



Does it take Two to Tango? The Importance of Coaggregation in Multi-Species Biofilms

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A well-choreographed tango requires more than simple proximity. Venue, mood, partner, and the presence of other dancers all influence the performance. If even one of these components is missing or altered, the dance can come to an end or become disjointed. Such orchestrated interactions are not just the preserve of human society; a type of dance arguably occurs in bacterial biofilm communities.

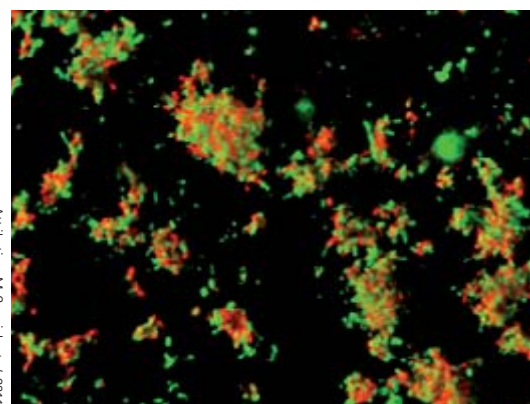
While bacteria obviously do not dance, there is increasing evidence that they specifically recognize partner species to which they adhere and with which they interact. The specificity of bacterial recognition is a hallmark of coaggregation: the specific recognition and subsequent attachment of different bacterial species to each other, which is mediated by complementary cell-surface-associated polymers (Figure 1). This short review will summarize the phenomenon and potential importance of coaggregation in the context of biofilm development.

The planktonic mode of bacterial life was once thought to dominate in most natural environments, but a growing body of evidence suggests that biofilms represent the predominant lifestyle of many microorganisms¹. Biofilms have gained notoriety in recent decades because bacteria within these communities can be up to 1000 times more resistant to antimicrobials, cause many persistent bacterial infections (such as periodontal disease), and contribute to environmental changes (such as the corrosion of water pipes)^{1,2,3,4}.

Of direct relevance, coaggregation is proposed to be an integral step in multispecies biofilm formation in certain environments. A proposed consequence of coaggregation is the enhanced sequential colonization and ordered development of multispecies biofilms⁵.

Historical overview

In 1970, Gibbons and Nygaard reported that different species of oral bacteria could specifically aggregate (coaggregate) to one-another⁶. In particular, strains of two bacterial species indigenous to dental plaque, *Streptococcus sanguis* (now *Streptococcus oralis*) and *Actinomyces naeslundii* (now *Actinomyces oris*) were found to coaggregate strongly. Consequently, these two species were the focus of several additional studies describing the mechanism mediating coaggregation. In particular, Ellen and Balcerzak-Raczkowski⁷ determined that this interaction was highly strain-specific and mediated by cell-surface molecules. Two groups expanded this observation in the late 1970s to demonstrate that coaggregation was mediated by the interaction of an *A. oris* cell-surface-associated protein with a *S. oralis* surface carbohydrate^{8,9}.



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Figure 1 Confocal laser scanning micrograph showing *Streptococcus gordonii* (green cells) coaggregating with *Streptococcus oralis* (red cells) after mixing in coaggregation buffer⁸. Coaggregates show a random assortment of each cell type with no defined structure and size.

Initial studies by Gibbons and Nygaard⁶ indicated that coaggregation was uncommon - it was observed in just 23 of 253 total pairs. However, as research progressed and techniques and systems to study coaggregation improved in sensitivity, the ubiquity of this phenomenon began to be appreciated. It is now believed that most isolated oral bacteria are able to coaggregate to at least one other bacterium from the oral cavity¹⁰. These interactions are often specific at the strain level and many interactions are intergeneric; between different species within different genera. One finding that further popularized the importance of coaggregation within the oral research community was the discovery that certain strict anaerobes coaggregate to aerobes/facultative anaerobes. For example, coaggregation of streptococci (facultative anaerobe) to *Fusobacterium nucleatum* (obligate anaerobe), was shown to allow for the survival of strict oral anaerobes in an aerobic environment¹¹. This is because *F. nucleatum* can coaggregate with many oral species, including facultative anaerobes, such as streptococci, and obligate anaerobes, such as *T. denticola*.

In particular, streptococci surround the *F. nucleatum* cells and form corn-cob-like aggregates¹², which likely deplete local oxygen levels and create a microenvironment suitable for the growth of obligate anaerobes. The promiscuity of coaggregation interactions displayed by *F. nucleatum* therefore enhances the integration and expansion of pathogenic populations (e.g. *Porphyromonas gingivalis* and *Treponema denticola*) in the oral dental plaque biofilm communities. In addition to serving as a mechanism to enhance the ability of bacteria to adhere to developing oral biofilms, many oral researchers consider coaggregation to be a process that enhances successional attachment of oral bacteria to dental plaque biofilms, and promotes the expansion of certain bacterial populations within multispecies dental plaque biofilm communities. It is also hypothesized that coaggregation may serve as a mechanism which contributes to the progression from health to periodontal disease¹³.

