

PS6.07 Moulds isolated from Norwegian dry cured meat products and their significance 124.00

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Abstract Unwanted mould growth on the surface of Norwegian dry-cured meat products may represent a significant quality problem for the producers. Consumers could be exposed to serious health risk too as some of the moulds are mycotoxigenic. In order to reduce mould problem and make risk assessment, it is important to start with a thorough identification of the moulds growing on the products. In total, 161 dry-cured meat samples were collected in order to identify and characterise the moulds associated with Norwegian dry cured meat products. Standard mycological procedures were followed in order to isolate and identify the mould contaminants. After identification, the ability of the dominant species to produce toxic secondary metabolites was investigated using bioassay analysis and HPLC. In total, 264 isolates belonging to 21 species and four fungal genera were identified. The genus *Penicillium* contributed to the total isolates by 88.3 %. *Penicillium nalgiovense* was the dominant species isolated from both smoked and unsmoked products and covered 38 % of the total isolates. *Penicillium solitum* and *Penicillium commune* were the next most frequently isolated species with a contribution of 13 % and 10 % respectively. Species of *Cladosporium* and *Eurotium* contributed to the total isolates by 6 % and 4.9 % respectively. Bioassay analysis performed on 58 *Penicillium nalgiovense* isolates indicated that 54 of them could produce penicillin. However, traces of penicillin were not obtained on the dry-cured meat samples contaminated with penicillin producing *P. nalgiovense* isolates. Similarly, HPLC analysis confirmed that *Penicillium crustosum* isolates that produced penitrem mycotoxins, which are neurotoxins, on agar media did not produce the toxins on slices of dry-cured meat products under a laboratory conditions. The survey showed that the species of *Penicillium* are associated with Norwegian dry

cured meat products. Many isolates of the dominating species have the ability to produce toxic secondary metabolites, but traces of the metabolites or their production on the products was not observed.

I. INTRODUCTION

Dry-cured meat production has been practiced in Norway since the Viking age. Raw meat develops distinctive flavour as it passes through different processing stages for several months under specific controlled environmental conditions. Variation in environmental factors at each production stage facilitate unwanted and uncontrolled fungal growth on the surface of the products [1].

Many studies showed that xerophilic species of *Aspergillus*, *Eurotium* and *Penicillium* are associated with dry-cured meat products in different parts of the world [2-4, 4-9]. The growth of some moulds could be beneficial for the development of characteristic flavour and aroma of dry-cured meat products due to their involvement in the degradation of lipids and proteins. Enzymes of *P. chrysogenum* and *P. nalgiovense* were reported to contribute for lipolytic and proteolytic activities that generated flavour precursors and improved the texture [10]. However, such positive contributions remain unclear. Rather, moulds are frequently referred as cause for food spoilage leading to quality reduction and a major economic loss for the producers [11-13].

Mould contamination is often associated with unpleasant appearance, odour and changes in taste and nutritional value of foods [4, 13]. Moreover, some moulds are able to produce mycotoxins and antibiotics, which represent a potential health hazard to consumers. Mycotoxins are secondary metabolites that are toxic to vertebrates when introduced via food through natural route [12]. Moreover, thousands of spores can be released from

moulds growing on the products in to the air of the production facilities. This can lead to allergic disorders or even chronic lung disease to the staffs of food processing plants in addition to increasing the risk of airborne food contamination. Identification and characterization of moulds associated with different food products is a starting point to understand their importance. Although dry-cured meat products have been a part of Norwegian food history, the significance of the moulds associated have been paid little attention. The aim of this study was, therefore, to identify moulds associated with the Norwegian dry-cured meat products and get an initial overview on their importance.

II. MATERIALS AND METHODS

2-1- Materials A total number of 161 dry-cured meat samples were collected from six Norwegian producers in 2007, out of which 110 were at the ripening stage and 51 were fully ripened products. The products sampled were lightly smoked dry-cured hams (103 in total), while the rest 58 were unsmoked Norwegian dry-cured meat speciality called “Fenalår” (dry-cured lamb leg). The age of the dry-cured meat products sampled ranged from 4-22 months, with 53 of them aged more than a year and the rest 108 were younger.

The temperature at the ripening stage varied from 12-16 °C. The samples collected have water activity (Aw) of 0.84-0.90. All samples were forwarded to the mycological laboratory at the National Veterinary Institute without temperature control by overnight mail.

