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► **To cite this version:**

Alexandre Esteban, M Lourdes Abarca, M Rosa Bragulat, F Javier Cabañes. Effect of pH on ochratoxin A production by *A. niger* aggregate species. *Food Additives and Contaminants*, 2006, 23 (06), pp.616-622. 10.1080/02652030600599124 . hal-00577290

HAL Id: hal-00577290

<https://hal.science/hal-00577290>

Submitted on 17 Mar 2011

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Journal:	<i>Food Additives and Contaminants</i>
Manuscript ID:	TFAC-2005-277.R2
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	10-Jan-2006
Complete List of Authors:	Esteban, Alexandre; Universitat Autònoma de Barcelona, Sanitat i Anatomia Animals Abarca, M Lourdes; Universitat Autònoma de Barcelona, Sanitat i Anatomia Animals Bragulat, M Rosa; Universitat Autònoma de Barcelona, Sanitat i Anatomia Animals Cabañes, F Javier; Universitat Autònoma de Barcelona, Sanitat i Anatomia Animals
Methods/Techniques:	Chromatography - HPLC
Additives/Contaminants:	Ochratoxin A
Food Types:	Coffee, Wine, Dried fruit

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Abstract

The effect of pH (2-10) on growth and ochratoxin A (OTA) production by twelve *Aspergillus niger* aggregate strains was studied in two culture media: Czapek Yeast Autolysate agar (CYA) and Yeast Extract Sucrose agar (YES), during a period of 30 days. The strains were selected to include different sources, different reported abilities to produce OTA and different ITS-5.8S rDNA RFLP patterns. YES was found to be a better culture medium than CYA for OTA production. In this medium, OTA was produced from pH 2 or pH 3 to pH 10 depending on the strain. Our results show the ability of *A. niger* aggregate strains not only to grow but also to produce OTA over a wide pH range. These results will lead to a better understanding of the role of *A. niger* aggregate strains in the OTA contamination of several food commodities.

Keywords: *Aspergillus niger*, coffee, grapes, raisins, ochratoxin A, pH.

Introduction

Among the different mycotoxins which merit special concern for the hazard they represent in food commodities, ochratoxin A (OTA) deserves particular attention. OTA is a nephrotoxic mycotoxin which also possesses carcinogenic, teratogenic, genotoxic and immunosuppressive potential (O'Brien and Dietrich 2005). The International Agency for Research on Cancer (IARC 1993) has classed OTA as a possible human carcinogen (group 2B). The European Commission has established limits for OTA in cereals, dried vine fruits, wine, coffee and infant foods. These limits will be reviewed in the short term and maximum limits for more products such as cocoa, beer, meat products, spices or liquorice will be included (Commission Regulation 2002, 2004, 2005).

OTA was until recently believed to be produced only by *Aspergillus ochraceus* and by *Penicillium verrucosum*. The description of OTA production by two species belonging to *Aspergillus* section *Nigri*, *Aspergillus niger* var. *niger* (Abarca et al. 1994) and *Aspergillus carbonarius* (Horie 1995), may contribute to explain the widespread OTA contamination reported in some food and feed products. This distribution was unclear only taking into account the incidence and natural occurrence of the previously described OTA-producing species.

Within *Aspergillus* section *Nigri* it is known that isolates included in the *A. niger* aggregate can produce a range of different toxic metabolites (Frisvad and Thrane 2000) but it is the ability of some of their members to produce OTA what deserves special concern. It is considered that in substrates such as grapes, raisins and wine

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2
3 the source of OTA contamination detected is due to *A. carbonarius* and some species
4 included in the *A. niger* aggregate (Abarca et al. 2004). Furthermore, it is worth noting
5 that *A. niger* products hold the GRAS (Generally Regarded as Safe) status from the
6 United States, Food and Drug Administration and are used as a source of enzymes
7 and organic acids for food processing, despite the reported ability to produce OTA
8 (Bigelis and Lausure 1987).
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20 The taxa included in the *A. niger* aggregate are extremely difficult to distinguish one
21 from each other by morphological means. In an attempt to solve the classification of
22 these fungi, several molecular techniques have been carried out in order to clarify the
23 taxonomy and divide these taxa into two or more species (Abarca et al. 2004). On the
24 basis of their ITS-5.8S rDNA restriction fragment length polymorphism (RFLP), the
25 strains were grouped in two patterns, designated N and T (Accensi et al. 1999). To
26 date the OTA-producing isolates with RFLP pattern known are classified as type N
27 (Accensi et al. 2001; Cabañes et al. 2002; Abarca et al. 2003; Leong et al. 2004;
28 Esteban et al. 2004).
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44 Little is known about the environmental factors which can affect the OTA production
45 by species of the *A. niger* aggregate. In a previous study on the effect of temperature,
46 the *A. niger* aggregate strains showed the highest levels of OTA in Yeast Extract
47 Sucrose (YES) agar at 20-25°C (Esteban et al. 2004).
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56 As little is known about the influence of pH, the aim of this study was to establish the
57 effect of pH on growth and OTA production by different species belonging to the *A.*
58 *niger* aggregate at 25°C over a period of 30 days. The strains were selected to
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3 include different sources, different reported ochratoxigenic ability and different ITS-
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5 5.8S rDNA RFLP patterns.
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10 **Material and methods**