Although the phenomenon of coaggregation was originally thought to be exclusive to the oral cavity¹⁰, reports describing coaggregation in environments other than the oral cavity began to appear in the late 1980s. Among the first reports was that of Reid and colleagues, who demonstrated that coaggregation occurs between specific strains of *Escherichia coli* and *Lactobacillus casei* isolated from the human urogenital tract¹⁴. The authors proposed that coaggregation interactions could reduce the pathogenicity of *E. coli* by bringing the two organisms into close proximity and allowing *L. casei* to inhibit the growth of *E. coli*. Soon after, Vandevoorde and coworkers reported upon coaggregation between lactobacilli in the chicken gut¹⁵. Other papers followed, including those that demonstrated that coaggregation occurs between bacteria isolated from the human gut and canine dental plaque. In the last decade, coaggregation has also been reported to occur in environmental ecosystems, including freshwater and wastewater biofilms. However, the mechanism mediating coaggregation between freshwater bacteria is more complicated than that mediating coaggregation between oral bacteria. Specifically, the ability of freshwater bacteria to coaggregate is influenced by nutrient availability¹⁶ and environmental conditions, such as pH and ionic strength of solutions¹⁷. It is possible that similar complications have slowed the identification of coaggregation between bacteria in other environmental samples. As in human societies, where dances only occur under particular circumstances, freshwater bacterial coaggregation occurs as a consequence of specific environmental cues and conditions.

Mechanism behind coaggregation partnerships

Most studies of the mechanisms mediating bacterial coaggregation have focused on human oral bacteria¹⁰. Interestingly, the limited studies of coaggregation between bacteria in environments other than the oral cavity have shown similarities. For example, studies of coaggregation between bacteria from both the human oral cavity and freshwater biofilms have shown that proteinaceous adhesins are often expressed on the cell-surface of one species, which recognizes and binds to the polysaccharide-containing receptor on the cell-surface of the partner species⁵.

Adhesins are proteins found either on the bacterial outer membrane, cell wall, or on surface appendages, such as fimbriae^{5,10,18}. These structures allow for bacterial surface attachment to host tissue, solid surfaces or other microorganisms. Bacteria can express multiple adhesins^{5,19}. In many instances, coaggregation is reversed by adding one or more simple sugars to a suspension of coaggregating bacteria, indicating that many bacterial receptor molecules contain a carbohydrate moiety and that attachment is mediated by lectin-like adhesins. This lectin-saccharide interaction between coaggregating partners is found in both oral¹⁰ and aquatic bacteria^{16,20}. However, it should be noted that there have also been reports of non-lectin adhesins (viz. protein-protein interactions) mediating coaggregation between pairs of bacteria^{5,10}.

In order for coaggregation to occur, there must be a complementary polymer, often a polysaccharide, on the coaggregation partner to which adhesins can bind. This polysaccharide possesses a specific binding site, or motif, that is recognized by the (lectin-like) adhesin of the complementary strain. It is important to note, however, that while the receptor is a polysaccharide, it could be a component or part of a complex with other molecules/polymers, e.g. lipopolysaccharides or glycoproteins. In the case of polysaccharide coaggregation receptor polymers presented by oral streptococci, studies have shown that subtly different receptor polysaccharides (RPS) can be expressed. As described by Yoshida *et al.*²¹, six different structural types of RPS in oral streptococci have been identified through their reactions with specific antibodies and lectin-like adhesins. Each RPS consists of unique units of repeating hexa- or heptasaccharides and contains a receptor to which adhesins of specific coaggregating partners are able to bind. Yoshida and coworkers²¹ suggest that coaggregation mediated by RPS adhesin interactions play a direct role in the formation of multispecies biofilms and allow for mutualistic/synergistic interactions, which require close contact between different species.

A commonly over-looked coaggregation phenomenon is the possibility that multiple adhesins and multiple receptors can mediate coaggregation between a particular pair of bacteria. Kolenbrander *et al.*²² addressed this question in 1985 and showed that a coaggregating pair should be defined as unimodal if coaggregation is inhibited by the heat treatment of one strain but not the other. This description suggests that a heat-sensitive protein adhesin- and a heat-insensitive polysaccharide receptor mediate the coaggregation interaction. Conversely, a coaggregation interaction should be defined as bimodal if coaggregation is only inhibited by the heat treatment of both strains (suggesting an adhesin-receptor and a receptor-adhesin interaction between the coaggregating pair or possibly a protein-protein interaction).

Unfortunately, the identification of bacterial coaggregation adhesins and complementary receptors has focused primarily on those species

that exist in the human oral cavity. However, as the identification of coaggregating bacteria in other environments continues, it is highly likely that the number of known proteins and carbohydrate moieties responsible for coaggregation will increase and their structures and functions determined.

