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Potential pathogenic fungi assessment through molecular biology in cork industry

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Introduction

Portugal has been the world leader in the cork sector in terms of exports, employing ten thousands of workers. In this working activity, the permanent contact with cork may lead to the exposure to fungi, raising concerns as potential occupational hazards in cork industry[1,2]. The application of molecular tools is crucial in this setting, since fungal species with faster growth rates may hide other species with clinical relevance, such as species belonging to *P. glabrum* and *A. fumigatus* complexes[3]. A study was developed aiming at assessing fungal contamination due to *Aspergillus fumigatus* complex and *Penicillium glabrum* complex by molecular methods in three cork industries in the outskirt of Lisbon city.

Fig. 1 - Equipment used for air samples collection to apply molecular identification

Materials and methods

Air samples of 250L were collected using the impinger Coriolis µ air sampler (Bertin Technologies), at 300 L/min airflow rate. Samples were collected onto 10ml sterile phosphate-buffered saline with 0.05% Triton X-100, and the collection liquid was subsequently used for DNA extraction using the ZR Fungal/Bacterial DNA MiniPrep Kit (Zymo Research) according to the manufacturer's instructions. Molecular identification of *P. glabrum* complex and *A. fumigatus* complex was achieved by quantitative PCR (qPCR) using the Rotor-Gene 6000 qPCR Detection System (Corbett). Reactions included 1× iQ Supermix (Bio-Rad), 0.5 µM of each primer and 0.375 µM of TaqMan probe in a total volume of 20 µl. Amplification followed a three-step PCR: 40 cycles with denaturation at 95°C for 30 s, annealing at 52°C for 30 s, and extension at 72°C for 30 s.

Fig. 2 – Equipment used for molecular identification

Results and discussion

Aspergillus fumigatus complex was not detected in any of the 12 samples collected. However, in ten of those samples *Penicillium glabrum* complex was detected, corroborating the high prevalence of this species in cork industries. Nevertheless, the fact that the DNA from *P. glabrum* complex was amplified at quite high Cq indicates that the fungal load is likely to be low. Fungal contamination by *P. glabrum* was detected in the three analyzed plants, suggesting that there might be an increased risk of development of fungi-related diseases, such as hypersensitivity pneumonitis (interstitial lung disorder), later called Suberosis.[4]

Sampling sites	Real Time PCR *P. glabrum* (Cq– Cycle threshold)
Tracing - Plant A	-
Cutting - Plant A	+ (37,07)
Baking - Plant A	+ (35,07)
Selecting - Plant A	+ (35,30)
Rectification - Plant B	+ (36,45)
Grinding of raw materials - Plant B	+ (34,71)
Grinding in mills - Plant B	+ (35,76)
Agglomeration - Plant C	+ (35,21)
Mixing - Plant C	-
Sawing - Plant C	+ (36,51)
Pressing - Plant C	+ (38,73)
Sanding - Plant C	+ (35,58)

Table 1 – Molecular detection of *P. glabrum*

Conclusions

With this study, it was possible to detect, at the molecular level (by qPCR), occupational fungal exposure due to *P. glabrum* in the cork industry. Therefore, it is recommended the application of preventive and protective measures in order to minimize fungal exposure preventing the workers from developing *P. glabrum* related diseases, such as Suberosis.

References

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