11 *Fungal strains and inocula preparation*

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13 Twelve isolates of the *A. niger* aggregate were used in this study (ten of N RFLP
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15 pattern and two of T pattern). The reported OTA production, source of isolation and
16
17 RFLP pattern is shown in Table I. The strains were grown on malt extract agar (Pitt
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19 and Hocking 1997) for 7 days at 25°C. Conidia suspensions of each isolate were
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21 prepared in an aqueous solution of 0.05% Tween 80. After filtering through sterile
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23 cheesecloth they were adjusted to approximately 10^6 - 10^7 conidia per ml as
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25 determined by a counting chamber.
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34 *Culture conditions*

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36 OTA production was determined on two culture media: yeast extract sucrose (YES)
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38 agar and Czapek yeast extract (CYA) agar (Pitt and Hocking 1997). The pH of the
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40 culture media was varied from 2 to 10. Adjustment of pH was performed before
41
42 autoclaving by adding HCl (1 and 10 N) and NaOH (1 N). The pH value was
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44 measured using a pHmeter GLP21 (Crison Strumenti S.p.A., Carpi, Italy). The
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46 sterilization of the media adjusted at pH 2, 3 and 4 was carried out by autoclaving the
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48 agar separately from the rest of components in order to avoid solidification problems.
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50 The pH of each media was checked after sterilization. Each plate was point
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52 inoculated with 1 μ l of the adjusted suspension. Plates were incubated at 25°C. Each
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54 assay was performed in duplicate.
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OTA production and quantification

OTA production was determined after 5, 10, 15, 20 and 30 days of incubation at each pH value following a previously described high-pressure liquid chromatography (HPLC) screening method (Bragulat et al. 2001), being efficient at the range of pH tested. On each sampling occasion, three agar plugs were removed from different points of the colony and extracted with 0.5 ml of methanol. The extracts were filtered and injected into the HPLC. OTA detection and quantification was made by a Waters LCM1 chromatograph with a fluorescence detector Waters 2475 (excitation wavelength: 330 nm/emission wavelength: 460 nm). Twenty μl of each extract were applied. The mobile phase, with a flow rate of 1 ml/min, consisted of the following isocratic program: acetonitrile, 57%; water, 41% and acetic acid, 2% (Bauer and Gareis 1987). The extracts with the same retention time as OTA (around 6.8 min), were considered positive. Confirmation was made through derivatization of OTA in its methyl-ester (Hunt et al. 1980). The detection limit of the extraction procedure and the HPLC technique was 0.02 ng OTA and the quantification limit of HPLC technique with the extraction procedure was 0.01 $\mu\text{g g}^{-1}$ for this mycotoxin.

Data analysis

Data obtained were analyzed statistically by means of the one-way analysis of variance test and Student's test. All statistical analyses were performed using SPSS software (Version 12.0).