Ecological relevance

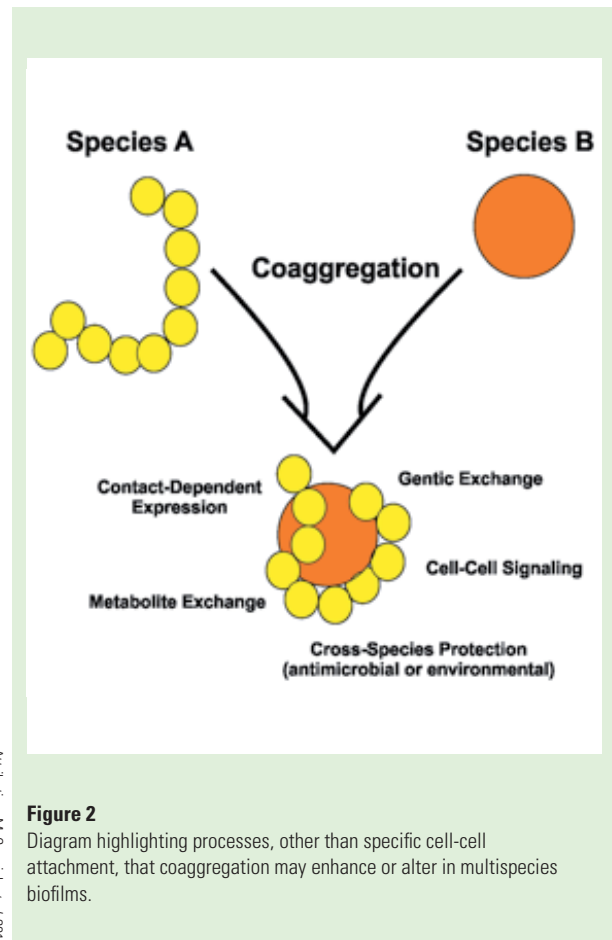
Natural flowing environmental conditions can impose strong selective pressure against planktonic growth. The oral cavity is an excellent example of such a selective pressure, as it is an environment with large amounts of fluid, a high shear rate, and prompt transfer to an unfavorable environment (the stomach) if microbes fail to adhere to surfaces. Surfaces to which bacteria can adhere include teeth, gingiva, or other cells within biofilms. In this environment, bacteria that have evolved the ability to coaggregate could potentially have a higher relative biofilm-fitness than those that are unable to coaggregate. Coaggregation could therefore be a primary determinant for the relatively similar oral microbiota observed between healthy individuals as well as the same reproducible and sequential development of multispecies biofilms after toothbrushing⁴.

Unlike the oral cavity, freshwater environments have highly variable flow rates, ranging from static glacial lakes to rapid rivers. Research has shown that different shear rates select for freshwater biofilms with differing species composition and also select for different proportions of intra-species aggregating bacteria (a phenomenon called autoaggregation) and coaggregating species of bacteria²³. A further consideration is that freshwater coaggregation ability varies with the metabolic status of the cells¹⁶. It is therefore feasible that coaggregation can be switched “on” or “off”, depending upon environmental conditions, and thereby contributes to biofilm development or biofilm dispersion, which would allow for bacteria to traverse freshwater environments more effectively.

With respect to ecological relevance, coaggregation is proposed to contribute towards enhanced biofilm colonization, community succession and interbacterial interactions within the biofilm^{4,5,19,24}. This phenomenon allows for the development of a complex multispecies community. These multispecies biofilms consequently function as highly structured communities, and interactions between biofilm bacteria can conceivably be commensal, synergistic or parasitic. Coaggregation likely has a multifaceted role in multispecies biofilm development.

An unquantified phenomenon that likely has a multifaceted role in multispecies biofilms

By virtue of cellular juxtaposition and species specificity, a number of interspecies interactions could occur as a consequence of coaggregation (Figure 2). These interactions include genetic exchange, cell-cell signaling, cross-species protection, metabolite exchange, and contact-dependent signaling. The vast majority of studies that have identified these possible types of interactions between coaggregating bacteria have focused on strains from the human oral cavity. Of particular interest is the potential role of coaggregation in enhancing cell-cell signaling. A recent review by Kolenbrander and coworkers highlighted the potential importance of coaggregation in bringing species in close proximity. Specifically, that bacterial juxtaposition within multispecies biofilms may enhance cell-cell signaling between the component species via the production and detection of autoinducer-2²⁴. Eglund *et al.* have also shown that metabolic communication (an outcome of metabolite exchange) may occur between *Streptococcus gordonii*-*Veillonella atypica* in dual-



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Figure 2

Diagram highlighting processes, other than specific cell-cell attachment, that coaggregation may enhance or alter in multispecies biofilms.

species oral biofilms²⁵. Not only has coaggregation been shown to facilitate metabolic communication between bacteria within the oral cavity, but coaggregation has been shown to facilitate metabolite transfer/exchange between methanogens and archaea in marine environments. Coaggregation in this environment was strongly dependent on the growth substrate, indicating that bacteria are able to selectively coaggregate in order to maximize metabolic potential under a specific condition²⁶. An intriguing, albeit unstudied, possibility is that coaggregation between microbes in marine snow helps to establish a parsimonious food chain and increase the energetic potential of metabolites.