2-2- Mould isolation and identification Standard mycological isolation and identification procedures described in [14] were followed.

2-3- Production of toxic secondary metabolites The ability of 58 *P. nalgiovense* isolates to produce penicillin and its occurrence on the dry-cured meat products was investigated by bioassay analysis outlined in [15] using a penicillin sensitive *Staphylococcus aureus* strain. HPLC was employed to investigate if four penitrem (neurotoxins) producing *P. crustosum* isolates can produce the mycotoxins on slices of ham up on artificial inoculation at a laboratory condition.

III. RESULTS AND DISCUSSION

In total 264 moulds were isolated from the dry-cured meat samples investigated. The isolates belonged to 20 species of four mould genera (Table 1). The genera were *Penicillium*, *Cladosporium*, *Eurotium* and *Aspergillus*. All the four genera were recovered from dry-cured hams (smoked), while three with the exception of *Aspergillus* did from Fenalår (unsmoked).

Species of *Penicillium* generally dominated the mycobiota of Norwegian dry-cured meat products by covering 88.3 % of the total isolates. *Penicillium nalgiovense*, in particular, seems to be associated with the dry-cured meat products of Norway by covering 38 % of the total isolates followed by *P. solitum* and *P. commune* with a contribution of 13 % and 10 % respectively.

These three mould species have been reported to be associated with dry-cured meat and cheese products by many [4-9, 16, 17]. It look like that *P. nalgiovens* *P. solitum* and *P. commune* are tolerant to high level of salt concentration. *Penicillium chrysogenum*, *P. atramentosum*, *P. crustosum* and *P. brevicompactum* had relatively similar isolation frequency and contributed by almost 21 % to the total mould isolates. The genus *Cladosporium* contributed to the total isolates by 6 %.

The remaining isolates belonged to species of *Eurotium* and *Aspergillus*, which contributed to the total isolates by 4.9 % and 0.8 % respectively. The number of species isolated from fully ripened products was lower than the number of species isolated from products at the ripening stage (Table 1).

However, this reduction in the total number of species is accompanied by an increase in the occurrence of the dominating species. For example, the contribution of *P. nalgiovense* to the total isolates of smoked products has increased from 44 % at the ripening stage to 57 % on fully ripened products. The increase for unsmoked products was from 15 % to 42 %. Some differences were observed as to the occurrences of the dominant species on smoked and unsmoked products. *Penicillium nalgiovense* dominated the mycobiota of both product types, but its contribution to the total isolates of smoked products (47 %) was twice of its contribution to the isolates of unsmoked

products (23 %). The contribution of *P. solitum* to the isolates of unsmoked products was twice of its contribution to the isolates of smoked products. Similarly, *P. commune* contributed by four folds for the isolates of unsmoked products compared to its contribution to the isolated smoked products (Figure 1).

Penicillium atramentosum and *P. brevicompactum* were frequently isolated from smoked dry-cured hams, while *P. commune*, *P. chrysogenum* with almost all the species of *Cladosporium* did from unsmoked ones. The isolation frequencies of *Eurotium* spp. from smoked and unsmoked products were relatively similar. Smoking is believed to be effective to prevent mould growth on the surface of dry-cured meat production [18], but it looks like that light smoking may not have a preventive effect on the growth of some species like *P. nalgioense*. All the dominant isolated species of *Penicillium*, with the exception of *P. solitum*, are known producers of toxic secondary metabolites [12, 19]. Their growth on dry-cured meats can possibly lead to the contamination of the products with mycotoxins, which can pose potential health risks. The bioassay analysis indicated that 54 out of 58 *P. nalgioense* can produce penicillin.

However, traces of penicillin were not obtained on the dry-cured meat samples contaminated with penicillin producing *P. nalgioense* isolates. Similarly, HPLC analysis confirmed that *P. crustosum* isolates that produced penitrem toxins on agar media did not produce the toxins on slices of ham placed on a similar agar media and inoculated with the isolates. Generally moulds were isolated from all the dry-cured meat samples collected. The most frequently isolated species, *P. nalgioense*, was isolated from 63 % of dry-cured hams at the ripening stage and 90 % of fully ripened products. The same species was recovered from 32 % Fenalår at the ripening stage, while it was isolated from 67 % of fully ripened Fenalår.

The present study is the first survey on moulds associated with Norwegian dry cured meat products. Further work should focus on identifying the most important contamination sources and possible preventive measures in the production process. It can serve as a starting point for the development of non toxic starter cultures, which can be important both for quality improvement and

reduction of unwanted fungal growth on dry-cured meat products.