Results and discussion

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3 The twelve strains grew in CYA and YES media adjusted from pH 2 to 10. According
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5 to our results, the pH range for growth in *Aspergillus niger* was previously reported to
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7 be from 1.4 to 9.8 (Panasenko 1967).
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12 Six out of the 12 strains tested produced detectable levels of OTA in both media.
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14 Tables II and III show the concentration detected at each pH value and incubation
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16 time in CYA and YES medium respectively. In CYA medium most of the strains
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18 produced OTA at all the pH values tested. The strain CBS 618.78 produced OTA
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20 from pH 4 to 8 and A-943 was a weak OTA-producing strain with detected values
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22 very close to the detection limit of the technique at some pH values. In YES medium,
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24 the strains produced OTA from pH 2 (strains A-942, A-943 and CBS 139.52) or pH 3
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26 (strains A-75 and A-136) to pH 10. However, this range was lower for the strain CBS
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28 618.78 which produced OTA from pH 5 to 8.
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37 Mean OTA concentration produced at each pH value by the six positive strains is
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39 shown in Figure 1. The concentration achieved by all the strains in YES was higher
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41 than in CYA medium except at pH 3 (Figure 1). At this pH value the strains showed a
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43 slight higher OTA production in CYA medium. The total mean OTA concentration
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45 produced in YES medium ($4.42 \mu\text{g g}^{-1}$) was significantly higher (Student's t $P < 0.01$)
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47 than in CYA medium ($1.38 \mu\text{g g}^{-1}$) which supports that YES is better than CYA
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49 medium for OTA production. This result was in accordance with previous studies
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51 which reported the suitability of YES medium to test OTA production by *A. niger*
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53 aggregate strains (Bragulat et al. 2001; Esteban et al. 2004). On the contrary, CYA
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55 medium has been reported to be more suitable than YES medium in *A. carbonarius*
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57 (Bragulat et al. 2001; Esteban et al. 2004; Esteban et al. 2005).
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In both media most of the strains produced OTA after only 5-10 days of incubation and showed different optimal values for OTA production. In CYA medium (Table 2) the strains showed the optimal OTA production within the range pH 3-8. In YES medium (Table 3) the optimal conditions for OTA production were reached within the range pH 5-10 and always after 5-10 days of incubation. In both media, the strain A-943, isolated from grapes, showed the lowest OTA production in comparison with the rest of strains studied.

In agreement with previous studies (Esteban et al. 2004) the OTA content decreased with increasing incubation time. It has been suggested that this may be due to the fact that the strains remove and assimilate the phenylalanine moiety from the OTA molecule as other nitrogen sources become exhausted (Téren et al. 1996).

It is not known yet why the percentage of OTA-producing strains in the *A. niger* aggregate is low (Abarca et al. 2004), nor if this is due to a genetic based feature. To clarify this fact it is necessary to study the effect of different environmental conditions on OTA production. In this study, the six strains initially considered as OTA negative did not produce the mycotoxin at any of the pH values tested for the period of 30 days. In a previous study, these six strains did not produce OTA at any of the temperatures tested (Esteban et al. 2004). The strains with the RFLP pattern of type T did not produce OTA in the range of pH values assayed. To date OTA production has never been demonstrated in type T strains (Accensi et al. 2001; Cabañes et al. 2002; Abarca et al. 2003; Leong et al. 2004; Esteban et al. 2004).

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3 There is no previous published work regarding the effect of pH on OTA production by
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5 *A. niger* aggregate strains. Nevertheless, in the case of *A. carbonarius* it has been
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7 recently reported that the strains are able to produce OTA in a broad pH range (pH 2
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9 to 10) (Esteban et al. 2005). The results obtained here with the *A. niger* aggregate
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11 strains state that they are also able to grow at a wide range of pH values (pH 2-10).
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13 This would explain why *A. niger* is so commonly isolated from a great variety of food
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15 commodities and is considered the most common *Aspergillus* species responsible for
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17 the post-harvest decay of fresh fruit (Pitt and Hocking 1997). The black aspergilli
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19 belonging to the *A. niger* aggregate are the predominant *Aspergillus* species isolated
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21 from grapes. They are isolated along the different developmental stages of berries
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23 although it is at harvesting when the highest levels are observed (Battilani et al. 2003;
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25 Serra et al. 2003; Bau et al. 2005). The black spores provide protection from sunlight
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27 and UV light, providing a competitive advantage in such habitats (Pitt and Hocking
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29 1997). It is worth mentioning that the pH of grapes increases during ripening,
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31 achieving a final pH range of 3-4 (Splittstoesser 1987; FDA 2003). *Aspergillus niger*
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33 has been also reported to be the predominant fungi in dried vine fruits (Heenan et al.
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35 1998; Abarca et al. 2003) which show pH ranges of 3.8 to 4.1 (FDA 2003).
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37 *Aspergillus niger* aggregate strains have been also isolated from different stages of
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39 coffee beans, before and after harvesting (Taniwaki et al. 2003). The pH of
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41 unfermented coffee beans can range from 5.4 to 6.4 (Thompson et al. 2001) and
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43 therefore, the time before the arrival of the cherries at the factory may represent a
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45 high risk for OTA production (Bucheli and Taniwaki 2002). The remarked conditions
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47 could support growth and also the production of OTA on these substrates.
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3 It is worth noting that our results show *A. niger* aggregate strains can grow and
4 produce OTA in a wide pH range. Moreover significant amounts of OTA can be
5 produced in only 5 days. This would lead to confirm their contribution in the OTA
6 contamination of substrates, either in the field or after harvesting, due to the presence
7 of the proper environmental conditions for the production of the mycotoxin.
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17 Further studies on the effect of environmental factors on growth and OTA production
18 are necessary. A better knowledge of the ecology of these species will provide us
19 with management strategies to reduce the OTA contamination of the substrates
20 where they are usually isolated.
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29 **Acknowledgements**

30 This research was supported by the Ministerio de Educación y Ciencia of the Spanish
31 Government (AGL2004-07549-C05-03) and by the grant 2002 SGR 00079 from the
32 DURSI, Generalitat de Catalunya.
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Table I. *Aspergillus niger* aggregate strains used in this study.

