Quantification of *Penicillium nalgiovense* on Dry-Cured Sausage 'Salchichón' Using a SYBR Green-Based Real-Time PCR

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Abstract

To evaluate the effective implantation of a specific protective culture of *Penicillium nalgiovense*, a real-time quantitative PCR (qPCR) using SYBR Green methodology was developed. Two specific primers were designed on the basis of the published partial sequences of the Internal Transcribe Spacer (ITS)1–5.8S-ITS2 region of various strains of *P. nalgiovense*. Using the developed method, a PCR product of 51 bp with a T_m value 81.34 °C was detected. T_m values of the amplified product allowed specific differentiation between *P. nalgiovense* and the remaining mould species tested. The developed qPCR method was tested on inoculated slices of dry-cured sausage ('salchichón') showing an efficiency of 97.24 %, a R^2 value of 0.99 and a detection limit of *P. nalgiovense* of 1 log colony-forming units (cfu)/cm^2. The qPCR method demonstrated that the protective strain of *P. nalgiovense* grew and competed against an ochratoxin A (OTA)-producing *Penicillium verrucosum* strain on commercial dry-cured sausage. This qPCR method provides a specific, accurate and sensitive detection and quantification of *P. nalgiovense* on dry-cured sausage salchichón in order to estimate its colonization during their processing. This assay will improve strategies to prevent and control unwanted mould colonization and OTA risk in dry-cured meat commodities.