

Prevention of Ochratoxin A Contamination of Food and Ochratoxin A Detoxification by Microorganisms – A Review

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Abstract

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Ochratoxin A is a mycotoxin produced by several fungal species of the genera *Aspergillus* and *Penicillium*. This mycotoxin is nephrotoxic, immunosuppressive, teratogenic, and carcinogenic to animals and has been classified as a possible human carcinogen. Human exposure to ochratoxin A is worldwide. Ochratoxin A occurs in a variety of foods. An ideal method for minimisation of the health risk that this mycotoxin poses is the prevention of food contamination. When the contamination occurs, the hazard associated with the mycotoxin presence in the food must be eliminated. Various microorganisms such as bacteria and microscopic fungi have been tested for their abilities to prevent ochratoxin A contamination or detoxify foods. Biological control by microorganisms is studied widely, therefore the objective of this article is to provide an overview of the recent development in the biological control of ochratoxin A contamination.

Keywords: ochratoxin A; prevention; detoxification; bacteria; microscopic fungi

Ochratoxin A (OTA) is the main mycotoxin in the group of ochratoxins (RINGOT *et al.* 2005) and is produced by several fungal species of the genera *Aspergillus* and *Penicillium* (MALÍŘ *et al.* 2003; VALERO *et al.* 2006; CAVIN *et al.* 2007). The major OTA-producing species are *Penicillium (P.) verrucosum*, *Aspergillus (A.) ochraceus*, and *A.* section *Nigri* (MAGAN & ALDRED 2005). *P. verrucosum* is the major OTA-producing fungus in northern Europe, while *A. ochraceus* is more important in warmer climatic zones (CAIRNS-FULLER *et al.* 2005). More *Aspergillus* species have been found to produce OTA, for example, *A. melleus*, *A. sulphureus*, *A. alliaceus*, *A. sclerotiorum* (MALÍŘ *et al.* 2003; BAYMAN & BAKER 2006; PALUMBO *et al.* 2007), *A. albertensis*, *A. lanosus* (BAYMAN & BAKER 2006; PALUMBO *et al.* 2007), *A. glaucus*,

A. ostianus, and *A. petrakii* (MALÍŘ *et al.* 2003; BAYMAN & BAKER 2006). In *Penicillium*, OTA has also been detected, for example, in *P. nordicum* (LARSEN *et al.* 2001; BAYMAN & BAKER 2006), *P. chrysogenum* (MALÍŘ *et al.* 2003; BAYMAN & BAKER 2006).

OTA has been detected, for example, in cereal grains, grapes, wine, grape juice, dried vine fruits, coffee, legumes, beer, nuts, cocoa, spices (BAYMAN & BAKER 2006; CLARK & SNEDEKER 2006). The occurrence of OTA in meat products is due to its transmission into tissues of animals fed with contaminated feed (GUILLAMONT *et al.* 2005). The highest reported occurrences of OTA contamination have been found in cereal grains, and to a lesser extent in grapes, wine, grape juice, and dried vine fruits (CLARK & SNEDEKER 2006). The

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highest OTA content, among grapes and their derivatives, has been found in dried vine fruits (OTA is a thermostable molecule) (BOUDRA *et al.* 1995; CZERWIECKI *et al.* 2002) that is not completely eliminated during the food processing operations and, therefore, it appears in the final products (BULLERMAN & BIANCHINI 2007). *P. verrucosum* and *A. ochraceus* are considered the main producers of OTA in cereals. BATTILANI *et al.* (2003) found that the most ochratoxigenic strains isolated from grapes belonged to *A. carbonarius*. Also, this species is the most probable source of OTA in dried vine fruits (ABARCA *et al.* 2003). In coffee, *A. carbonarius* and *A. ochraceus* are the most potent OTA producers (BUCHELI & TANIWAKI 2002).