Species	Strain (source) ^a	OTA production reported (Ref.)	RFLP pattern (Ref.)
<i>A. niger</i> var. <i>niger</i>	A-75 (feedstuffs, CCFVB)	+ (Abarca et al. 1994; Esteban et al. 2004)	N (Accensi et al. 2001)
<i>A. niger</i> var. <i>niger</i>	A-136 (soy, CCFVB)	+ (Abarca et al. 1994; Esteban et al. 2004)	N (Accensi et al. 2001)
<i>A. niger</i> var. <i>niger</i>	A-942 (raisins, CCFVB)	+ (Abarca et al. 2003; Esteban et al. 2004)	N (Abarca et al. 2003)
<i>A. niger</i> var. <i>niger</i>	A-943 (grapes, CCFVB)	+ (Esteban et al. 2004)	N (Esteban et al. 2004)
<i>A. awamori</i>	CBS 139.52 (kuro-koji)	+ (Ono et al. 1995; Esteban et al. 2004)	N (Accensi et al. 2001)
<i>A. foetidus</i>	CBS 618.78 (unknown)	+ (Téren et al. 1996; Esteban et al. 2004)	N (Accensi et al. 2001)
<i>A. niger</i>	CECT 2088 (unknown)	- (Varga et al. 2000; Esteban et al. 2004)	N (Esteban et al. 2004)
<i>A. niger</i>	CBS 554.65 (tannin-gallic acid fermentation)	- (Accensi et al. 2001; Esteban et al. 2004)	N (Accensi et al. 2001)
<i>A. niger</i> var. <i>niger</i>	A-946 (coffee, CCFVB)	- (Esteban et al. 2004)	N (Esteban et al. 2004)
<i>A. niger</i>	CBS 121.55 (otomycosis)	- (Esteban et al. 2004)	N (Esteban et al. 2004)
<i>A. tubingensis</i>	CBS 134.48 (unknown)	- (Accensi et al. 2001; Esteban et al. 2004)	T (Accensi et al. 2001)
<i>A. niger</i> var. <i>niger</i>	A-947 (grapes, CCFVB)	- (Cabañes et al. 2002; Esteban et al. 2004)	T (Cabañes et al. 2002)

^a Abbreviations: CCFVB, Culture Collection of Veterinary Faculty of Barcelona, Bellaterra (Barcelona), Spain; CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CECT, Colección Española de Cultivos Tipo, Valencia, Spain.

Table II. OTA concentration (mean and mean standard error) produced by the six OTA-producing strains of *A. niger* aggregate in CYA medium at 25°C at each pH value tested.

