

Antifungal proteins from molds

Some molds produce various peptides and proteins with antifungal properties, including a group of small, basic, and cysteine-rich antifungal proteins (AFPs) that have been reviewed recently by different authors [48,49••]. AFPs are a group of 5.5–10 kDa proteins produced by molds from different genera (Table 2). AFPs are assumed to share a compact β-barrel structure stabilized by 3 or 4 internal disulphide bridges, which makes them highly stable to low pH, heat and proteolysis. The proteins tested withstand temperatures up to 100 °C, pH values from 1 to 12, and proteases usually added to foods [50•,51].

Table 2

Antifungal proteins produced by molds [49••].

Producer mold	Antifungal proteins
Aspergillus clavatus	AcAFP, AcAMP
Aspergillus giganteus	AFP, AFP_{NN5353}
Aspergillus niger	AnAFP
Fusarium polyphilaidicum	FPAP
Monascus pilosus	MAFP1
Neosartorya fischeri	NFAP
Penicillium brevicompactum	BP
Penicillium chrysogenum	PAF, Pc-Arctin, PgAFP
Penicillium citrinum	PcPAF
Penicillium digitatum	AfpB
Penicillium nalgiovense	NAF

The mode of action of AFPs from molds is multifactorial, following two different pathways [49••]. First, AFPs can either bind the cell wall or be actively internalized by sensitive fungi. Then, AFPs can alter chitin synthesis leading to cell death, or increase intracellular reactive oxygen species (ROS) levels, which permeabilize cell membrane and trigger programmed cell death and apoptosis.

AFPs have shown mainly fungistatic activity against sensitive molds. The short-length effect results in lower mycotoxin production due to retarded growth. In fact, proteomic studies with the PgAFP-sensitive *A. flavus* revealed a lower relative quantity of some enzymes involved in aflatoxin biosynthesis upon PgAFP exposure [52•]. However, ROS induction may increase mycotoxin production in the long term in partially inhibited molds, given that oxidative stress activates mycotoxins biosynthesis [53,54]. In addition, naturally resistant fungi may respond against cellular stress caused by AFPs increasing Rho protein levels, which via Pkc/Mpk signaling activates cell wall synthesis to successfully overcome inhibition [55]. An overexpression of Rho protein, as a stress response, has been linked to a higher mycotoxin production [56]. Hence, more studies are required to unveil the role of AFPs on mycotoxin production.

The sole use of AFPs instead of the producing mold could be desirable for surface mold-free foods. However, the information on the antifungal effect of AFPs in foods is quite limited. PgAFP retarded growth of *Aspergillus* and *Penicillium* strains on dry-fermented sausages, but the antifungal effect was time-limited [50•]. Repeated applications might be required to accomplish a long-lasting effect during ripening. Conversely, high extracellular cation concentrations minimize AFPs antifungal activity, but particularly divalent calcium triggers an adaptive response mediated via Ca^{2+} signaling leading to increased chitin synthesis in the sensitive mold [49••,57]. For these reasons, the sole use of AFPs is not expected to be very active on cheese.

Conclusion

Due to consumers' concerns about additives, reputable dry-ripened meats and cheese manufacturers must consider the ecological approach to control unwanted mold growth and mycotoxin production. AFPs are able to inhibit mycotoxigenic molds, but they usually have a rather time-limited effect and their antifungal effect is minimized by the presence of high concentrations of divalent cations. Thus, complementary strategies are required to effectively control unwanted molds in foods. These strategies should be based on synergistic agents to AFPs. Some microorganisms usually found in dry-ripened foods such as LAB and yeasts have been proposed for this purpose [33,40•]. Therefore, the combined use of AFPs and these microorganisms seems to be a potential strategy that deserves to be further studied. Taking advantage of the antifungal potential of the various microorganisms that grow on dry-ripened foods, including LAB, yeasts and fungi, is proposed as a natural cost-effective means to obtain safe and full-flavored products.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Hymery N, Vasseur V, Coton M, Mounier J, Jany JL, Barbier G, Coton E: **Filamentous fungi and mycotoxins in cheese: a review**. *Compr Rev Food Sci Food Saf* 2014, **13**:437-456.