Coaggregation also likely plays a role in cross-species protection (Figure 2). As described earlier, coaggregation between streptococci and *F. nucleatum* probably contributes to the protection of other anaerobic species from oxygen¹¹. Coaggregation may also confer cross-species protection to antimicrobials. The potential for such a role was first put forward by Gilbert and coworkers³. Another interesting possibility is that coaggregation enhances genetic exchange between bacteria²⁷, for example, through conjugation or the release and uptake of extracellular DNA during biofilm growth or cell death²⁸. Finally, an intriguing recent study by Inagaki *et al.* indicates that contact-dependent expression may occur in the oral pathogen *Tannerella forsythia* as a consequence of coaggregation with other oral species²⁹. This finding opens up the possibility that the actual act of coaggregation alters the expression of certain genes in response to physical contact.

Coaggregation as a common biofilm phenomenon

One of the most famous images in microbiology is Anton van Leeuwenhoek's drawing of human oral bacteria. Using his revolutionary microscope, he visualized and drew the oral bacterial cells he observed. However, a key facet of these organisms' lifestyle was not highlighted in these early images: bacteria are social entities, and they have the ability to aggregate and specifically coaggregate. In order to convey this message, we drew the analogy of coaggregation to a dance and asked, "Does it take two to tango?" Simply put, the answer is no - it likely takes more, and these interactions predominantly occur in complex multispecies biofilm communities. Combinations of specific species in close proximity could conceivably provide for a biofilm that possesses a greater combined phenotypic, genetic, metabolic and/or signaling potential

than the individual component species alone³⁰. Coaggregation likely plays a role in forming some of these associations and mediating interactions both within the oral cavity and in the broader environment. As to whether coaggregation is a ubiquitous phenomenon and what other the roles it plays in biofilm development, only more research studies will reveal the answer. Indeed, as coaggregation receives more attention (Figure 3) and as the microbial properties and composition of biofilms in other environments are investigated, the role of coaggregation in other environmental biofilms will be clarified. Such an understanding would not only improve our fundamental understanding of multispecies biofilm development, but it could aid in the development of novel approaches to control multispecies biofilms.