The fungi imperfecti are a major group of fungi in soil, the isolation of fungi such as *Penicillium* and *Aspergillus* is common (NESCI *et al.* 2006). According to VISCONTI *et al.* (2008) the main source of OTA in the wine food chain is the infection of grapes by *Aspergillus* spp. belonging to *A.* section *Nigri* in the field. Cereals can also be infected with ochratoxigenic fungi in the field (MILLER 1995). Post harvest contamination of food by ochratoxigenic fungi and sequential production of OTA will occur, if environmental conditions are favourable. For example, harvesting of cereals with a high content of water or their inefficient drying or storage under humid conditions may result in higher levels of OTA in them (JØRGENSEN & JACOBSEN 2002). Out of cereals, for example rice is an aquatic plant and is usually harvested at high moisture levels (ZINEDINE *et al.* 2007). If rice crop is infected by ochratoxigenic fungi and if the environmental conditions during storage are favourable, OTA will be produced (PENA *et al.* 2005).

Penicillium spp. and *Aspergillus* spp. are typical storage fungi that are able to develop at low water activity (a_w) (RAMAKRISHNA *et al.* 1996; WALDEMARSON *et al.* 2005), for example, the minimal water activity for the growth of *P. verrucosum* is about 0.80 (CAIRNS-FULLER *et al.* 2005). SWEENEY and DOBSON (1998) reported that significant levels of OTA production by *P. verrucosum* can occur at the a_w as low as 0.86 and at 4°C. According to CAIRNS-FULLER *et al.* (2005), the highest production of OTA by this fungus was at 0.93–0.98 a_w at 10–25°C. *A. ochraceus* produces OTA at minimal water activity of 0.80 (ADEBAJO *et al.* 1994; RIBEIRO *et al.* 2006). In the investigation by RAMOS *et al.* (1998), optimal water activity for OTA production by this fungus was 0.98. PARDO *et*

al. (2004) found optimal water activity as 0.99. OTA is produced by *A. ochraceus* at 12°C to 37°C with an optimum at 31°C (SWEENEY & DOBSON 1998). According to PARDO *et al.* (2004), optimal temperature is 30°C. VARGA and KOZAKIEWIC (2006) reported that the climatic and geographic differences influence fungal contamination and OTA contamination of grapes. For example, in Europe an increase in OTA levels occurred in wines coming from southern regions of Europe.

Aspergillus spp. are opportunistic pathogens and are responsible for several disorders in various plants (VARGA *et al.* 2004). In grapes, *Aspergillus* spp. belonging to *A.* section *Nigri* develop particularly in damaged grapes during ripening although they may occur and form OTA on grapes from veraison to harvest (VISCONTI *et al.* 2008). OTA concentrations tend to increase with the grape maturity (ROUSSEAU 2004). Berry damage is the primary factor affecting the disease development and OTA accumulation in berries. The damage may be due to birds, insects, infection by other fungi, or rain (LEONG *et al.* 2006; VISCONTI *et al.* 2008). Some grape varieties may display greater susceptibility than others to *Aspergillus* bunch rots. Data obtained in the investigations suggested that varieties had no direct effect on the incidence of black *Aspergillus* spp. on undamaged bunches, however, some varieties were more susceptible to berry splitting and hence would be at a greater risk of *Aspergillus* rots (LEONG *et al.* 2006).

The type of agricultural practices involved in the crop production also influences the amount of OTA. In the study of JUAN *et al.* (2008), the organic cereal samples showed a highest incidence of contamination. CZERWIECKI *et al.* (2002) analysed over 200 samples of Polish cereal grain from the 1997 harvest obtained from conventional and ecological farms. OTA contamination of rye from ecological farms was over six times more frequent than that from conventional cultivation. The OTA content in wheat and barley samples from ecological farms was also higher. JØRGENSEN and JACOBSEN (2002) also found that a multiyear mean concentration of OTA was higher in organically grown rye than in conventionally grown rice. The concentration of OTA was higher in rye than in wheat with both conventionally and organically grown rye and wheat. In a Danish survey, ELMHOLT and RASMUSSEN (2005) analysed organically cultivated grains and found that most of the harvested samples contained *P. verrucosum* prior to drying.

Other factor affecting the mycotoxin production by toxigenic fungus is the presence of other microorganism nonproducing the mycotoxin (MALÍŘ *et al.* 2003).

