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Effect of the Antifungal Protein PgAFP on Mycotoxin Production by Moulds in Food Matrices

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Received: July 19, 2019; **Published:** November 28, 2019

Abstract

Moulds are able to colonise a huge variety of substrates, in particular, foods are prone to be contaminated with these microorganisms. Some of these moulds are able to synthetize secondary metabolites, which are hazardous for animal and human health, mycotoxins. They have very different effects, but probably the most concern is the carcinogenic potential. Thus, strategies to control the proliferation and production of mycotoxins in foods are required. The use of synthetic antifungal compounds entails the generation of residues as well as the raise of resistance to these compounds, then, it would be interesting to explore environmentally friendly alternatives. The antifungal protein PgAFP, produced by *Penicillium chrysogenum*, inhibits a broad range of undesired moulds, being stable against a wide range of pH values and temperatures. Thus, the effect of this protein on mycotoxin production was evaluated on *Penicillium nordicum*, *Alternaria tenuissima sp. grp.*, *Aspergillus carbonarius* and *Penicillium expansum* grown on four food matrices: based on dry-cured ham, wheat, raisin and apple, respectively. PgAFP has shown a different pattern depending on the mould and the matrix. It has been reported to provoke a reduction in the mycotoxin production on *Alternaria tenuissima sp. grp.* and *Aspergillus carbonarius*, no effect on *Penicillium nordicum* mycotoxin production and an overproduction on *Penicillium expansum*. Therefore, the use of this antifungal protein must be assayed in every combination of food matrix - mould species before applying it, in order to ensure the correct control of the presence of these hazardous metabolites in foods.

Keywords: *PgAFP; Mycotoxins; Moulds; Food Safety*

Abbreviations

A_w: Water Activity; OTA: Ochratoxin A; AOH: Alternariol; AME: Alternariol Monomethyl Ether; TeA: Tenuazonic Acid

Introduction

Moulds have great adaptability to extreme environments, growing over a wide range of pH and temperature, and competing very advantageously with other microorganisms in substrates with low a_w. One of the most easily colonized substrates is food, like cured meat products where moulds play a key role in the flavour and aroma development [1]. However, the mould growth is undesirable in fruits, vegetables or cereals. Regardless of whether their growth is desirable or not in each type of food, some of the mould species have the

capacity to produce mycotoxins. They are secondary metabolites with very diverse chemical structure and different effects on the human health [2]. Amongst these effects, the most worrying is their carcinogenic potential; in fact, some of them have been included in a list of potential carcinogenic agents by the International Agency for Research on Cancer [3]. These toxins are highly stable against temperature or any decontaminating procedure [4], thus, prevention is the only alternative to ensure safe foods. Therefore, it is necessary to implement strategies that avoid the proliferation of toxigenic moulds, and more specifically the production of mycotoxins in foods.

The use of synthetic antifungal compounds is a strategy, which has been commonly used for decades, but implies the generation of resistances. In addition, they leave residues in the environment. Therefore, consumers demand more respectful strategies with the environment where they are applied. An interesting strategy to apply could be antifungal proteins produced by moulds, which are described as small, basic and rich in cysteine [5]. They are very stable against a wide range of pH values and temperatures [6]. In addition, they exhibit a broad spectrum of inhibition against filamentous fungi, thus offering new opportunities to control unwanted moulds in food.

The antifungal protein PgAFP, produced by *Penicillium chrysogenum*, has a broad spectrum of inhibition on toxigenic moulds, is stable at temperatures up to 100°C and at pH values 1 - 12 [6]. This protein offers an additional advantage in comparison with other similar, given that their activity is not dependent of the pH, as stated for others [7]. These characteristics make it an ideal tool to be tested in food. Different food matrices, inoculated with the mould species of interest in each case, have been supplemented with PgAFP to evaluate the impact on fungal development and mycotoxin production for each of them. The results discussed in this review come from studies designed to test different incubation temperatures, depending on the critical range in which every mould is able to produce mycotoxins in each food matrix, in which the authors simulated the environment where the mould grows and produce mycotoxins.

Results and Discussion

Amongst the different foods, There are some of them prone to be contaminated with a particular mould species which advantageously colonise that particular niche. The works aiming to test the ability of antifungal compounds to control toxigenic moulds on food have focused on the most worrying mould species - food matrix combinations, as they are the most concern for food safety. Ochratoxin A has a legal limit set, both European for raisins (10 µg OTA/kg) and Italian for fresh meats products (1 µg OTA/kg). The mycotoxins tested on wheat do not have any legal limit, although the European Food Safety Authority has recently highlighted that cereal-based foods for infants and young children are one of the main contributors of *Alternaria* spp. toxins to the diet. Apple derivatives have a limit, set by the European Union as 50 µg patulin/kg. In general, these low limits are related to the pathogenicity of these toxins. Then, low- environmental impact strategies and affordable strategies to control mycotoxin production are required.