	days	OTA concentration($\mu\text{g/g}$)								
		2	3	4	5	6	7	8	9	10
A-75	5	nd ^a	1.78 \pm 1.02 ^a	1.92 \pm 1.12 ^a	7.82 \pm 0.55 ^b	1.47 \pm 0.27 ^a	0.74 \pm 0.07 ^a	1.33 \pm 0.77 ^a	0.93 \pm 0.42 ^a	0.64 \pm 0.16 ^a
	10	1.70 \pm 0.49 ^a	1.57 \pm 0.66 ^a	1.59 \pm 0.49 ^a	1.19 \pm 0.03 ^a	0.82 \pm 0.13 ^a	0.69 \pm 0.08 ^a	0.79 \pm 0.23 ^a	0.39 \pm 0.03 ^a	0.41 \pm 0.06 ^a
	15	0.30 \pm 0.06 ^a	3.09 \pm 0.89 ^b	1.38 \pm 0.08 ^a	1.10 \pm 0.31 ^a	0.89 \pm 0.01 ^a	0.81 \pm 0.01 ^a	0.89 \pm 0.06 ^a	0.35 \pm 0.05 ^a	0.21 \pm 0 ^a
	20	0.57 \pm 0 ^a	1.09 \pm 1.01 ^a	1.44 \pm 0.07 ^a	0.41 \pm 0.06 ^a	0.92 \pm 0.02 ^a	0.62 \pm 0.27 ^a	1.00 \pm 0.30 ^a	0.32 \pm 0.04 ^a	0.31 \pm 0.01 ^a
	30	0.18 \pm 0.05 ^a	1.22 \pm 1.20 ^a	1.55 \pm 0.04 ^a	0.95 \pm 0.60 ^a	1.21 \pm 0.91 ^a	0.61 \pm 0.13 ^a	0.87 \pm 0.13 ^a	0.45 \pm 0.08 ^a	0.21 \pm 0.06 ^a
A-136	5	0.03 \pm 0.04 ^a	4.00 \pm 1.13 ^b	2.79 \pm 0.35 ^{ab}	9.31 \pm 2.12 ^c	2.44 \pm 0.47 ^{ab}	1.92 \pm 0.82 ^{ab}	1.54 \pm 0.20 ^{ab}	1.50 \pm 0.13 ^{ab}	1.67 \pm 0.12 ^{ab}
	10	3.68 \pm 0.71 ^a	2.93 \pm 0.20 ^a	1.98 \pm 0.03 ^b	2.01 \pm 0.57 ^b	0.77 \pm 0.23 ^c	1.17 \pm 0.03 ^{bc}	0.90 \pm 0.21 ^c	0.43 \pm 0.19 ^c	0.58 \pm 0.06 ^c
	15	1.14 \pm 0.28 ^a	4.16 \pm 0.54 ^b	2.00 \pm 0.05 ^c	1.83 \pm 0.28 ^c	1.06 \pm 0.12 ^a	0.67 \pm 0 ^a	0.69 \pm 0.27 ^a	0.54 \pm 0.26 ^a	0.51 \pm 0.11 ^a
	20	1.50 \pm 1.08 ^{ab}	2.86 \pm 0.19 ^c	1.98 \pm 0.09 ^{bc}	0.71 \pm 0.06 ^{ab}	1.09 \pm 0.01 ^{ab}	1.02 \pm 0.01 ^{ab}	0.82 \pm 0.25 ^{ab}	0.50 \pm 0.27 ^a	0.46 \pm 0.17 ^a
	30	1.24 \pm 0.71 ^{ab}	2.80 \pm 0.05 ^c	1.80 \pm 0.20 ^b	1.67 \pm 0.31 ^{ab}	1.41 \pm 0.78 ^{ab}	0.81 \pm 0.18 ^{ab}	0.59 \pm 0.13 ^{ab}	0.33 \pm 0.09 ^a	0.30 \pm 0.06 ^a
A-942	5	0.42 \pm 0.18 ^a	7.24 \pm 1.66 ^b	4.23 \pm 0.43 ^c	3.86 \pm 2.28 ^{ac}	1.21 \pm 0.04 ^{ac}	0.88 \pm 0.09 ^{ac}	1.32 \pm 0.34 ^{ac}	0.99 \pm 0.31 ^{ac}	0.44 \pm 0.14 ^a
	10	3.51 \pm 1.84 ^{ab}	4.90 \pm 0.66 ^b	2.91 \pm 0.64 ^{ac}	1.04 \pm 0.02 ^c	0.86 \pm 0.05 ^c	1.00 \pm 0.17 ^c	1.10 \pm 0.37 ^c	0.84 \pm 0.11 ^c	0.52 \pm 0.10 ^c
	15	0.22 \pm 0.01 ^a	8.53 \pm 0.29 ^b	1.92 \pm 0.21 ^c	0.89 \pm 0.06 ^{de}	1.06 \pm 0.13 ^e	0.92 \pm 0.36 ^{de}	1.07 \pm 0.07 ^e	0.91 \pm 0.15 ^{de}	0.37 \pm 0.04 ^{ad}