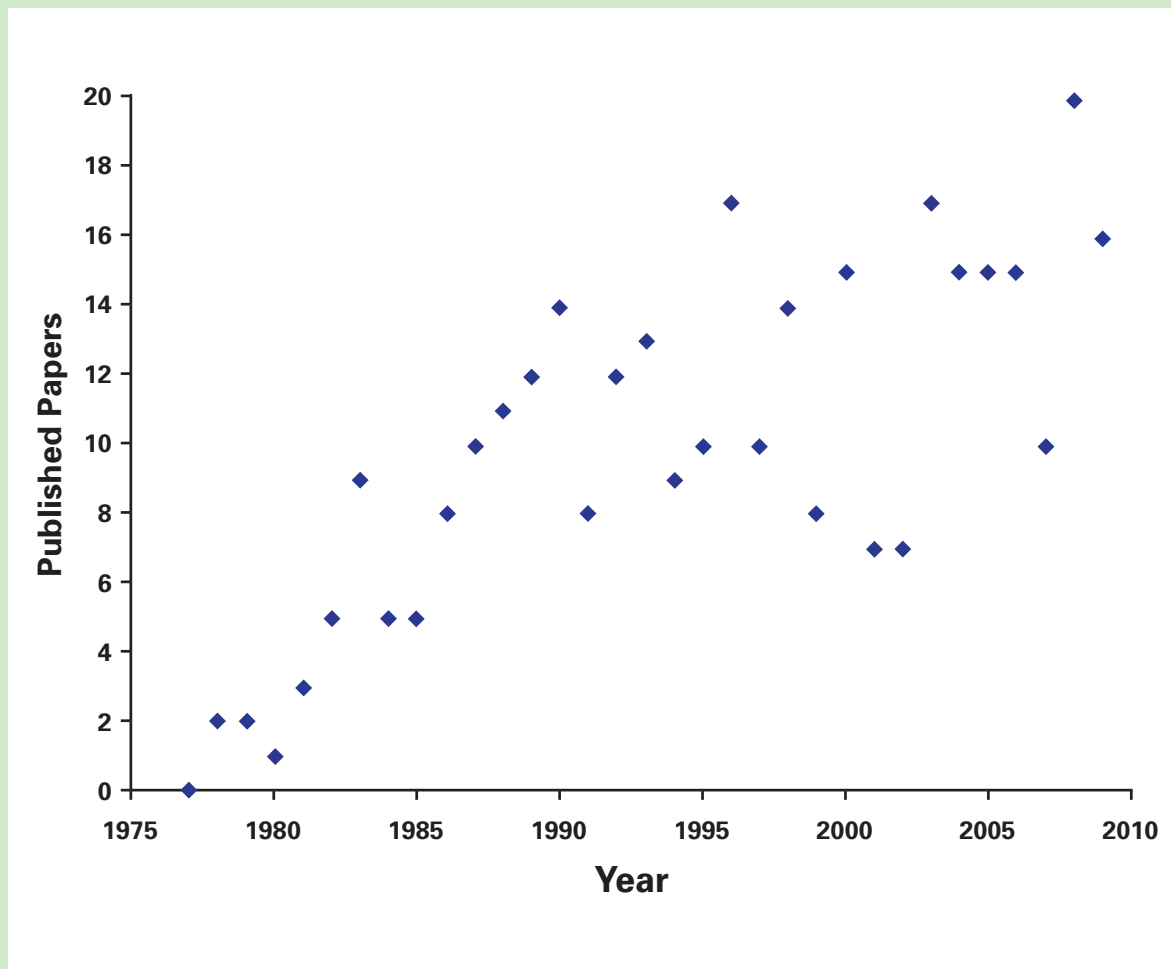


Figure 3

Graph showing the increase in research and review publications that report upon or consider aspects of bacterial coaggregation. Enumeration of publications per year was performed on Pubmed (www.ncbi.nlm.nih.gov/pubmed) using the search terms "coaggregation" and "bacteria" for each year up to 2009. Note: the first paper to show coaggregation was by Gibbons and Nygaard⁶, but this paper was not included in this graph because the authors did not describe the interaction as "coaggregation".

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Food, Fungi and Mycotoxins: an update

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Introduction

Despite advances in harvesting, storage and processing technologies, fungal spoilage still has a major economic impact on world food supplies. Even though we have a much greater understanding of the causal factors, fungal spoilage of commodities, fruits and vegetables seems to be as great a problem now as it has ever been. A recent report by FAO¹ estimates that, in areas such as Asia and Africa, 8–18% of commodities (cereals, root and tubers, oilseeds and pulses, and fruit and vegetables) are lost during postharvest handling and storage. In sub-Saharan Africa, postharvest grain losses alone have recently been estimated to be valued at US\$1.6 billion per year, or about 13.5% of the total value of grain production². The majority of these losses can be attributed to fungal growth and contamination with mycotoxins. The most common and destructive food spoilage fungi belong to the genera *Aspergillus*, *Penicillium*, *Eurotium* and *Fusarium*, although other genera are significant in particular foods and ecological niches.

In processed foods, fungal spoilage still causes significant losses, although the use of preservatives, more effective processing and packaging has probably decreased the wastage in this sector. However, in advanced economies, consumer pressure for fewer additives (particularly preservatives), reduced salt levels, less aggressive heat processes and a demand for 'fresh-like' foods has rendered some types of foods more susceptible to spoilage.

In recent years, we have seen an increase in spoilage, particularly in shelf-stable beverages, due to heat-resistant moulds, such as *Byssoschlamys*, *Talaromyces* and *Neosartorya* species, and even relatively heat-sensitive moulds, such as *Fusarium oxysporum*, *Mucor* and *Rhizopus* species.

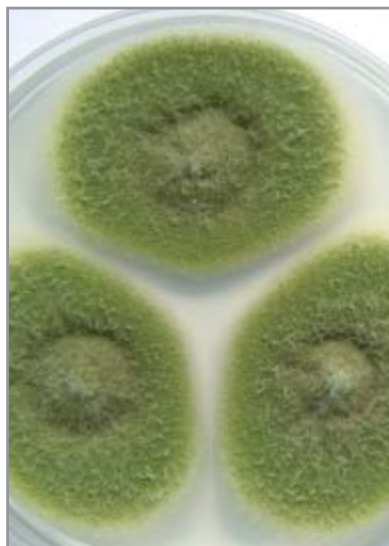
While fungal spoilage will continue to cause economic losses to food processors and consumers, the main concern arising from fungal contamination of food supplies comes from mycotoxin contamination and the consequent health effects to consumers. We have known about mycotoxins for a long time. It is almost 50 years since aflatoxins were discovered, and in the twenty or so years after that, many other toxic metabolites were described from the fungi that spoil our foods. New mycotoxins continue to be reported in the literature, but the most significant advances in mycotoxin research in the last decade or so have been in our understanding of which fungi produce which mycotoxins. As more fungal genomes become available, it is possible to search for the genetic machinery of mycotoxin production, the synthetic and regulatory genes.

