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**Penicillia and Aspergilli associated to Grana cheese**

**Multiphasic identification approach, ecological needs and toxins production**

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# **SUMMARY**

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Grana Padano and Parmigiano Reggiano, two Italian PDOs of grana cheese, account for millions of Euros of exports per year. Recently, ochratoxin A (OTA) was detected in packed grated cheese, supposed to be produced by fungal growth during cheese ripening. Some fungi are known as OTA producers, but few studies were published regarding the mycobiota associated to grana cheese and the knowledge regarding potential toxigenic fungi is limited. Therefore, getting more information on that topic became urgent.

The works managed and presented in this thesis aimed to improve knowledge about mycotoxigenic *Aspergilli* and *Penicillia* associated to Italian grana cheese. A multiphasic approach was applied for their identification and ecological trials were organised to define fungal needs of the expected dominant species in the ecological condition of grana cheese production. The overall objective was to predict the risk of mycotoxin contamination in the ripening condition of grana cheese and suggest mitigation actions.

Several mycotoxin producing *Aspergilli* and *Penicillia* may grow on Italian grana cheese surface and the latter resulted prevalent. A multiphasic approach is confirmed as mandatory for *Penicillia* identification at species level: morphological, chemical and molecular characterisation contributed altogether to reach this goal.

*Aspergillus puulaauensis* and some *Penicillium* Section *fasciculata* (*P. crustosum* and *P. solitum*) were the prevalent species associated to grana cheese, while neither *P. nordicum*, nor *P. verrucosum*, the two main OTA producers reported in literature, were detected; however, *P. verrucosum* was isolated from air.

Regarding ecological needs, sporulation, fungal growth and mycotoxin production were the considered variables. Relying on the results obtained, the environmental conditions of the ripening rooms (15-22°C; 72-88% RH) are more favourable to *P. nordicum* and *P. verrucosum*, rising concern about possible OTA contamination: in fact, 20°C was the optimum temperature for growth, sporulation and for OTA production by *P. nordicum*, a bit higher for *P. verrucosum*. Nevertheless, *P. crustosum*, penitrem A (PA) and roquefortine C (ROQ-C) producer, must not be neglected: its optima for growth and mycotoxin production were both around 22-23°C, and 20°C for sporulation.

Actions against mycotoxigenic *Penicillia* during ripening and ageing should mainly reduce total inoculum in the environmental air. Ozone is reported to be effective in this sense; moreover, blue light may significantly reduce fungal growth on cheese and shelves surfaces and mycotoxin production on cheese rind.

All information provided therein will be useful for producers and stakeholders to perform a better risk management, and guarantee a safe, high-quality product to consumers