	20	0.16 \pm 0.01 ^a	7.00 \pm 0.38 ^b	2.62 \pm 0.61 ^c	1.28 \pm 0.21 ^d	1.07 \pm 0.01 ^{de}	0.87 \pm 0.07 ^{adc}	1.22 \pm 0.20 ^d	0.65 \pm 0.04 ^{adc}	0.31 \pm 0.06 ^{ac}
	30	0.36 \pm 0.01 ^{ab}	4.59 \pm 0.31 ^c	1.37 \pm 0.10 ^d	0.49 \pm 0.06 ^{ab}	0.76 \pm 0.21 ^b	0.81 \pm 0.11 ^b	1.28 \pm 0.05 ^d	0.52 \pm 0.16 ^{ab}	0.25 \pm 0.04 ^a
A-943	5	nd ^a	0.14 \pm 0.01 ^b	nd ^a	nd ^a	0.05 \pm 0 ^c	nd ^a	nd ^a	nd ^a	nd ^a
	10	0.10 \pm 0.01 ^a	0.26 \pm 0.06 ^b	nd ^c	nd ^c	0.03 \pm 0.04 ^c	nd ^c	nd ^c	nd ^c	nd ^c
	15	0.19 \pm 0.04 ^a	0.22 \pm 0 ^a	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	0.09 \pm 0.05 ^c	nd ^b
	20	nd ^a	0.07 \pm 0.09 ^a	nd ^a	nd ^a	0.03 \pm 0.04 ^a	nd ^a	0.06 \pm 0.01 ^a	0.08 \pm 0.04 ^a	nd ^a
	30	nd ^a	0.08 \pm 0.11 ^a	nd ^a	nd ^a	0.05 \pm 0.06 ^a	nd ^a	nd ^a	nd ^a	nd ^a
CBS 139.52	5	4.87 \pm 1.99 ^{ab}	10.50 \pm 0.21 ^c	1.21 \pm 1.03 ^a	3.85 \pm 0.62 ^{ab}	2.90 \pm 1.02 ^{ab}	2.06 \pm 0.41 ^{ab}	2.68 \pm 1.47 ^{ab}	5.69 \pm 1.4 ^b	3.81 \pm 0.42 ^{ab}
	10	14.58 \pm 2.02 ^a	7.77 \pm 2.90 ^b	0.65 \pm 0.91 ^c	2.35 \pm 0.90 ^c	1.87 \pm 0.48 ^c	1.87 \pm 0.33 ^c	2.02 \pm 0.52 ^c	3.24 \pm 1.34 ^c	3.06 \pm 0.57 ^c
	15	2.07 \pm 0.54 ^{ab}	8.63 \pm 1.22 ^c	0.42 \pm 0.28 ^a	1.55 \pm 0.22 ^{ab}	2.30 \pm 0.86 ^{ab}	1.95 \pm 0.25 ^{ab}	3.14 \pm 0.53 ^b	2.79 \pm 0.78 ^b	1.95 \pm 0.12 ^{ab}
	20	1.80 \pm 1.37 ^a	15.35 \pm 4.59 ^b	0.63 \pm 0.80 ^a	1.41 \pm 0.43 ^a	2.04 \pm 0.37 ^a	2.02 \pm 0.42 ^a	2.57 \pm 1.22 ^a	3.64 \pm 1.71 ^a	3.78 \pm 1.58 ^a
	30	2.14 \pm 0.59 ^a	7.69 \pm 2.23 ^b	0.39 \pm 0.55 ^a	3.01 \pm 0.37 ^a	1.76 \pm 0.02 ^a	1.56 \pm 0.18 ^a	2.56 \pm 0.50 ^a	4.16 \pm 2.79 ^a	2.82 \pm 0.15 ^a
CBS 618.78	5	nd ^a	nd ^a	nd ^a	0.12 \pm 0.01 ^{ab}	0.17 \pm 0.01 ^b	0.28 \pm 0.12 ^c	0.08 \pm 0.01 ^{ab}	nd ^a	nd ^a
	10	nd ^a	nd ^a	nd ^a	1.04 \pm 0.50 ^a	0.25 \pm 0.09 ^a	0.52 \pm 0.39 ^a	1.84 \pm 2.47 ^a	nd ^a	nd ^a
	15	nd ^a	nd ^a	nd ^a	0.68 \pm 0.15 ^b	0.73 \pm 0.14 ^b	0.78 \pm 0.34 ^b	0.46 \pm 0.35 ^{ab}	nd ^a	nd ^a
	20	nd ^a	nd ^a	nd ^a	0.07 \pm 0.02 ^a	0.49 \pm 0.11 ^b	0.55 \pm 0.03 ^b	0.25 \pm 0.20 ^a	nd ^a	nd ^a
	30	nd ^a	nd ^a	0.05 \pm 0.06 ^a	0.96 \pm 0.56 ^b	0.27 \pm 0.06 ^a	0.31 \pm 0.09 ^a	0.04 \pm 0.06 ^a	nd ^a	nd ^a