We have also gained a greater understanding of how mycotoxins enter the food chain. In the early days of mycotoxin research, it was thought that mycotoxin formation occurred mainly during storage of commodities. Although mycotoxin contamination can and does occur during transport and storage, we now know that mycotoxin formation in food crops immediately pre- and post-harvest is a significant source of these toxins in our food supply. Genera, such as *Fusarium*, *Alternaria* and *Aspergillus*, can infect grains, nuts and even fruit crops, such as grapes and figs, while still in the field, with formation of mycotoxins in the ripening crops. *Penicillium* spp. are more likely to affect crops in the immediate post-harvest period and during storage.

In developed economies, mycotoxins may contribute to underlying health issues but are unlikely to be a direct cause of death. However, in developing countries, mycotoxins are certainly of much greater concern, contributing to the burden of disease, particularly through their carcinogenic and immunosuppressive activities. In times of severe drought, when food supplies are limited, mycotoxins become an even greater threat. The most important mycotoxins are still aflatoxins, ochratoxin A, fumonisins and trichothecene toxins, particularly deoxynivalenol.

Aflatoxins

Aflatoxins are carcinogenic mycotoxins produced mainly by *A. flavus* and *A. parasiticus*. *Aspergillus flavus* occurs in many food crops in the tropical and warm temperate zones of the world, particularly peanuts, maize and cottonseed. *Aspergillus flavus* also occurs in tree nuts, especially pistachios, almonds and brazil nuts, and less commonly in hazelnuts, walnuts, coconut, copra and pecans³, while *A. parasiticus* occurs where peanuts are grown and appears to be uncommon in other natural environments.



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Figure 1

Aspergillus flavus is the species responsible for aflatoxin contamination in many crops. (a) characteristic green colonies on Czapek Yeast Extract agar; (b) photomicrograph of *A. flavus*.

The four naturally occurring aflatoxins are aflatoxins B1 and B2, (they fluoresce blue under UV light) and aflatoxins G1 and G2, (greenish yellow fluorescence). The liver is the primary target for aflatoxins. In high doses, they can cause acute toxicity, resulting in liver failure. In lower doses, aflatoxins can cause liver cirrhosis and liver cancer. However, more insidious effects of aflatoxins are growth retardation in children and immunosuppression. Children with compromised immune systems are more susceptible to many other diseases, and may be unable to mount a proper immune response to vaccination.

Toxicity of aflatoxins

Acute toxicity from aflatoxin can occur when high levels (milligram quantities) of aflatoxins are ingested as a result of consumption of highly contaminated commodities. The most recently reported outbreak of aflatoxicosis occurred in Kenya in 2004, where contaminated maize caused 317 cases of hepatitis and 125 deaths⁴ with estimated daily ingestion of up to 6mg of aflatoxins⁵. Generally, aflatoxicosis only occurs when drought or famine causes exceptionally high levels of aflatoxins in the diet, and people are forced to eat poor quality food.

Liver cancer can develop from consuming much lower levels of aflatoxin over a protracted period. The International Agency for Research on Cancer (IARC) classifies aflatoxin B1 and naturally occurring mixtures of aflatoxins as Class 1 carcinogens, i.e. they are recognized as carcinogenic to humans. In the past 20 years, studies have identified that hepatitis B virus also causes human liver cancer, and it is synergistic with aflatoxin. The two agents together are about 30 times as potent as aflatoxin alone⁶ according to risk assessments carried out by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). JECFA estimates the liver cancer rate for aflatoxin alone to be 0.01 cases per 100,000 people per annum per nanogram of ingested aflatoxin per kg body weight per day, but for those also infected with hepatitis B, this cancer rate rises to 0.30 cases.

There is evidence from a number of studies that aflatoxin exposure before birth and in early childhood is associated with stunted growth. It is apparent from the high numbers of people believed to be consuming uncontrolled levels of aflatoxin that stunting is an important disease burden only recently recognised⁷. Aflatoxins also suppress the cell-mediated immune response, increasing susceptibility to infection and reducing response to vaccines⁸. These immunosuppressive effects of aflatoxins probably have very wide implications for human health.

Occurrence of aflatoxins in foods, and regulation

Peanuts, maize and cottonseed are the main commodities at serious risk of aflatoxin contamination. Other affected commodities include tree nuts, especially pistachios, almonds and Brazil nuts, figs and spices. If sorghum, rice, coffee and cocoa are poorly stored, they may become contaminated with aflatoxins.

