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Review

Self mutagens affect detrimentally PCR analysis of food fungi by creating potential mutants

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ABSTRACT

Analysing fungi from food by PCR is increasing rapidly. However, food fungi produce mutagens which may mutate the fungi in culture so that fungi which produce mycotoxins may have a negative PCR result for genes in the mycotoxin metabolic pathway and *vice versa*: It is impossible to state unequivocally that the current PCR results obtained are accurate. For example, food containing a mycotoxin fungus may be considered safe if the isolates from the food were mutated into being negative for the mycotoxin (or other) gene and *vice versa*. Growth conditions affect which mutagens are produced and the conditions used by authors are assessed for the first time in the current report. Previous research assumed that NA was unaffected by how fungi were grown despite no supporting evidence. Individual research groups used similar growth conditions for disparate fungi for PCR analysis which were different from methods used by alternative workers. Rationales for using particular growth methods are unexplained. The fungi will be in almost continuous contact for long periods with various biochemical mutagens at high concentrations. Only partial solutions can be provided by suggesting alternative methods. Future methods need to state why particular conditions are employed when growing fungi and what was done to avoid mutagens.

Contents

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1. Introduction

PCR methods to detect microorganisms from food are extensive. However, a firm grounding in basic methods is required before the more novel procedures can be applied accurately (Editorial, 2013). PCR is used to analyse isolated fungi from food, or the food itself for particular fungi and much concern about fungi in food is from the production of mycotoxins which have powerful mutagenic activities causing cancers in animals and humans (Table 1) (Luch, 2006; Paterson & Lima, 2010). Interpretation of PCR results is equivocal because mutagenic secondary metabolites are produced by the target fungi in the growth media (Paterson & Lima, 2009, 2013; Paterson, Sariah, Lima, Zainal Abidin, & Santos, 2008) and, for example, fungi which are normally positive for a mycotoxin, or other, gene may be mutated to negative. This makes it possible that analysis of food by PCR may yield false results for fungi (Paterson 2012a, 2012b) and this is discussed herein.

A mutagen is a physical or chemical agent that changes the genetic material, usually DNA, of an organism and increases the frequency of mutations above the natural background level. Mutations occur due to spontaneous hydrolysis, and errors in DNA replication, repair and recombination. Mutagens are likely to be carcinogens as many cause cancer. Most genotoxic organic carcinogens require metabolic activation to exert detrimental effects on DNA. The parent compounds are considered as pre-carcinogens bioactivated into carcinogenic forms (Luch, 2006). Hence, some mycotoxins will not bind to DNA without activation. Many are not mutagenic, but can form mutagenic metabolites through cellular processes and such mutagens are called pro-mutagens (e.g. aflatoxin). Chemicals may interact directly with DNA: Others (e.g. PAHs, aromatic amines, and benzene) are not necessarily mutagenic *per se*. Mutagens may modify the DNA sequence which includes substitution of nucleotide base-pairs and insertions and deletions of one or more nucleotides in DNA sequences (Table 1). The effect of mutagens may not be obvious in, for example, fungi because mutations can (a) have minor effects, as they do not result in residue changes with significant effects on proteins and (b) be silent because they occur in non-coding or non-functional sequences, or do not change the amino-acid sequence due to the redundancy of codons (Burnett, 2003). For example, *Blastomyces dermatitidis*, *Coccidioides immitis*, *Histoplasma capsulatum* and *Paracoccidioides brasiliensis* have particularly large genomes but small concentrations of coding DNA (Clutterbuck, 2011). Nevertheless, mutagens may act directly on DNA, causing direct damage, and most often result in replication error; others may act on the replication mechanism and chromosomal partition. Intercalating agents (e.g. ethidium bromide and proflavine) may insert between bases in DNA, causing frameshift mutation during replication (e.g. the mycotoxin alternariol (DiCosmo & Straus, 1985)). Base analogue mutagens can substitute for DNA bases and cause transition mutations (e.g. cordycepin (see later)). Finally, mutagenesis is the driving force of evolution (Burnett, 2003; Luch, 2006). How mutations within fungi are manifest is considered in the following section with relevance to our discussion.

2. Mutation in Fungi

Mutation is the sole source of variation which occurs in nDNA and mtDNA where the latter are common in fungi: Recombination mainly generates novel multi locus genotypes. Furthermore, phenotypic detection of mutations can be rapid in fungi, as in new virulent mutants (Joosten, Cozijnsen, & De Wit, 1994) and fungicide resistant mutants of crop pathogens, or in selective situations (e.g. industrial processes) and so could occur during growth of food fungi for PCR analyses. Interestingly, industrial fungal production (e.g. growth in bioreactors) is similar to the conditions employed to grow fungi for PCR analysis (e.g. pure culture, sterile growth conditions, nutrients supplied in batch form). Whether a new mutation persists and is beneficial to the fungus depends on the mutation rate, the genome in which it is located and the size of the population. A mutant or rare allele has a better chance of eventual survival if the mutation rate is high and reverse mutation is low. Thus, the potentially advantageous mutants, regarded as the most significant by some even at the molecular level, probably become established in a population only through recurrent mutation: Any gene present in a fungal population at a low frequency can be lost or fixed from (a) the inevitable random sampling of conjugating gametes or individuals as in sexual reproduction, or (b) if the population is maintained predominantly by asexual spores, when these are dispersed and germinate and persist or perish. Such stochastic changes occur regardless of whether or not the gene confers a potential selective advantage (Burnett, 2003). The present authors are considering mutants in pure culture where the spores can only disperse within the confines of the growth vessel and mutants are likely to accumulate much quicker.

Mutations in *Neurospora crassa* increased by 0.3 per cent per week at 32 °C but at 0.1 per cent per week at 4 °C (indicating temperature dependent enzymatic activity (see Section 3). Presumably these reflected mutations at a number of unspecified loci on non replicating nuclei. Other spontaneous mutation rates appear in the range of ca. 1 in 10^6 to 10^7 (Burnett, 2003). Mutation frequency could be affected by conditions of starvation or stress which arise when nutrients become depleted and this can stimulate the production of secondary metabolites. Hence, the stress may increase mutations *per se* and this could be compounded by the production of mutagenic secondary metabolites.

Table 1
Known (a) mutagenicity of various mycotoxins and (b) damage to DNA (see Paterson & Lima 2013).

Mycotoxin	Mutagenicity	Known damage to DNA in general
Aflatoxins	Most carcinogenic natural compounds; induce DNA damage; affect negatively the amelioration of damage; alter DNA base composition of genes.	Intercalations, intra and inter-strand cross links; Apyrimidinic sites; Apurinic sites;
Sterigmatocystin	Covalent binding to DNA; DNA adduct formation; carcinogenic.	Hydrolytic deamination;
Ochratoxin A	Potent carcinogen; DNA single strand breaks; Forms DNA adducts; Mutagenic activity; induces base substitutions; increased mutation frequency.	Single strand breaks; Radical formation; Double strand breaks;
Patulin	Induces DNA–DNA crosslinks; mutagenicity; reactivity to DNA.	DNA-protein cross links;
Deoxynivalenol	DNA damage; genotoxic.	Pyrimidine dimmers;
Nivalenol	Direct mutagen; DNA damage.	Base damage;
Fusarenon X	DNA damage; increases DNA strand breaks.	Alkylation;
Fusarin C	Mutagenic.	6-4 photoproducts;
Altertoxin I, Alternariol, *Alternaria* extracts	Mutagenic.	Bulky adducts; Loss of bases.