2. Spinnler H-E, Gripon J-C: **Surface mould-ripened cheeses**. In *Cheese: Chemistry, Physics and Microbiology*. Edited by Fox P, McSweeney P, Cogan T, Guinee T. Academic Press; 2004: 157-174.

3. Cantor MD, van den Tempel T, Hansen TK, Ardö Y: **Blue cheese**. In *Cheese: Chemistry, Physics and Microbiology*. Edited by Fox P, McSweeney P, Cogan T, Guinee T. Academic Press; 2004: 175-198.

4. Castellari C, Quadrelli AM, Laich F: **Surface mycobiota on Argentinean dry fermented sausages**. *Int J Food Microbiol* 2010, **142**:149-155.

5. Comi G, Iacumin L: **Ecology of moulds during the pre-ripening and ripening of San Daniele dry cured ham**. *Food Res Int* 2013, **54**:1113-1119.

6. Iacumin L, Chiesa L, Boscolo D, Manzano M, Cantoni C, Orlic S, Comi G: **Moulds and ochratoxin A on surfaces of artisanal and industrial dry sausages**. *Food Microbiol* 2009, **26**:65-70.

7. Núñez F, Rodríguez MM, Bermúdez ME, Córdoba JJ, Asensio MA: **Composition and toxigenic potential of the mould population on dry-cured Iberian ham**. *Int J Food Microbiol* 1996, **32**:185-197.

8. Bruna JM, Hierro EM, De La Hoz L, Mottram DS, Fernández M, Ordóñez JA: **Changes in selected biochemical and sensory parameters as affected by the superficial inoculation of *Penicillium camemberti* on dry fermented sausages**. *Int J Food Microbiol* 2003, **85**:111-125.

9. Martín A, Córdoba JJ, Núñez F, Benito MJ, Asensio MA: **Contribution of a selected fungal population to proteolysis on dry-cured ham**. *Int J Food Microbiol* 2006, **110**:8-18.

10. Song H, Cadwallader KR: **Aroma components of American country ham**. *J Food Sci* 2008, **73**:C29-C35.

11. Sunesen LO, Stahnke LH: **Mould starter cultures for dry sausages – selection, application and effects**. *Meat Sci* 2003, **65**:935-948.

12. Martín A, Córdoba JJ, Benito MJ, Aranda E, Asensio MA: **Effect of *Penicillium chrysogenum* and *Debaryomyces hansenii* on the volatile compounds during controlled ripening of pork loins**. *Int J Food Microbiol* 2003, **84**:327-338.

13. Lozano-Ojalvo D, Rodríguez A, Cordero M, Bernáldez V, Reyes-Prieto M, Córdoba JJ: **Characterisation and detection of spoilage mould responsible for black spot in dry-cured fermented sausages**. *Meat Sci* 2014, **100**:283-290.

14. Marcellino SN, Benson DR: **The good, the bad, and the ugly: tales of mold-ripened cheese**. *Microbiol Spectr* 2013, **1** CM-0005-2012.

15. Merget R, Sander I, Rozynek P, Heinze E, Imoehl M, Raulf-Heimsoth M, Bruening T: **Occupational immunoglobulin E-mediated asthma due to *Penicillium camemberti* in a dry-sausage packer**. *Respiration* 2008, **76**:109-111.

16. Wantke F, Simon-Nobbe B, Pöll V, Götz M, Jarisch R, Hemmer W: **Contact dermatitis caused by salami skin**. *Contact Dermatitis* 2011, **64**:111-114.

17. Boisnault A, Cassaing S, Morelle K, Dubarry A, Berry A, Linas MD, Rancé F: **Allergie alimentaire à une moisissure présente sur une croûte de fromage: À propos d'une observation pédiatrique**. *Rev Fr d'Allergol d'Immunol Clin* 2005, **45**:597-600.

