Development of a multiplex qPCR method for simultaneous quantification in dry-cured ham of an antifungal-peptide *Penicillium chrysogenum* strain used as protective culture and aflatoxin-producing moulds

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Abstract

A multiplex qPCR method to quantify the implantation of the antifungal-peptide-producing *Penicillium chrysogenum* RP42C and aflatoxin-producing moulds including a non-competitive IAC in dry-cured ham was developed. For this, the primer pairs F/R-Pc, F/R-omt and Tub-F1/R1 and the TaqMan probes, P-Pc, *OMTprobe* and *Tubprobe* targeting the *pgafp*, *omt*-1 and *β-tubulin* genes, respectively, were used. The ability of the optimized qPCR method to quantify simultaneously both non-toxigenic and toxigenic moulds in inoculated dry-cured ham was demonstrated since efficiency values ranged from 102.1 to 111.1%, and the limit of detection was between 2 and 3 log cfu/cm^2 for both antifungal-peptide- and aflatoxin-producing moulds. In addition, suitability of multiplex qPCR to test implantation of protective *P. chrysogenum* as well as growth of aflatoxigenic strain in a controlled model system was carried out successfully. The multiplex qPCR allowed demonstrating that the protective strain of *P. chrysogenum* RP42C limits growth of the aflatoxin-producing *Aspergillus flavus* strain on dry-cured ham. These findings were confirmed by the results of aflatoxin analysis, since aflatoxin B1 was not detected when the protective *P. chrysogenum* RP42C was inoculated. Furthermore, the multiplex qPCR including an IAC quantified efficiently the implantation of protective culture *P. chrysogenum* RP42C in dry-cured ham after 6 months of incubation. Thus, the multiplex qPCR should be considered a sensitive and rapid tool to monitor the implantation of fungal protective cultures and to determine growth of aflatoxin-producing moulds in dry-cured ham as well.