Regulation of aflatoxin levels in foods started as early as about 1970, using the

then limit of detection, 5µg/kg, as the permitted limit, with a higher limits (15µg/kg) allowed for peanuts. This higher limit has now been agreed for maize, peanuts and other foods in international trade. Although safe levels of aflatoxins have now been established, and aflatoxin levels are closely controlled in developed countries, it has been estimated that up to 5 billion people worldwide are at risk from exposure to uncontrolled levels of aflatoxins in their diets⁹.



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Figure 2

(a) peanuts infected with *Aspergillus flavus*, plated on Dichloran Rose Bengal Chloramphenicol (DRBC) agar; (b) maize cobs showing *A. flavus* infected kernels. Both crops are often contaminated with aflatoxins before harvest or during drying.

Control of aflatoxins in foods

Control of aflatoxins is difficult, as invasion by *A. flavus* (and *A. parasiticus* in peanuts) can occur before harvest in all of the major crops affected³ if there are high spore numbers in soil, insect damage and/or drought stress. Irrigation and improved harvesting and drying practices can minimize aflatoxin contamination. However, in many areas where peanuts and maize are grown, irrigation is not an option. In tree nuts, irrigation and insect control are important in aflatoxin minimization.

One of the most useful approaches to reduce aflatoxin appears to be biocontrol by competitive exclusion for both peanut and cotton crops, and possibly also for maize. This involves addition of high numbers of spores of selected nontoxigenic strains of *A. flavus* into soils in peanut or cotton fields^{9,10}. These nontoxigenic spores compete with the existing toxin-producing spores in the soil for infection sites on developing plants. This process is used commercially in the USA for peanuts and cotton^{11,12}. Pilot scale work is currently in progress on maize crops and under development for some tree nuts.

Further controls are achieved during processing. In peanuts, colour sorting can reduce aflatoxin levels by removing discoloured kernels. Maize and fig samples can be screened for the presence of aflatoxin by the examination of cracked kernels or fruit by ultraviolet light. However, end-product testing remains an important control step in limiting aflatoxin-contaminated commodities entering the food chain.

Ochratoxin A

Ochratoxin A (OTA) is unusual in that it is produced by three groups of fungi: first, the ochre- coloured Aspergilli, comprising mainly *Aspergillus ochraceus* (after which the toxin is named), *A. westerdijkiae* and *A. steynii*; second, the black Aspergilli, *A. carbonarius* and the closely related and common species *A. niger*, which produces OTA only infrequently; and, third, the *Penicillium* spp, *P. verrucosum* and the closely related species *P. nordicum*^{13,14}.

The ochre-coloured Aspergilli are xerophilic and occur in stored commodities, with *A. westerdijkiae* recognized as a major source of OTA in coffee¹⁵. *Aspergillus carbonarius* and *A. niger* are the primary source of OTA contamination in grapes and grape products, including wines and dried grapes (sultanas, raisins), throughout the world^{16,17}. These black aspergilli can also produce OTA in coffee and cocoa beans. Figs, peanuts, maize, and paprika may also occasionally be contaminated with OTA.

In cereals grown in cool temperate climates, *Penicillium verrucosum* is the major OTA producer¹⁸. OTA is frequently present in cereal products in these areas, especially bread and flour, and in the meat of animals fed cereals as a major part of their diet. *Penicillium verrucosum* is not found in warmer climates, so small grains from the tropics and warm temperate zones do not contain OTA³. *Penicillium nordicum* occurs on manufactured meat products, such as salami, and ham, where it can occasionally produce OTA³.

Toxicity of ochratoxin A

Ochratoxin A is a chronic nephrotoxin, affecting kidney function in all animal species tested. People in Europe and northern North America are exposed to ochratoxin A in barley and wheat and their products, especially bread, and also from meat, especially pork, from animals fed contaminated feed. Low levels also occur in beer, wines, coffee, cocoa and chocolate, and dried vine fruits. OTA has a long half life, so in areas where it occurs in the diet, the blood of healthy humans regularly contains detectable amounts of ochratoxin A¹⁹. Ochratoxin A also has carcinogenic properties, but its effects in humans remain unclear.

Fumonisin

Fumonisin are produced by *Fusarium verticillioides* (previously known as *F. moniliforme*) and some closely related species, in particular *F. proliferatum*. These species are systemic in maize worldwide, even in healthy kernels. Fumonisin are structurally similar to the sphingoid base backbone of sphingolipids, important constituents of membranes²⁰. The most important of this family of compounds is fumonisin B₁.

It has been shown recently that *A. niger* can produce some fumonisins. Frisvad *et al.*²¹ reported for the first time the production of fumonisin B₂ in cultures of three full-genome-sequenced strains of *A. niger*, including the ex-type culture *A. niger*. Isolates of *A. niger* were able to produce FB₂ and FB₄ on grapes and raisins²², and FB₂ was produced on coffee²³. *Aspergillus niger* is among the fungi most commonly reported from foods, and the possibility of co-occurrence of ochratoxin A and fumonisin B₂ in foods is of concern.

