MOULDS ISOLATED FROM RIPENED FERMENTED SALAMI: BIODIVERSITY AND OCHRATOXIN A PRODUCTION

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Moulds play an important role in the manufacture and ripening of fermented meat products, since they contribute to the development of a characteristic flavour, due to the decomposition of fat, proteins and lactic acid. Furthermore, moulds have an antioxidative effect, protecting from rancidity and keeping the colour. On the other hand, the presence of species or strains with the ability to produce mycotoxins, in particular ochratoxin A (OTA), could represent a risk for the consumer's health. The aims of this work were to evaluate the biodiversity of moulds isolated from traditional fermented salami produced in Veneto region (Italy) and to verify their ability to produce OTA.

MATERIALS AND METHODS

A total of 62 isolates were recovered from the environment (air) of two plants, situated respectively in mountain and plain areas of Veneto region and from the casing of fermented salami at different ripening stages (7, 15, 25, 60 and 90 days). Moulds were isolated in Dichloran Rose Bengal Chloramphenicol Agar incubated at 25℃ for 5 days.

Isolates were characterized at genetic level by means of RAPD-PCR with primer M13 and by sequencing the ITS1-5.8S rDNA-ITS2 region and the D1/D2 26S rDNA domain.

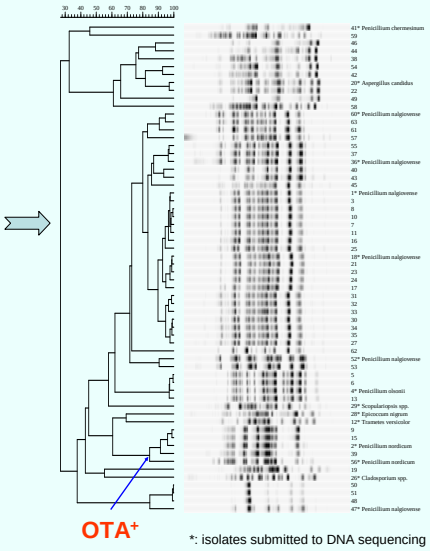
The presence of the non-ribosomal peptide synthetase "*otanps*" gene, involved in OTA biosynthetic pathway in *Penicillium*, was determined by PCR. Positive isolates were confirmed in their ability to produce ochratoxin A by means of HPLC analysis.

RESULTS

Cluster analysis performed on RAPD-PCR profiles revealed the presence of a significant level of biodiversity, with the presence of 13 different biotypes among the 62 moulds collected. The majority of isolates were identified by DNA sequencing as belonging to the species *Penicillium nalgiovense* (59.7%), *P. nordicum* (8.0%), *P. olsonii* (6.4%) and *Aspergillus candidus* (12.9%). The remaining 5 isolates were identified as *P. chermesinum*, *Trametes versicolor*, *Epicoccum nigrum*, *Cladosporium* sp., *Scopulariopsis* sp., while 3 isolates were not identified.

ITS and D1/D2 rDNA sequencing did not allow to assign unambiguously *Penicillium* isolates at species level. A reliable species assignment was obtained only after sequencing a portion (500 bp) of β-tubulin gene.

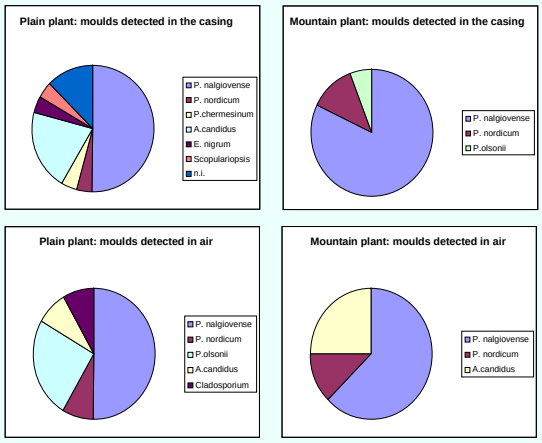
P. nalgiovense resulted the dominant species isolated both from salami casings and plant air. A higher species biodiversity was detected in the plain-situated plant (both on salami surface and air) compared to the plant situated in the mountain.



*: isolates submitted to DNA sequencing

Among *Penicillium* isolates, "*otapns*" gene was detected in all the 5 isolates identified as *P. nordicum*. HPLC analysis confirmed the production of ochratoxin A in synthetic medium by these isolates, although in different amounts.

Spores of a *P. nordicum* OTA+ isolate were spread on the surface of a salami, which was stored in laboratory for 25 days under conditions simulating ripening and then evaluated for ochratoxin A production by HPLC. Preliminary data showed the presence of OTA at a concentration of 5.8 µg/kg in the casing, while in the edible inner part the concentration was always below 1 µg/kg, which is the limit set by Italian Ministry of Health guidelines.



CONCLUSIONS

In the present study RAPD-PCR revealed to be a reliable method for detecting the biodiversity among moulds isolated from the surface and environment of fermented salami.

An accurate species identification of *Penicillia* was achieved by sequencing a region of β-tubulin gene, while ITS and D1/D2 rDNA sequencing allowed identification only at genus level.

The presence of moulds producing ochratoxin A resulted limited in the samples examined in this work. However, considering the possible presence of OTA strains and the possible production of toxins during ripening, the inoculum of selected non-toxinogenic mould starters could be advisable in order to prevent the presence of the toxin at concentrations potentially harmful for consumer's health.

AKNOWLEDGEMENTS
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