18. González-de-Olano D, Gandolfo-Cano M, González-Mancebo E, Meléndez-Baltanás A, Juárez-Guerrero R, Bartolomé B: **Different patterns of sensitization in allergy to dry fermented sausage**. *J Invest Allergol Clin Immunol* 2012, **22**:152-153.

19. Asefa DT, Kure CF, Gjerde RO, Omer MK, Langsrud S, Nesbakken T, Skaar I: **Fungal growth pattern, sources and factors of mould contamination in a dry-cured meat production facility**. *Int J Food Microbiol* 2010, **140**:131-135.

20. Papagianni M, Ambrosiadis I, Filiousis G: **Mould growth on traditional greek sausages and penicillin production by *Penicillium* isolates**. *Meat Sci* 2007, **76**:653-657.

21. Rodríguez A, Medina A, Córdoba JJ, Magan N: **Relationship between ecophysiological factors, growth and ochratoxin A contamination of dry-cured sausage based matrices**. *Int J Food Microbiol* 2015, **194**:71-77.

22. Bailly JD, Tabuc C, Quérin A, Guerre P: **Production and stability of patulin, ochratoxin A, citrinin, and cyclopiazonic acid on dry cured ham**. *J Food Prot* 2005, **68**:1516-1520.

23. Pleadin J, Malenica M, Vah N, Milone S, Safti L: **Survey of aflatoxin B_1 and ochratoxin A occurrence in traditional meat products coming from Croatian households and markets**. *Food Control* 2015, **52**:71-77.

24. Rodríguez A, Rodríguez M, Martín A, Delgado J, Córdoba JJ: **Presence of ochratoxin A on the surface of dry-cured Iberian ham after initial fungal growth in the drying stage**. *Meat Sci* 2012, **92**:728-734.

25. Sengun IY, Yaman DB, Gonul SA: **Mycotoxins and mould contamination in cheese: a review**. *World Mycotoxin J* 2008, **1**:291-298.

26. Crowley S, Mahony J, Van Sinderen D: **Current perspectives on antifungal lactic acid bacteria as natural bio-preservatives**. *Trends Food Sci Technol* 2013, **33**:93-109.

27. Sangmanee P, Hongpattarakere T: **Inhibitory of multiple antifungal components produced by *Lactobacillus plantarum* K35 on growth, aflatoxin production and ultrastructure alterations of *Aspergillus flavus* and *Aspergillus parasiticus***. *Food Control* 2014, **40**:224-233.

28. Fuchs S, Sontag G, Stidl R, Ehrlich V, Kundi M, Knasmüller S: **Detoxification of patulin and ochratoxin A, two abundant mycotoxins, by lactic acid bacteria**. *Food Chem Toxicol* 2008, **46**:1398-1407.

29. Pizzolitto RP, Salvano MA, Dalcero AM: **Analysis of fumonisin B_1 removal by microorganisms in co-occurrence with aflatoxin B_1 and the nature of the binding process**. *Int J Food Microbiol* 2012, **156**:214-221.

30. Garcha S, Natt NK: ***In situ* control of food spoilage fungus using *Lactobacillus acidophilus* NCDC 291**. *J Food Sci Technol* 2012, **49**:643-648.

31. • Sedaghat H, Eskandari MH, Moosavi-Nasab M, Shekarforoush SS: **Application of non-starter lactic acid bacteria as biopreservative agents to control fungal spoilage of fresh cheese**. *Int Dairy J* 2016, **56**:87-91.

This paper explores the use of lactic acid bacteria on fresh cheese as antifungal strategy. It studies the stability of the antifungal ability and the mechanism of action. These bacteria could be used together with AFPs.

32. Stoyanova LG, Ustyugova EA, Sultimova TD, Bilanenko EN, Fedorova GB, Khatrukha GS, Netrusov AI: **New antifungal bacteriocin-synthesizing strains of *Lactococcus lactis* ssp. *lactis* as the perspective biopreservatives for protection of raw smoked sausages**. *Am J Agric Biol Sci* 2010, **5**:477-485.