^{a,b,c,d,e} Values with the same superscript within each strain and incubation time are not significantly different ($p < 0.05$); nd: not detected (limit of detection 0.01 $\mu\text{g g}^{-1}$).

Table III. OTA concentration (mean and mean standard error) produced by the six OTA-producing strains of *A. carbonarius* in YES medium at 25°C at each pH value tested.

	days	OTA concentration($\mu\text{g/g}$)								
		2	3	4	5	6	7	8	9	10
A-75	5	nd ^a	0.67±0.11 ^a	7.27±0.24 ^{ab}	16.82±0.39 ^b	18.22±3.01 ^b	14.82±7.45 ^b	12.89±2.45 ^b	15.46±3.79 ^b	8.46±0.52 ^{ab}
	10	nd ^a	0.69±0.01 ^a	4.43±0.04 ^{abc}	4.80±1.84 ^{abc}	9.72±1.34 ^c	9.01±1.18 ^c	15.02±4.14 ^d	7.24±0.02 ^{bc}	2.54±0.35 ^{ab}
	15	nd ^a	0.27±0.12 ^a	2.20±0.87 ^{ab}	4.19±1.03 ^{bc}	4.75±0.45 ^{bc}	8.15±2.36 ^d	6.49±0.55 ^{cd}	3.72±1.25 ^{bc}	0.50±0.11 ^a
	20	nd ^a	0.52±0.15 ^a	1.81±0.37 ^{ab}	0.09±0.12 ^a	3.53±0.34 ^b	5.91±0.46 ^c	3.53±1.33 ^b	2.93±0.86 ^b	0.91±0.23 ^a
	30	nd ^a	0.15±0.02 ^a	0.96±0.13 ^{ab}	1.57±0.54 ^{bc}	2.24±0.62 ^c	2.65±0.26 ^c	3.56±0.78 ^d	1.60±0.21 ^{bc}	0.44±0.15 ^{ab}
A-136	5	nd ^a	5.91±0.71 ^a	26.78±4.51 ^{bc}	37.93±1.22 ^d	34.69±1.18 ^{cd}	34.66±2.08 ^{cd}	20.27±0.90 ^{bc}	19.90±1.06 ^{bc}	15.94±6.93 ^e
	10	nd ^a	0.91±0.43 ^{ab}	6.19±0.43 ^{ab}	11.55±3.92 ^{ab}	6.39±0.39 ^{ab}	6.55±0.23 ^{ab}	7.08±0.76 ^{ab}	13.98±9.57 ^b	2.62±0.21 ^{ab}
	15	nd ^a	1.04±0.20 ^a	2.34±0.40 ^a	1.75±1.05 ^a	5.33±1.10 ^a	3.55±2.27 ^a	6.37±5.10 ^a	6.15±0.26 ^a	0.29±0.01 ^a
	20	nd ^a	0.39±0.13 ^a	1.56±0.32 ^{ab}	0.18±0.10 ^a	1.54±0.49 ^{ab}	3.84±1.77 ^b	1.83±0.91 ^{ab}	3.43±0.16 ^b	0.69±0.37 ^a
	30	nd ^a	0.22±0.03 ^{ab}	0.73±0 ^{ab}	0.69±0.27 ^{ab}	2.44±0.71 ^c	1.52±0.35 ^{bc}	1.02±0.28 ^{ab}	2.45±0.69 ^c	0.28±0.06 ^{ab}
A-942	5	nd ^a	2.06±0.47 ^{ab}	10.89±1.87 ^{bcd}	5.38±1.09 ^{abc}	12.90±6.56 ^{cd}	11.66±2.36 ^{bcd}	5.49±0.90 ^{abc}	18.23±1.44 ^d	11.38±3.59 ^{bcd}
	10	3.25±3.00 ^a	0.15±0.03 ^a	2.29±1.39 ^a	1.32±0.38 ^a	2.34±0.08 ^a	2.85±0.73 ^a	1.26±0.07 ^a	4.97±1.54 ^a	5.05±2.62 ^a
	15	1.18±0.44 ^{abc}	0.33±0.18 ^a	0.89±0.38 ^{ab}	0.58±0.02 ^a	1.91±0.42 ^c	1.54±0.13 ^{bc}	2.86±0.05 ^d	0.39±0.02 ^a	1.70±0.19 ^{bc}
	20	1.48±1.57 ^a	nd ^a	0.61±0.04 ^a	0.35±0.18 ^a	1.29±0.02 ^a	1.19±0.50 ^a	1.00±0.18 ^a	1.13±0.39 ^a	0.87±0.19 ^a
	30	0.39±0.26 ^a	0.14±0.01 ^a	0.33±0.02 ^a	0.36±0.11 ^a	1.25±0.54 ^a	0.76±0.08 ^a	1.01±0.04 ^a	0.84±0.20 ^a	1.27±0.64 ^a
A-943	5	nd ^a	nd ^a	0.23±0.09 ^a	0.12±0.01 ^a	0.16±0.05 ^a	0.16±0.01 ^a	nd ^a	0.57±0 ^a	1.39±0.48 ^b
	10	0.11±0.15 ^a	0.18±0.26 ^a	nd ^a	nd ^a	0.15±0.02 ^a	nd ^a	nd ^a	0.20±0.06 ^a	0.13±0 ^a
	15	0.12±0.02 ^a	0.17±0.24 ^a	nd ^a	nd ^a	nd ^a	nd ^a	nd ^a	0.07±0.03 ^a	nd ^a
	20	nd ^a	0.12±0.16 ^a	nd ^a	nd ^a	nd ^a	nd ^a	0.08±0.01 ^a	0.09±0 ^a	nd ^a
	30	nd ^a	0.09±0.12 ^a	nd ^a	nd ^a	nd ^a	nd ^a	nd ^a	nd ^a	nd ^a
CBS 139.52	5	0.03±0.04 ^a	16.82±3.14 ^{bc}	17.37±7.70 ^{bc}	58.00±1.34 ^d	10.25±7.95 ^{abc}	14.74±0.28 ^{bc}	19.65±1.80 ^c	19.03±7.65 ^c	1.88±0.23 ^{ab}
	10	0.12±0.16 ^a	3.58±0.75 ^a	1.11±0.79 ^a	17.12±5.13 ^a	9.54±10.88 ^a	11.97±9.62 ^a	16.96±4.77 ^a	42.27±4.59 ^b	18.74±3.28 ^a
	15	nd ^a	3.43±3.29 ^a	9.60±5.33 ^a	16.38±4.24 ^a	13.69±2.76 ^a	9.05±4.79 ^a	8.92±5.58 ^a	7.87±9.38 ^a	5.73±0.86 ^a
	20	nd ^a	4.61±0.70 ^a	4.14±2.49 ^a	4.88±2.61 ^a	10.01±0.40 ^a	12.56±1.92 ^a	12.43±4.50 ^a	17.98±17.78 ^a	5.88±1.21 ^a
	30	nd ^a	2.70±0.08 ^a	3.29±0.02 ^a	11.66±1.34 ^a	6.46±1.87 ^a	7.66±0.70 ^a	6.03±4.05 ^a	11.59±12.43 ^a	2.32±0.11 ^a
CBS 618.78	5	nd ^a	nd ^a	nd ^a	24.56±15.90 ^b	5.49±7.50 ^a	0.25±0.35 ^a	4.12±2.01 ^a	nd ^a	nd ^a
	10	nd ^a	nd ^a	nd ^a	1.17±0.55 ^a	0.95±0.59 ^a	0.21±0.29 ^a	30.56±18.72 ^b	nd ^a	nd ^a
	15	nd ^a	nd ^a	nd ^a	nd ^a	1.63±2.22 ^a	0.40±0.02 ^a	0.95±0.08 ^a	nd ^a	nd ^a
	20	nd ^a	nd ^a	nd ^a	nd ^a	0.12±0 ^a	0.99±0.059 ^b	1.21±0.04 ^b	nd ^a	nd ^a
	30	nd ^a	nd ^a	nd ^a	nd ^a	0.51±0.06 ^b	0.63±0.03 ^c	0.10±0.07 ^a	nd ^a	nd ^a