Toxicology

A wide range of effects has been reported for fumonisins in animals and man. Because they inhibit ceramide synthase, fumonisins cause accumulation of intermediates of sphingolipid metabolism, and also depletion of complex sphingolipids. These effects interfere with the function of some membrane proteins, including folate binding. The most dramatic effect occurs in horses, where the disease called equine leucoencephalomalacia occurs if horses consume feed containing >10mg/kg fumonisin B₁. It is a rapidly progressing disease that liquefies equine brains. In pigs, fumonisins cause pulmonary oedema, while in rats, the primary effect is liver cancer¹³. In humans, fumonisins and *F. verticillioides* are associated with oesophageal cancer, a connection established by extensive studies in areas of low and high maize consumption in South Africa^{13,24}. The disease is also prevalent in areas of China²⁵, in parts of Iran, northern Italy, Kenya, and a small area of the southern USA¹³. In all of those areas, consumption of maize and maize products is very high. High exposure to fumonisins from maize may be associated with neural tube defects, such as spinal bifida, in areas of Guatemala, South Africa, China and a population along the Texas-Mexican border²⁶.

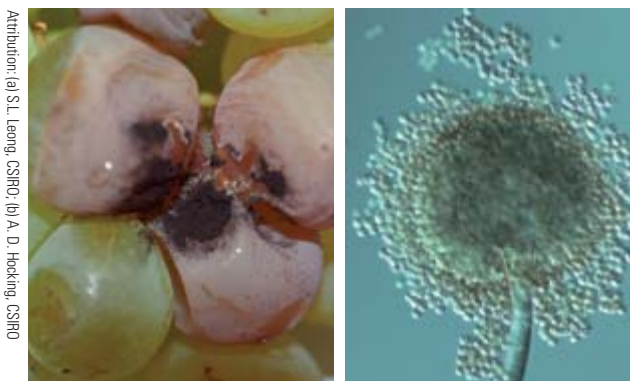


Figure 3

Ochratoxin A in grapes is caused by infection with black *Aspergillus* species. (a) Semillon grapes showing black *Aspergillus* infection; (b) photomicrograph of *Aspergillus carbonarius*, the main species responsible for production of ochratoxin A in grapes.

Occurrence, regulation and control

The major source of fumonisins in foods is maize, though other small grains, particularly sorghum, occasionally are affected. With the discovery that *A. niger* can produce some fumonisins, the range of foodstuffs where fumonisins may be found has become much wider. In the United States, dry milled grain products should contain no more than 2mg/kg of total fumonisins²⁷. In the European Union, the maximum limit is 1mg/kg for maize flour, semolina, germ and oil, and 0.4mg/kg for products ready for consumption; except for a limit of 0.2mg/kg for babies and children²⁸.

Fumonisin are not destroyed during wet or dry milling of maize and are found in all fractions, with higher concentrations in bran and germ. Fumonisin levels are reduced by processing at temperatures above 150°C, including extrusion processes used extensively in the production of breakfast cereal, snack and textured foods²⁹. In Central America, the process of nixtamalization removes almost all fumonisins as well as aflatoxins, resulting in tortillas and other maize based foods being substantially free of these mycotoxins³⁰.

Deoxynivalenol

The other important mycotoxins formed by *Fusarium* spp. belong to a class of compounds known as trichothecenes. Deoxynivalenol (DON) is the most commonly occurring. DON is produced by *F. graminearum*, *F. culmorum* and occasionally some related species. These fungi cause head blight in wheat and cob rot in maize, with consequent formation of DON³.

DON inhibits protein synthesis, and although DON can cause gastrointestinal problems and immunotoxicity, toxicoses in humans appear to be rare³¹. DON toxicity is more significant in domestic animals, particularly pigs, where it causes feed refusal and vomiting.

The future

Genomic studies on economically important fungi should improve our understanding of mycotoxin biosynthesis, the biology, evolution, biochemical function and genetic regulation of the genes in these fungal systems. The genomes of several toxigenic fungi, including *A. flavus*, *A. niger*, *F. graminearum*, and *F. verticillioides* have been sequenced^{32,33}.

The threat to human health from mycotoxins is likely to increase as global warming causes more severe weather patterns, protracted droughts and extensive floods. In many parts of the world, particularly Africa and northern Asia, drought will render crops more susceptible to mycotoxin formation, and famine will force people to eat poor quality grains. There is urgent need for strategies to reduce mycotoxin contamination in these countries, particularly those mycotoxins that enter the food supply before harvest.

Fungal spoilage will continue to take its tithe of human and animal food supplies, but better storage systems and supply chains can minimize these losses. There is a constant search for new and 'natural' preservatives to protect processed foods from fungal attack, but the best solutions will still rely on the combination of adequate processing, good manufacturing practice and stable formulations.

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