33. Andrade MJ, Thorsen L, Rodríguez A, Córdoba JJ, Jespersen L: **Inhibition of ochratoxigenic moulds by *Debaryomyces hansenii* strains for biopreservation of dry-cured meat products**. *Int J Food Microbiol* 2014, **170**:70-77.

34. Rodríguez A, Bernáldez V, Rodríguez M, Andrade MJ, Núñez F, Córdoba JJ: **Effect of selected protective cultures on ochratoxin A accumulation in dry-cured Iberian ham during its ripening process**. *LWT Food Sci Technol* 2015, **60**:923-928.

35. Virgili R, Simoncini N, Toscani T, Camardo Leggieri M, Formenti S, Battilani P: **Biocontrol of *Penicillium nordicum* growth and ochratoxin A production by native yeasts of dry cured ham**. *Toxins* 2012, **4**:68-82.

36. Masih EI, Paul B: **Secretion of ß-1,3-glucanases by the yeast *Pichia membranifaciens* and its possible role in the biocontrol of *Botrytis cinerea* causing grey mold disease of the grapevine**. *Curr Microbiol* 2002, **44**:391-395.

37. Laitila A, Sarlin T, Kotaviita E, Huttunen T, Home S, Wilhelmson A: **Yeasts isolated from industrial maltings can suppress *Fusarium* growth and formation of gushing factors**. *J Ind Microbiol Biotechnol* 2007, **34**:701-713.

38. Hernández-Montiel LG, Ochoa JL, Troyo-Diéguez E, Larralde-Corona CP: **Biocontrol of postharvest blue mold (*Penicillium italicum* Wehmer) on Mexican lime by marine and citrus *Debaryomyces hansenii* isolates**. *Postharvest Biol Technol* 2010, **56**:181-187.

39. Taczman-Brückner A, Mohácsi-Farkas C, Balla C, Kiskó G: **Mode of action of *Kluyveromyces lactis* in biocontrol of *Penicillium expansum***. *Acta Aliment* 2005, **34**:153-160.

40. • Núñez F, Lara MS, Peromingo B, Delgado J, Sanchez-Montero L, Andrade JM: **Selection and evaluation of *Debaryomyces hansenii* isolates as potential bioprotective agents against toxigenic penicillia in dry-fermented sausages**. *Food Microbiol* 2015, **46**:114-120.

This paper provides noteworthy information about some antifungal mechanisms which could be used together with antifungal proteins as a synergistic strategy.

41. Wheatley RE: **The consequences of volatile organic compound mediated bacterial and fungal interactions**. *Antonie van Leeuwenhoek Int J Gen Mol Microbiol* 2002, **81**:357-364.

42. Coelho AR, Tachi M, Pagnocca FC, Nobrega GMA, Hoffmann FL, Harada K-I, Hirooka EY: **Purification of *Candida guilliermondii* and *Pichia ohmeri* killer toxin as an active agent against *Penicillium expansum***. *Food Addit Contam A Chem Anal Control Expo Risk Assess* 2009, **26**:73-81.

43. Weiler F, Schmitt MJ: **Zygocin, a secreted antifungal toxin of the yeast *Zygosaccharomyces bailii*, and its effect on sensitive fungal cells**. *FEMS Yeast Res* 2003, **3**:69-76.

44. Izgu DA, Kepekci RA, Izgu F: **Inhibition of *Penicillium digitatum* and *Penicillium italicum* in vitro and in planta with Panomycocin, a novel exo-β-1,3-glucanase isolated from *Pichia anomala* NCYC 434**. *Antonie van Leeuwenhoek Int J Gen Mol Microbiol* 2011, **99**:85-91.

45. Gil-Serna J, Patiño B, Cortés L, González-Jaén MT, Vázquez C: **Mechanisms involved in reduction of ochratoxin A produced by *Aspergillus westerdijkiae* using *Debaryomyces hansenii* CYC 1244**. *Int J Food Microbiol* 2011, **151**:113-118.

