Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Food Science

Mutagens affect food and water biodeteriorating fungi

Robert Russell Monteith Paterson and Nelson Lima

Many areas of food mycology could be affected detrimentally by mutation of wild type fungi. Some of these will contact mutagens from pre-isolation to experimentation and the effect on fungi isolated from mycotoxin-contaminated food is assessed for the first time in this review. However, this mutagen issue is not considered by other authors in primary research papers, which is relevant to molecular biology techniques for gene sequencing, phylogenetics, diagnostics and mycotoxin production. The presence of mutagens is anathema to methods for DNA analysis at the experimental design level and concepts such as cryptic species and correlating anamorphs with teleomorphs are affected. Strains held in culture collections may be artifacts. Methods to ameliorate the problem are provided herein.

Address
CEB - Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal

Corresponding author: Paterson, Robert Russell Monteith (russell.paterson@deb.uminho.pt)

Current Opinion in Food Science 2015, **5**:8–13

This review comes from a themed issue on **Food mycology**

Edited by **Russell Paterson**

For a complete overview see the Issue and the Editorial

Available online 10th June 2015

http://dx.doi.org/10.1016/j.cofs.2015.06.004

Introduction

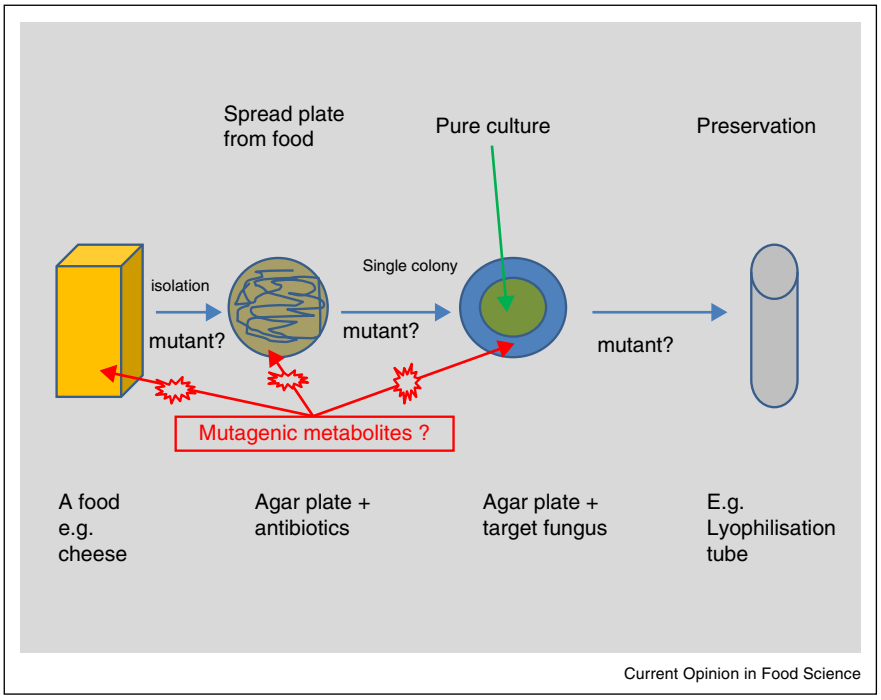
Wild type strains are employed when fungi from food and potable water are studied [1]. They are investigated to, *inter alia*, (a) determine taxonomic relationships, (b) undertake whole genome sequencing, (c) create diagnostic methods and/or (d) determine mycotoxin production. However, some of the wild types will contact mutagens, from pre-isolation to analysis, raising doubt as to the validity of data (Figures 1 and 2) [2••,3••]. The following discussion highlights this fundamental problem still unconsidered by other researchers in the primary research papers.

Certain fungi grow and deteriorate food, some of which are mycotoxin producing species (Figure 1). Many mycotoxins are known mutagens (Table 1) [2••,3••] and are tested for mutagenicity because they are found in food intended for humans and animals which may cause cancers: there are numerous other similar fungal secondary products which have not been tested as they are detected infrequently, or not at all, in food. More information is provided on the mutagenicity of these compounds in [2••,3••,4], together with appropriate references. However, it is worth discussing fusarenon X as there is some confusion as to whether it is causes only apoptosis rather than DNA breaks [4]. Nuclear DNA double strand breaks are highly deleterious because they interfere with transcription or replication: Genes are disrupted, leading to hybrid proteins or inappropriate activation of genes (see Clancy [5]). Bony *et al.* [6] mention that fusarenon X had been described by others as a potent apoptosis inducer and mention evidence for this activity in their research as being very scarce and demonstrated clear results of DNA strand breaks, although fusarenon X could cause apoptosis and DNA breaks as the two may be compatible in different systems.

