COMPOSITION OF MOULDS POPULATION AND THEIR SAFETY ASSESSMENT ON JINHUA HAM DURING RIPENING

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Introduction

Jinhua ham, one of the three famous hams in China, is produced in Jinhua district of Zhejiang province with a long history，which has manufactured by traditional way that maturing under natural environmental condition for a long time. These characteristics, together with suitable temperature and relative humidity, favour the development of uncontrolled fungal population. Abundant moulds growth is often observed on the surface of country cured hams which provides a "microclimate".

Moulds on the surface of dry-cured ham have been studied in great details (Leistner et al., 1968; Sosa et al., 2002). The *Penicillium* spp., *Aspergillus* spp., *Eurotium* spp., *Rhizopus* spp., *Mucor* spp., *Cladosporium* spp., *Alternaria* spp., *Scopulariopsis* spp. and *Paecilomyces* spp. et al. seem to be present during all stages of ham production. Among them, the moulds microflora are dominated by the genera of *Penicillium, Aspergillus* and *Eurotium*. However, the moulds growth is so high that there is a real problem with the presence of toxigenic moulds (Nunez et al., 1996). Mycotoxin production by moulds isolated from meat products is well established (Leistner and Ayer, 1968; Sosa et al., 2002; Bailly et al., 2005). To prevent the growth of undesirable moulds, it is suggested to use the non-toxigenic strains as starters, such as *Penicillium nalgiovense* which is used to produce dry-cured hams and sausages in European countries (Leistner and Ayer, 1968). The usage of controlled strains can avoid the development of toxinogenic moulds and ensure the safety of hams and stability of their quality.

The objective of the present study was to define the moulds population growing on the surface during ripening of Jinhua ham and their safety, a control product (naturally maturing hams) and a treatment inoculated *P. nalgiovense* were assayed.

Materials and Methods

Strain 3.4357 of *Penicillium nalgiovense*, provided from China General Microbiological Culture Collection Center, were used. To obtain the inoculum, sterile water was added to the grown culture media, the spores and colonies were gently rubbed, approximately 10^6 spores/ml suspension were taken for inoculation. Another group without inoculation naturally ripened as a control. Each group was consisted of 5 legs selected randomly. The legs were then processed according to traditional procedure.

Samples were taken from a layer 5mm under the surface at five different positions of each ham aseptically. After dispersion, homogenization and dilution, samples were infused into dichloran rose bengal chloramphenicol agar (DBRC Agar) which were incubated for 5 d at 28□. Then, the different colonies of the moulds were selected and inoculated in three points on potato dextrose agars (PDA). Isolates were identified to genus and species level on their morphological characteristics by standard methods currently used according to Pitt and Hocking (1997). Mycotoxin were extracted by chloroform: methanol (2:1, V:V) solvent system, and their toxicity were assessed by the test of inhibiton of *Staphylococcus aureus* (American Type Culture Collection 25923) according to Nurez et al. (1996).

Results and Discussion

Moulds population on the surface of Jinhua ham. A total of 72 strains were isolated mainly represented by five genera *Alternaria* (5.56 %), *Aspergillus* (34.72 %), *Penicillium* (43.06 %), *Mucor* (9.72 %), *Rhizopus* (6.94 %) (Table 1). It is found the prevalence of *Penicillium* at early-ripening phase and occasionally contamination of *Mucor* and *Rhizopus*. The frequency of *Aspergillus* strains was low in the early stage of ripening and its percentage increased during mid-ripening phase till the end. The reason for the evolution of diverse moulds population could be due to the production area, which related to local uncontrolled conditions, such as temperature, the relative humidity and other natural climate factors.

Penicillium citrinum, P. crustosum, P. chrysogenum, P. decumbens, P. expansum and *P. islandicum* were the most frequently isolated *Penicillium* strains in the whole stages of ham maturation. *P. funiculsom* and *P. italicum* were only occasionally found. All the species are quite xerophilic. In particular, *P. citrinum* and *P. chrysogenum* were isolated at the end-ripening stage which can grow and predominated in food with low a_w and RH (Pitt and Hocking, 1997). According to this study, *Penicillium* strains were most frequency found on the surface of hams at early-ripening stage when the RH was higher than 80-85 %. *Aspergillus flavus, A. fumigatus, A. fumisynnematus, A. parasiticus, A. sydowii, A. tamari, A. versicolor* and *A. zhaoqigensis* were also found on

the surface of the tested hams whose frequencies were quite high in contrast to other genera. The increasing temperature at the ripening chamber could be a favourable condition for *Aspergillus* growth, in the later stages of the process.