46. • Pfliegler WP, Pusztahelyi T, Pócsi I: **Mycotoxins – prevention and decontamination by yeasts**. *J Basic Microbiol* 2015, **55**:805-818.
This paper reviews the potential of yeast as biocontrol agents to reduce mycotoxin contamination, considering strain improvement strategies and genetic modification for improved detoxifying properties or mycotoxin resistance.

47. Bernáldez V, Córdoba JJ, Rodríguez M, Cordero M, Polo L, Rodríguez A: **Effect of *Penicillium nalgiovense* as protective culture in processing of dry-fermented sausage "salchichón"**. *Food Control* 2013, **32**:69-76.

48. Hegedüs N, Marx F: **Antifungal proteins: more than antimicrobials?** *Fungal Biol Rev* 2013, **26**:132-145.

49. •• Delgado J, Owens RA, Doyle S, Asensio MA, Núñez F: **Antifungal proteins from moulds: analytical tools and potential application to dry-ripened foods**. *Appl Microbiol Biotechnol* 2016, **100**:6991-7000.
This paper is an excellent review of antifungal proteins, including the mechanism of action of these proteins, the potential analytical tools to study their mechanism of action, as well as some strategies to apply them on foods.

50. • Delgado J, Acosta R, Rodríguez-Martín A, Bermúdez E, Núñez F, Asensio MA: **Growth inhibition and stability of PgAFP from *Penicillium chrysogenum* against fungi common on dry-ripened meat products**. *Int J Food Microbiol* 2015, **205**:23-29.
This paper studies the activity of an antifungal protein after being treated with different enzymes, pH, and temperatures, and demonstrates the activity of the protein on dry-fermented sausages.

51. Skouri-Gargouri H, Jellouli-Chaker N, Gargouri A: **Factors affecting production and stability of the AcAFP antifungal peptide secreted by *Aspergillus clavatus***. *Appl Microbiol Biotechnol* 2010, **86**:535-543.

52. • Delgado J, Owens RA, Doyle S, Asensio MA, Núñez F: **Impact of the antifungal protein PgAFP from *Penicillium chrysogenum* on the protein profile in *Aspergillus flavus***. *Appl Microbiol Biotechnol* 2015, **99**:8701-8715.
This paper studies the mechanism of action of an antifungal protein against a toxigenic mold.

53. Reverberi M, Punelli M, Smith CA, Zjalic S, Scarpari M, Scala V, Cardinali G, Aspite N, Pinzari F, Payne GA *et al.*: **How peroxisomes affect aflatoxin biosynthesis in *Aspergillus flavus***. *PLoS ONE* 2012, **7**:e48097.

54. Schmidt-Heydt M, Stoll D, Schütz P, Geisen R: **Oxidative stress induces the biosynthesis of citrinin by *Penicillium verrucosum* at the expense of ochratoxin**. *Int J Food Microbiol* 2014, **192C**:1-6.

55. Delgado J, Owens RA, Doyle S, Asensio MA, Núñez F: **Increased chitin biosynthesis contributes to the resistance of *Penicillium polonicum* against the antifungal protein PgAFP**. *Appl Microbiol Biotechnol* 2016, **100**:371-383.

56. Hayes BME, Anderson MA, Traven A, Van Der Weerden NL, Bleackley MR: **Activation of stress signalling pathways enhances tolerance of fungi to chemical fungicides and antifungal proteins**. *Cell Mol Life Sci* 2014, **71**:2651-2666.

57. Binder U, Bencina M, Eigentler A, Meyer V, Marx F: **The *Aspergillus giganteus* antifungal protein AFP_{NN5353} activates the cell wall integrity pathway and perturbs calcium homeostasis**. *BMC Microbiol* 2011, **11**:209.

58. Schmitt MJ, Klavehn P, Wang J, Schönig I, Tipper DJ: **Cell cycle studies on the mode of action of yeast K28 killer toxin**. *Microbiology* 1996, **142**:2655-2662.