The effect of mycotoxins on other fungi has been reported in terms of model systems, such as the reported mutagenicity of AFB1 on *Neurospora crassa* [7], indicating that there is not a barrier to the mutagenicity of these compounds in fungi *per se* and these studies are particularly relevant to the present discussion and are of outstanding interest. Cytochrome P-450 in the cells of *Saccharomyces cerevisiae* was investigated where cells were capable of metabolizing AFB1 to products active genetically in the same cells [8]. The formation of convertants, revertants and other types of mitotic segregants were induced in *S. cerevisiae* upon incubation with AFB1 [9]. Furthermore, AFB1, G1 and G2 were mutagenic in *N. crassa* [10,11], whereas AFB2 was not [11]. The genetic activity of PR toxin caused (a) gene conversion and mitotic crossing-over in *S. cerevisiae*, and (b) reverse mutation in *S. cerevisiae* and *N. crassa* [12] without enzymatic activation; the mycotoxin was not mutagenic in the forward mutation system of *Schizosaccharomyces pombe* [13]. Patulin was investigated in an extrachromosomal mutation system of a haploid strain of *S. cerevisiae* and mutation from wild type to petite form was observed [14], although the mechanism of mutation was not discussed.

How could extracellular metabolites interact physically with DNA in the cell [2••,3••,4]? These compounds may accumulate in the environment, to the extent that excretion could be affected and allowing them into intracellular space to interact with DNA. However, there are secondary metabolites which are already strictly intracellular, as determined most clearly within the terverticillate penicillia [15••], although they may be secured in compartments. This segregation may break down as

Figure 1



How fungi could be mutated from growth on the foodstuff, isolation in a mixture, and purification. The fungus would be subjected to further mutagens from re-growth after preservation.

the metabolites accumulate to high concentrations when growth continues. Autolysis is another factor which will allow the metabolites to interact with the DNA of the cells, or intracellular metabolites may interact directly with DNA if they are unconstrained. Finally, many of the metabolites are enzyme inhibitors which will inhibit processes such as active secretion and enzymatic degradation of toxic compounds.

Figure 2

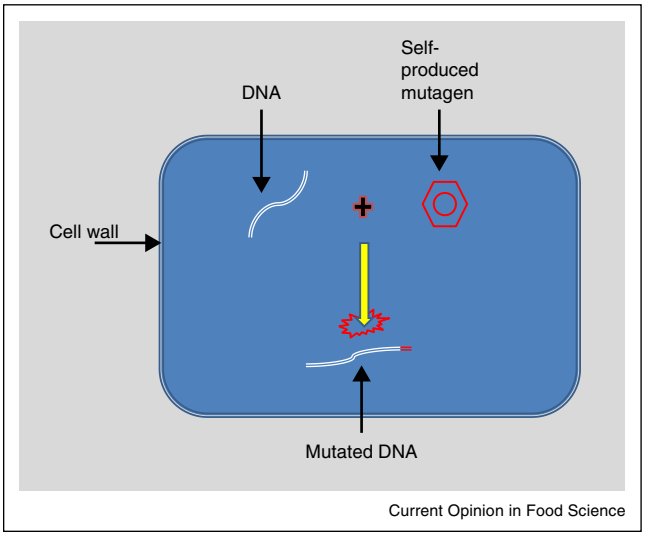


Diagram of a fungal cell containing self produced mutagens in the cytoplasm and affecting mutations in DNA.

All these may be self-mutagenic toward fungi in culture (Figure 2) and/or the environment (e.g. from food). Paterson and Lima [2••] estimated 200 000 mutagenic compounds produced from all fungi. Furthermore, DNA in general may sustain 50 000 damages per cell per day and 150 000 oxidative adducts per cell generated through reactive oxidative species, which can cause mutations if uncorrected. The repair mechanisms are enzyme-based and many fungal secondary metabolites are enzyme inhibitors which may inhibit DNA repair, creating greater mutagenic pressure. How do the mutagens occur?

Pre-isolation

Fungi may be in contact with mutagens before isolation. For example, they are exposed to UV irradiation [16•], and they can be isolated from agricultural areas contaminated with mutagenic pesticides, for example, *Aspergillus fumigatus* mutants resistant to azoles [17•]. Paterson and Lima [18,19] discussed mutants in the environment caused by increases in mutagenic fungal metabolites and UV irradiation from climate change. Fungi are often isolated from foodstuffs containing mycotoxins which may be mutagenic and the fungi could become mutated (Figure 2), and this possibility has not been considered