In this study, it is showed that inoculation strain grew quite well (about 10^3cfu/g virus 10^4cfu/g from aging 0 d to 30 d) which can isolated from hams of treatment group, rather than the control one. In addition, the mould population were quite simple on the surface of hams inoculated by *P. nalgiovense*, chiefly found strains of *Penicillium* and occasionally of *Aspergillus*. The usage of controlled strains can avoid the development of toxigenic moulds, which can grow on the hams during maturation. The moulds present on the surface could contribute to the taste, aroma and storage quality, so it is not a sensible way to inhibit the growth of moulds (Leistner and Ayer, 1968). Consequently, it is possible that selecting non-toxigenic strain used in the manufacture of ham in order to assure the safety of the final product.

Table 1. Number of moulds strains isolated on Jinhua ham surfaces during ripening

Moulds	Maturation Time (d)					Moulds	Maturation Time (d)				
	0	30	60	90	120		0	30	60	90	120
Penicillium chrysogenum	0	1	2	1	0	*Aspergillus fumigatus*	0	0	0	1	1
Penicillium citrinum	1	0	1	2	1	*Aspergillus fumisynnematus*	0	0	0	1	1
Penicillium crustosum	0	2	2	0	1	*Aspergillus parasiticus*	0	0	2	3	3
Penicillium decumbens	1	0	1	1	0	*Aspergillus sydowii*	0	0	0	1	1
Penicillium expansum	0	0	1	1	1	*Aspergillus tamarii*	0	1	1	2	0
Penicillium funiculosum	0	0	0	1	0	*Aspergillus versicolor*	0	1	0	1	0
Penicillium islandicum	1	0	1	1	0	*Aspergillus zhaoqigensis*	0	1	0	1	0
Penicillium italicum	0	1	1	0	0	*Alternaria*	2	1	1	0	0
*Penicillium nalgiovense**	0	2	2	1	0	*Mucor*	4	1	1	1	0
Aspergillus flavus	0	0	0	1	2	*Rhizopus*	3	2	0	0	0

*Only found in hams of treatment group, others were from control one.

Antimicrobial activity against Staphylococcus aureus. Chloroform extracts from 60 moulds isolates were able to inhibit *S. aureus*. A high percentage of active isolates was found in *Alternaria* and *Aspergillus* than in *Penicillium* species. However, the highest levels of inhibition were recorded with extracts obtained from *P. islandicum*, *P. crustosum* and *Aspergillus parasiticus*. In addition, extracts with larger inhibition zones (> 100 mm^2) were obtained from strains isolated at the last sampling. And the lowest levels of inhibition were from *P. nalgiovense*, the inhibition zones (< 100 mm^2) were obtained from 5 strains of it.

There were less toxigenic strains of *Aspergillus spp.* in hams with *P. nalgiovense*, only found one strain of *A. parasiticus* and one of *A. tamari*. But 23 strains of *Aspergillus spp.* were isolated from control product and 78% of them were potentially toxigenic strains through the test of inhibiton of *Staphylococcus aureus*. Therefore, hams with *P. nalgiovense* surpassed control product of their safety by the number of potentially toxigenic strains and the toxicity of metabolites.

Conclusions

Totally 72 strains were isolated belonging to the genera *Alternaria*, *Aspergillus*, *Penicillium*, *Mucor* and *Rhizopus.* Among them, the mould microflora was dominated by *P. chrysogenum*, *P. citrinum* and *P. crustosum* of *Penicillium spp.* and *A. parasiticus*, *A. tamarii* of *Aspergillus spp.*. There were less toxigenic strains of *Aspergillus spp.* in hams with *P. nalgiovense*, but 23 strains of *Aspergillus spp.* were isolated from control product and 78% of them were potentially toxigenic strains through the test of inhibiton of *Staphylococcus aureus*. Therefore, hams with *P. nalgiovense* surpassed control product of their safety by the number of potentially toxigenic strains and the toxicity of metabolites.

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