Human exposure to OTA is worldwide (GUILLAMONT *et al.* 2005; CLARK & SNEDEKER 2006). OTA has been detected in human serum and milk in different countries (CLARK & SNEDEKER 2006). The contaminated foods have been recognised as a possible threat to human health (JODLBAUER *et al.* 2002). Toxic effects of OTA are various, the most relevant being nephrotoxicity and nephrocarcinogenicity in rodents (CAVIN *et al.* 2007). In 1993, the International Agency for Research on Cancer classified OTA as a possible human carcinogen (VALERO *et al.* 2006). It has been speculated that OTA may be associated with the human disease Balkan endemic nephropathy (BEN) and the onset of urinary tract tumours. However, in the workshop organised by the European branch of the International Life Sciences Institutes, it was concluded that there is no convincing evidence from human epidemiology to confirm the association between OTA exposure and the prevalence of BEN or Urinary Tract Tumours. In 2006, the Scientific Panel on Contaminants in the Food Chain of the European Food Safety Authority stated that the epidemiological data on human carcinogenicity are incomplete and did not justify OTA classification as a human renal carcinogen (CAVIN *et al.* 2007). OTA has also been reported to be immunosuppressive and teratogenic in animals (BAYMAN & BAKER 2006; BEJAOUÍ *et al.* 2006).

Because of the detrimental effects of mycotoxins, some strategies have been developed to prevent the growth of mycotoxigenic fungi and also to decontaminate and/or detoxify foods and feeds (KABAK *et al.* 2006). These strategies include:

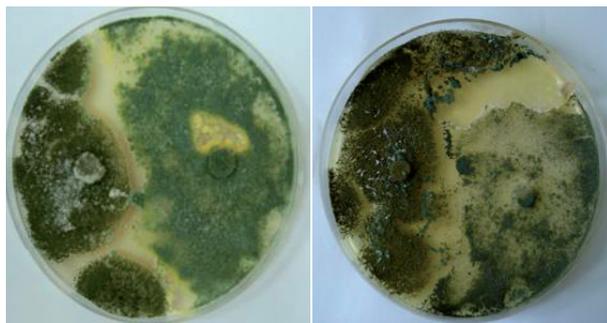
- the prevention of mycotoxin contamination,
- the detoxification of mycotoxins present in foods and feeds,
- inhibition of mycotoxin absorption in the gastrointestinal tract.

Prevention of ochratoxin A contamination

Various control strategies including pre-harvest (e.g., field management, use of biological and chemical materials), harvest management, post-harvest strategies (e.g., improving drying and storage conditions) are important in the prevention of the

fungus growth and mycotoxin formation (KABAK *et al.* 2006; AMÉZQUETA *et al.* 2009). Some microorganisms have been found to control *Aspergillus*, *Penicillium* infections and OTA production. For example, lactic acid bacteria produce antifungal substances. CORSETTI *et al.* (1998) found antifungal effect of the mixture of short-chained organic acids that were produced by *Lactobacillus (Lb.) sanfranciscensis*. This bacterium inhibited the growth of *A. niger* and *P. expansum* on malt agar medium. Cell-free supernatant from *Lb. casei* inhibited spore germination of the investigated *Penicillium* spp. on potato dextrose agar medium. Antimycotic activity of the supernatant was sensitive to proteolytic enzymes (GOURAMA 1997). Also *Bacillus subtilis* produced a peptidolipid that inhibited *A. ochraceus* (KLICH *et al.* 1991). Other *Bacillus* sp., *B. thuringiensis* used as a commercial insecticide during the cultivation of wine grapes inhibited the growth of *A. carbonarius* on potato dextrose agar medium (BAE *et al.* 2004).

Also yeasts and moulds influence the growth of ochratoxigenic microorganisms. For example, BLEVE *et al.* (2006) reported that yeasts isolated from grapes *Issatchenkia orientalis*, *Metschnikowia pulcherrima*, *Issatchenkia terricola*, and *Candida incommunis* reduced the *A. carbonarius* and *A. niger* colonisation on grape berry. The best antagonistic activity was shown by *I. orientalis* isolates. LEE and MAGAN (1999) investigated the effect of water activity on the interactions between OTA-producing strain of *Aspergillus ochraceus* and other fungi in dual culture experiments on maize meal-based agar medium. For example, at 0.995 a_w and 18–30°C, *Alternaria (Al.) alternata* and *A. niger* were dominant. At 0.90 a_w and 18–25°C there was mutual antagonism between *A. ochraceus* and the two *Eurotium* spp. According to GACHOMO and KOTCHONI (2008), *A. ochraceus* and *A. niger* were overgrown by *Trichoderma (T.) harzianum* when they were grown in paired cultures on potato dextrose agar medium. *Trichoderma* spp. are plant symbionts and parasites of other fungi (HARMAN *et al.* 2004). They produce enzymes that degrade cell walls of other fungi (BENÍTEZ *et al.* 2004). ABOU-ZEID *et al.* (2008) found that all isolates studied (two isolates of *Trichoderma* spp. and two isolates of *Gliocladium* spp.) inhibited the growth of *P. chrysogenum* on potato dextrose agar medium. In dual culture experiments the fungi *T. harzianum*, *Al. alternata*, *Cladosporium (Cl.) herbarum*, *Eurotium (E.) amstelodami*, *P. janthinellum*, *P. decum-*