ACKNOWLEDGEMENTS

This work was financed by the Norwegian research council and Stranda Spekemat AS.

REFERENCES

- [1.] Rodriguez M, Nunez F, Cordoba JJ, Bermudez ME, Asensio MA. (1998). Evaluation of proteolytic activity of micro-organisms isolated from dry cured ham. *Journal of Applied Microbiology*, 85 (5):905-912.
- [2.] Comi G, Orlic S, Redzepovic S, Urso R, Iacumin L. (2004). Moulds isolated from Istrian dried ham at the pre-ripening and ripening level. *International Journal of Food Microbiology*, 96 (1):29-34.
- [3.] Lopez-Diaz TM, Santos JA, Garcia-Lopez ML, Otero A. (2001). Surface mycoflora of a Spanish fermented meat sausage and toxigenicity of *Penicillium* isolates. *International Journal of Food Microbiology*, 68 (1-2):69-74.
- [4.] Papagianni M, Ambrosiadis I, Filiouis G. (2006). Mould growth on traditional Greek sausages and penicillin production by *Penicillium* isolates. *Meat Science*, 76 (4):653-657.
- [5.] Nunez F, Rodriguez MM, Bermudez ME, Cordoba JJ, Asensio MA. (1996). Composition and toxigenic potential of the mould population on dry-cured Iberian ham. *International Journal of Food Microbiology*, 32 (1-2):185-197.
- [6.] Battilani P, Pietri VA, Giorni P, Formenti S, Bertuzzi T, Toscani T, Virgili R, Kozakiewicz Z. (2007). *Penicillium* populations in dry-cured ham manufacturing plants. *Journal of Food Protection*, 70 (4):975-980.
- [7.] Tabuc C, Bailly JD, Bailly S, Querin A, Guerre P. Toxigenic potential of fungal mycoflora isolated from dry cured meat products: preliminary study. (2004). *Revue de medecine veterinaire*, 156 (5):287-291.
- [8.] Peintner U, Geiger J, Poder R. (2000). The Mycobiota of Speck, a Traditional Tyrolean Smoked and Cured Ham. *Journal of Food Protection*, 63:1399-1403.
- [9.] Sorensen LM, Jacobsen T, Nielsen PV, Frisvad JC, Koch AG. (2008). Mycobiota in the processing areas of two different meat products. *International Journal of Food Microbiology*, 124 (1):58-64.
- [10.] Martin A, Cordoba JJ, Aranda E, Cordoba MG, Asensio MA. (2006). Contribution of a selected fungal population to the volatile compounds on dry-cured ham. *International Journal of Food Microbiology*, 110 (1):8-18.
- [11.] Pitt JJ, Hocking AD. (1999). *Fungi and food spoilage*, 2nd ed. Maryland: Aspen publisher, Inc, (593 pp).
- [12.] Samson RA, Frisvad JC, Hoekstra ES. (2004). *Introduction to Food- and Airborne fungi Centraalbureau voor Schimmelcultures*, Utrecht.

[13.] Filtenborg O, Frisvad JC, Thrane U. (1996). Moulds in food spoilage. *International Journal of Food Microbiology*, 33 (1):85-102.

[14.] Asefa DT, Gjerde RO, Sidhu MS, Langsrud S, Kure CF, Nesbakken T, Skaar I. (2009). Moulds contaminants on Norwegian dry-cured meat products. *International Journal of Food Microbiology*, 128 (3):435-439.

[15.] Andersen SJ, Frisvad JC. (1994). Penicillin production by *Penicillium nalgiovense*. *Letters in Applied Microbiology*, 19 (6):486-488.

[16.] Kure CF, Skaar I. (2000). Mould growth on the Norwegian semi-hard cheeses Norvegia and Jarlsberg. *International Journal of Food Microbiology*, 62 (1-2):133-137.

[17.] Lund F, Filtenborg O, Frisvad JC. (1995). Associated mycoflora of cheese. *Food Microbiology*, 12:173-180.

[18.] FAO. (1990). Manual on simple methods of meat preservation. Rome.

[19.] Frisvad JC, Samson RA. (2004). Polyphasic taxonomy of *Penicillium* subgenus *Penicillium*: A guide to identification of food and air-borne terverticillate *Penicillia* and their mycotoxins. *Studies in Mycology*, 49:1-251.