The effect of PgAFP (10 µg/g and/or 40 µg/g) on mycotoxin production by four mould strains: *Penicillium nordicum*, *Alternaria tenuissima sp. grp.*, *Aspergillus carbonarius* and *Penicillium expansum* grown on four food matrices: based on dry-cured ham, wheat, raisin and apple, respectively [8-11]. The inhibition caused by PgAFP on the mycotoxin production by moulds in the different food matrices did not follow the same pattern was tested. Depending on the matrix and the mould to be tested, there was a greater or lesser reduction in the production of toxins. Even in one case, there was a mycotoxin overproduction due to the PgAFP effect.

In general, less complex food matrices (raisin and wheat) achieved a satisfactory inhibition on the mycotoxin production. In these cases, the mycotoxin inhibition achieved was at least of 74.8% for OTA in *A. carbonarius* in raisin simulating media [10] and 58.3% for the three mycotoxins produced by *A. tenuissima* on wheat-based media [9] (Table 1). These are promising outcomes, given that opposite results were obtained by the reduction in the dose of synthetic antifungals, such as potassium sorbate and the commercial mix fludioxonil 2.5% and metalaxyl-M 1%, which provoked a mycotoxin overproduction on *A. carbonarius* and *A. tenuissima* under different combinations of a_w. In spite of the fact that consumers demand products free of synthetic preservatives, the strategy based on the single reduction in the synthetic antifungal dose should be ruled out, given this risk. From the results obtained from these particular food matrices, PgAFP seems to be a tool which deserves to be further studied on raisins and wheat.

Mould species	Food matrix	Evaluated mycotoxin	% Inhibition
Aspergillus carbonarius	Raisin simulating culture medium	Ochratoxin A	≤ 74.8
Alternaria tenuissima sp. grp.	Wheat-based culture medium	Tenuazonic acid Alternariol Alternariol monomethyl ether	65.5 - 84 ≥ 60 ≥ 58.3
Penicillium nordicum	Dry-cured ham-based culture medium	Ochratoxin A	p > 0.05
Penicillium expansum	Apple-based culture medium	Patulin	↑Production

Table 1: *Effect of PgAFP on the inhibition of different mycotoxins produced by moulds inoculated in different food matrices.*

However, in a more complex matrix, such as dry-cured ham-based medium, PgAFP did not exert any effect on mycotoxin production by *P. nordicum*. In addition, its impact on the proteome of the producer mould was very limited, according to the data obtained from the analysis carried out [8]. It can be hypothesised that the high content of proteins from dry-cured ham in the culture medium can hamper or block the contact of PgAFP with the ochratoxigenic mould. However, there were proteome changes in P. nordicum, meaning that any kind of interaction of PgAFP and *P. nordicum* was achieved.

Finally, PgAFP caused an overproduction of patulin on *P. expansum* inoculated on apple agar. This increase in mycotoxin production occurred in dose-response mode with regard to PgAFP concentration assayed [11]. This response was also parallel to an increase in oxidative stress suffered by *P. expansum* in relation to the effect of PgAFP. Since apple agar is also a relatively simple matrix in terms of chemical composition, pH could have played a key role in the mould-PgAFP interaction. However, low pH values, such as that regarding to apple, are linked with greater antifungal activity because of the net positive charge of these cationic proteins, which increases their ability to bind to cell membranes [7]. It is interesting to highlight that until now, no patulin determination has been evaluated when any substance or bioprotective agent was used for *P. expansum* control [12-14], being this mould not completely inhibited, which means that it could be underwent to any kind of stress and produce higher patulin quantities. Therefore, additional studies are necessary to reveal the cause of the increase in toxin production linked to the higher reactive oxygen species levels.

Conclusion

The antifungal protein PgAFP is a non-synthetic antifungal protein with the ability to control mycotoxin production in foods. However, the antifungal protein mechanism of action could entail an overproduction of mycotoxins in particular cases. Therefore, the effect of PgAFP on the production of these toxins must be evaluated for every combination of mould species present in the type of food to protect. Additionally, the evaluation of the effect of PgAFP on the mycotoxin production should be carried out on the food matrix of interest, to extract the most accurate results.

Conflict of Interest

No financial interest or any conflict of interest exists.

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Volume 14 Issue 12 December 2019