P. chrysogenum and *G. virens* *P. chrysogenum* and *G. deliquescens*

Figure 1. Growth inhibition of *P. chrysogenum* by *Gliocladium* spp. (ABOU-ZEID *et al.* 2008)

bens, and *Candida* sp. influenced the growth of grape-associated fungi *A. carbonarius*, *A. niger*, and *A. japonicus* on a medium similar to the grape composition. For example, at 0.97 a_w the growth rates of all *Aspergillus* spp. were clearly inhibited by *T. harzianum* and almost all moulds reduced *A. niger* growth. *Candida* sp. stimulated the growth of *Aspergillus* spp. At 0.92 a_w , *Penicillium* spp. and *Cl. herbarum* stimulated the growth of OTA-producing *A. carbonarius* (VALERO *et al.* 2007a).

Some authors demonstrated the inhibition of OTA production. For example, MEDINA *et al.* (2007) reported that OTA production by *A. carbonarius* was inhibited by natamycin produced by *Streptomyces natalensis*. Yeast *Pichia anomala* inhibited OTA production by one isolate of *P. verrucosum* in malt extract agar medium as well as in wheat (PETERSSON *et al.* 1998). MASOUD *et al.* (2005) found that *Pichia anomala* and *Pichia kluyveri* inhibited the production of OTA by *A. ochraceus* on malt extract agar medium and on coffee agar medium. According to VALERO *et al.* (2007b), the amount of OTA was reduced in healthy grapes during its dehydration when OTA-nonproducing *A. niger* aggregate was added to the inoculum of OTA-producing *A. carbonarius*. In the investigation conducted by VALERO *et al.* (2007c), grapes were inoculated with *A. carbonarius*, *A. niger* aggregate, *E. amsterodam*, and *P. janthinellum* in different combinations. Coinoculation of *P. janthinellum* with *A. carbonarius* resulted in the reduction of OTA amount in grapes during its dehydration. The effects of fungi (*P. janthinellum*, *P. decumbens*, *T. harzianum*, *Al. alternata*, *Cl. herbarum*, *E. amstelodami*, and *Candida* sp.) on OTA production by *A. carbonarius* were studied on a medium similar to the grape composition by VALERO *et al.* (2006). From the tenth day, all species inhi-

bited OTA accumulation at 0.97 a_w and 30°C. At 0.97 a_w and 20°C, there was no distinct effect of the interacting species on OTA accumulation; in genera, *P. decumbens*, and *T. harzianum* seemed to inhibit OTA production where as *E. amstelodami*, and *Candida* sp. stimulated it. VALERO *et al.* (2007b) found that *E. amstelodami* increased OTA production by *A. carbonarius* in grapes during dehydration. LEE and MAGAN (1999) reported that the interactions between *A. ochraceus* and the species such as *Eurotium* spp. (0.950–0.995 a_w and 25°C) and *Al. alternata* (0.995 a_w and 18°C) resulted in the stimulation of OTA production.

The interactions between microbial species influence the amount of ochratoxigenic fungi and OTA production in substrate. LEE and MAGAN (1999) reported that the ability of *A. ochraceus* to colonise maize and produce mycotoxins is primarily determined by its competitive capabilities relative to other species. According to VALERO *et al.* (2006), the reduction of OTA amount may be due to: (i) limitation of the growth of OTA-producing fungus, (ii) antagonistic fungi consumption of specific nutrients that are necessary for OTA synthesis, (iii) OTA degradation by other fungi.