^{a,b,c,d,e} Values with the same superscript within each strain and incubation time are not significantly different ($p < 0.05$); nd: not detected (limit of detection 0.01 $\mu\text{g g}^{-1}$).

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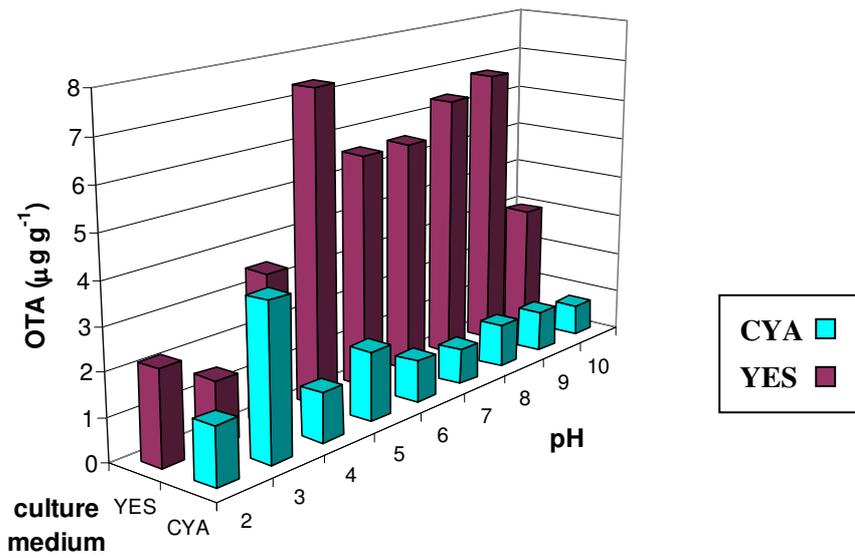


Fig.1. Mean OTA concentration produced by the OTA-producing strains at each pH value and culture media tested.