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> ONE MONTH, ONE PROTEIN <

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small poison

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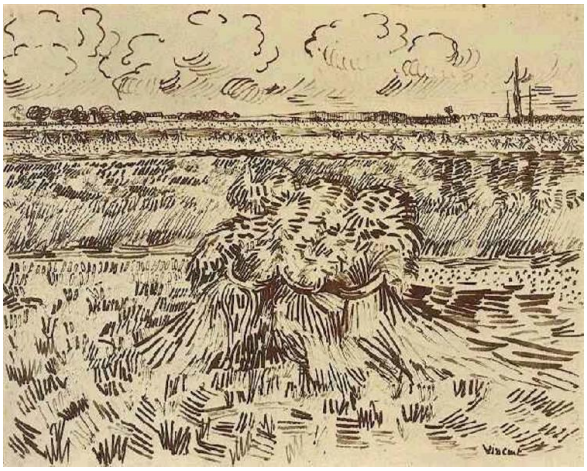
Who has not seen mould spread in the corner of a bathroom or on the edges of wallpaper in a damp house? Or winced at the green growth on the edges of a jam jar or on yoghourt left in the open air too long? Moulds are also sure to flourish on harvested crops that have not been kept dry, while the toxins they produce may wind up in water we drink. Moulds, or fungi, are as much part of our lives as insects or plants but they are not always so easy to see. Sometimes they end up inside us where they can produce poisons known as mycotoxins which may be detrimental to our health. Or we may simply ingest only the mycotoxins themselves. In the past 60 years, hundreds of different kinds of mycotoxins have been characterized, several of which, depending on their nature and concentration, can cause severe damage. Ochratoxin – in particular ochratoxin A (OTA) – is a mycotoxin which is secreted by several species of *Aspergillus* and *Penicillium* and frequently detected in popular food and beverages such as coffee, cow's milk, pork, cereals, wine and beer. In order for food items to remain safe for human consumption, OTA levels are constantly monitored to keep them as low as possible. One way of doing this would be to use an OTA biodegrading enzyme, or ochratoxinase.



Wheat Field With Sheaves (1888)

Vincent Van Gogh

Moulds – and their potential toxicity – have been familiar to humans for centuries if not more. However, it was only in the early 1960s that the term 'mycotoxin' was actually coined following the death of about 100,000 young turkeys near London in the UK. The cause of their death was finally tracked down to peanut meal they had been given, and which had been contaminated with toxins from the fungus *Aspergillus flavus*. This particular incident made scientists wonder whether other fungal toxins could be just as deadly, and generous funds were granted to unveil them. This sparked off what has been called the 'mycotoxin gold rush' that lasted for about 15 years – from 1960 to 1975 – and resulted in the isolation of hundreds of different mycotoxins, several of which did indeed turn out to be dangerous for humans and animals.

The circumstances which encourage mycotoxin production are of great concern to researchers and economical actors alike. Today we know that they occur in various crops intended for human or animal consumption such as barley, wheat, rye and oats. This may happen where storage and food-handling are poor, notably in countries where regulations are not strict or disregarded. Mycotoxin contamination can also arise following a chain of unfortunate events where environmental conditions – namely moisture and temperature but also the presence of other microorganisms – encourage fungal growth and toxin synthesis. The simple shift of food stuffs from one storage point to another is enough to create surroundings which, unless closely monitored, can cause fungus to spread.

Mycotoxins are low-molecular weight secondary metabolites secreted by filamentous fungi, and their chemical structures are as numerous and diverse as their biological effects. Ochratoxin A (OTA) is one of

the most harmful mycotoxins known – active in only parts-per-billion. Chlorinated, it consists of an isocoumarin moiety (more commonly known as a 1H-2-benzopyran-1-one skeleton) and a phenylalanine moiety, linked to one another by an amide bond. Why is OTA toxic? Because it intervenes with protein synthesis, thus disturbing vital cellular metabolic processes. OTA is known to cause liver or kidney failure as well as being teratogenic and carcinogenic.

Ochratoxin takes its name from *Aspergillus ochraceus*, where it was first discovered in South Africa in 1962 – although it is produced by other *Aspergillus* species too. OTA is regularly found in food items dear to humans like barley, wheat and rye but also maize, rice, legumes, grapes, raisins, wine, nuts, beer, cacao, spices, cow's milk, pork and coffee – and perhaps many more. It is hardly surprising then that OTA represents a challenge both to a country's all-round health as well as its economy, and regulations regarding the amounts of OTA allowed in foods are strict – at least in developed countries. For this, however, you need the technology to detect and measure OTA which is active in near trace amounts; in humans, levels have been set to less than 5ng/kg of body weight per day. While current preventive measures include the careful supervision of crop handling and storage conditions, yet other methods could involve the actual biodegradation of OTA, either by micro-organisms or even enzymes – such as ochratoxinase.

Ochratoxinase (OTase) is not the only OTA biodegrading enzyme known to date but it does perform 600 times faster than any other, thus making it a choice enzyme. It so happens that OTase is synthesized by *Aspergillus* itself, which may strike one as being a tad odd. Why degrade what you have just made? It may be one way for fungi to keep their own secreted toxins at a concentration that does not become harmful to them. How does OTase do it? By destroying the amide bond between the two mycotoxin moieties. OTase is a homo-octamer – in fact, a tetramer of dimers – which, viewed from above, resembles a twin propeller. The active site is formed by a large cavity filled with water molecules, which awaits its substrate (OTA) with its mouth, so speak, open wide. Once the substrate has slipped inside, the cavity closes and OTA is neutralized by a zinc-coordinated water molecule that cleaves the mycotoxin's amide bond.

Mycotoxins, in particular OTAs, are frequently found in crops on which a country's economy depends – not to mention the nation's health – which is why it is so important to find ways of monitoring OTA concentration. Keeping this in mind and with the benefit of hindsight, OTA may well have been the cause of thousands of deaths in the past. How is this explained? In the 1750s, mortality rates in Great Britain and France had decreased. Health and nutrition had indeed improved by then, however, potatoes were also becoming popular at about the same time, gradually replacing wheat, barley and rye – all prone to OTA contamination, unlike potatoes. Unknowingly, the advent of potatoes on the kitchen table may well have saved many lives. It is an intriguing theory, which is backed up by mass mortality events that had occurred before the 1750s, during times when climate conditions would have been favourable to OTA production. There certainly seems to an ongoing battle between humans and fungi. Save perhaps for one famous mould: *Penicillium* which, thanks to the Scottish microbiologist Alexander Fleming, has been providing us with precious antimicrobial mycotoxins for almost exactly a century.

Cross-references to UniProt

Ochratoxinase, *Aspergillus niger (*strain CBS 513.88 / FGSC A1513*)*: A2R2V4

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