Additionally, plant substances have been proven to prevent fungal growth and OTA production. For example, essential oils of thyme and anise (500 ppm), cinnamon (1000 ppm) and spearmint (2000 ppm) inhibited *A. ochraceus* growth. 1% oils of thyme and anise and 2% oil of cinnamon completely inhibited OTA production in wheat (SOLIMAN & BADEAA 2002). Fungicidal activity of thyme essential oil against *A. ochraceus* and *P. verrucosum* was reported by NGUEFACK *et al.* (2009). Cinnamon essential oil was highly effective against *A. niger* (SINGH *et al.* 2007). REDDY *et al.* (2007) found that garlic bulb extract completely inhibited the growth of *A. ochraceus*.

Ochratoxin A detoxification and inhibition of ochratoxin A absorption from gastrointestinal tract

Detoxification of mycotoxins is achieved by the removal or elimination of the contaminated commodities or by inactivation of mycotoxins present in these commodities (KABAK *et al.* 2006). In recent years, there has been increasing interest in the use of microorganisms to detoxify OTA. ÖZPINAR *et al.* (1999) reported that the rumen microorga-

nisms hydrolyse OTA into a less toxic metabolite, ochratoxin α . Microorganisms responsible for this transformation were mainly protozoa (XIAO *et al.* 1991). Also *Bacillus licheniformis* isolated from soybean hydrolysed OTA and removed 92.5% of OTA (PETCHKONGKAEW *et al.* 2008). Some *Lactobacillus* spp. and *Bifidobacterium* spp. degraded OTA. The most effective *Lb. acidophilus* VM 20 caused a decrease of OTA by 95% or more. Also two *Bifidobacterium longum* strains (LA 02, VM 14) were highly effective and caused a decrease of OTA by approximately 50%. OTA was degraded more efficiently by viable bacteria. The influence of the pretreatment of OTA with *Lactobacillus* strain on micronucleus induction and cell division rates in human derived hepatoma cells was investigated. *Lactobacillus* strain caused a reduction in OTA-induced micronucleus formation. The inhibition of cell division by OTA was reduced by *Lactobacillus* strain (FUCHS *et al.* 2008). SKRINJAR *et al.* (2002) found the reduction of OTA amount by *Lb. acidophilus* in yoghurt.

OTA degradation was also due to yeasts and filamentous fungi. For example, *Phaffia rhodozyma* degraded more than 90% of OTA (PÉTERI *et al.* 2007). Ochratoxin α (OT α) was detected in malt yeast medium. OTA was degraded by yeast *Aureobasidium pullulans* in grape must (FELICE *et al.* 2008) and yeast *Botrytis cinerea* in grape-like synthetic medium (VALERO *et al.* 2008). VARGA *et al.* (2000) tested the abilities to degrade OTA of seventy *Aspergillus* isolates representing six sections of the *Aspergillus* genus. Atoxigenic strain *A. niger* CBS 120.49 completely converted OTA to OT α within five days in a yeast extract medium. OT α was further degraded to an unknown compound within seven days. Also ABRUNHOSA *et al.* (2002) and BEJAOU *et al.* (2006) reported that *Aspergillus* spp. isolated from grapes and belonging to *Aspergillus* section *Nigri* degraded OTA. Other filamentous fungus *Rhizopus stolonifer* variety *stolonifer* TJM 8A8 could degrade 96.5% of OTA in wheat while *A. niger* CBS 120.49 was not able to degrade OTA in wheat (VARGA *et al.* 2005). Only 23% of the initial OTA amount were detected after the incubation of barley with *Pleurotus ostreatus* (ENGELHARDT 2002). It has been suggested that a carboxypeptidase enzyme is responsible for the conversion of OTA to OT α (BEJAOU *et al.* 2006; PÉTERI *et al.* 2007).

Microorganisms are also able to remove OTA by adsorption onto the cell surface components.

Biosorption is generally based on a set of chemical and physical mechanisms leading to the immobilisation of a solute component on the microbial cell wall components and is not dependent on metabolism (RINGOT *et al.* 2007). PIOTROWSKA and ZAKOWSKA (2005) found the adsorption of OTA onto some *Lactobacillus* and *Lactococcus* strains. All strains caused the reduction of OTA amount. The highest decrease, exceeding 50% of the original amount, was caused by *Lb. acidophilus* CH-5, *Lb. rhamnosus* GG, *Lb. plantarum* BS, *Lb. brevis*, and *Lb. sanfranciscensis*. According to TURBIC *et al.* (2002), viable and nonviable cells of *Lb. rhamnosus* reduced OTA amount.

Also *Saccharomyces* strains were able to adsorb OTA. The percentages of OTA removal were between 11% and 45% in yeast pepton medium, depending on the strain used. OTA adsorption by heat-treated *S. cerevisiae* LALVIN Rhône 2056 was rapid and total within 5 min and took up to 2 h in grape juice (BEJAOU *et al.* 2004). *S. sensu stricto* adsorbed OTA in grape must (CARIDI *et al.* 2006). According to CECCHINI *et al.* (2006), the yeast strains responsible for alcohol fermentation influenced the concentration of OTA in wine. The percentages of OTA removal were 46.83–52.16% and 53.21–70.13% during the fermentation of white must and red must, respectively. No degradation products were found. RINGOT *et al.* (2007) reported the adsorption of OTA onto yeast industry by-products: a vinasse containing yeast cell walls, a purified yeast beta glucan, and a yeast cell wall fraction. The cell wall fraction was able to bind 95–100% of OTA. The adsorption of OTA onto yeast cells and yeast cell walls was also reported by HUWING *et al.* (2001) and NUNEZ *et al.* (2008).

According to RINGOT *et al.* (2007), yeast biomass may be regarded as a good source of adsorbent material, due to the presence in the cell wall of some specific macromolecules, such as mannoproteins and beta glucans. The differences in the binding activity of wine yeasts towards OTA may be explained by the structural variability of the mannoproteins of each wine yeast (CARIDI *et al.* 2006). It is possible to reduce greatly the OTA content of grape must during winemaking by using expressly selected wine yeasts. Additionally, mannoproteins may well induce chemical and sensorial benefits, thus improving the wine quality (CARIDI 2006).

BEJAOU *et al.* (2005) found the removal of OTA by conidia of *A. niger*, *A. carbonarius*, and

A. japonicus in grape juice. OTA removal was a two-stage phenomenon. In the first stage, viable and nonviable conidia were able to remove OTA. No degradation products having been detected, the adsorption of OTA on conidia was supposed. In the second stage, OTA was degraded by live conidia only. *A. carbonarius* detoxified grape juice most effectively. However, this species often produces OTA. *A. niger* was also effective. In a study of LEONG *et al.* (2007), none of the tested isolates of *A. niger* produced OTA. SCHUSTER *et al.* (2002) stated that only 3–10% of *A. niger* strains examined for OTA production were toxinogenic under favourable conditions. *A. niger* is generally regarded as a safe microorganism and is one of the most important microorganisms used in biotechnology, being utilised for the production of enzymes and citric acid. Many *A. niger* enzymes are considered GRAS (generally recognised as safe) by the United States Food and Drug Administration. *A. niger* could be interesting in further use for the biological elimination of OTA.

The contamination of animal products occurs either as a result of direct fungal contamination or indirectly via contaminated feed (CLARK & SNEDEKER 2006). One of the methods for the prevention of contamination is the addition of adsorbents to feeds that bind mycotoxins in the gastrointestinal tract (KABAK *et al.* 2006). Various types of adsorbents have been used in feeds to assess the ability of these substances to bind OTA (DENLI *et al.* 2008). For example, in broilers, the inhibition of OTA absorption from the digestive tract into blood was due to the addition of yeast *S. boulardii* (AGAWANE & LONKAR 2004) or *Trichosporon mycotoxinivorans* (HANIF *et al.* 2008) to feeds.

CONCLUSION

Various strategies have been developed to prevent OTA contamination of foods. Some microorganisms have been proven to prevent the growth of ochratoxigenic fungi and OTA production. They could be used as natural control material. Further research is needed to examine the effects of microbial interactions on OTA production *in situ*, for example in vineyards. The impact of the treatment on food quality should also be investigated. Thermal treatment does not completely eliminate OTA, therefore the contaminated food contains, after thermal treatment, OTA remnant. Some microor-

ganisms have been detected to reduce the amount of OTA in cultivating media.

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