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Impact of some environmental factors on growth and ochratoxin A production by *Aspergillus niger* and *Aspergillus welwitschiae*

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ABSTRACT

Ochratoxin A (OTA) is a nephrotoxic mycotoxin which may contaminate various foods and feed products worldwide. *Aspergillus niger* is one of the species responsible for OTA contamination in grapes and derived products. This species has recently been split into *A. niger* and *Aspergillus welwitschiae*. Both species can not be distinguished by phenotypic or extrolite profiles and to date there is no ecophysiological information of *A. welwitschiae*.

The aim of this study was to determine the effects of water activity (a_w) (0.90; 0.95 and 0.98–0.99), culture media (Yeast Extract Sucrose Broth (YESB); Synthetic Grape Juice Medium (SGM); White grape juice (WGJ)) and temperature (15 °C, 25 °C and 35 °C) on the growth and OTA production of four strains of *A. niger* and six strains of *A. welwitschiae*. The assay was performed in microtiter plates, determining the absorbance at 530 nm and the concentration of OTA at 1, 2, 4 and 10 days.

No significant differences were observed in absorbance and OTA values between the two species under study. The highest absorbance values were recorded in YESB, followed by SGM and WGJ. Absorbance values increased with increasing a_w and temperature. The highest OTA values were obtained at 0.98–0.99 a_w and the best culture media for OTA production was YESB, followed by WGJ and SGM. The studied strains of *A. niger* produced the highest mean OTA level at 25 °C whereas *A. welwitschiae* strains produced the highest mean OTA concentration at 15 °C, although not differing significantly from concentration produced at 25 °C.

To our knowledge, this is the first report on the impact of some environmental factors on growth and OTA production by *A. welwitschiae*.

1. 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 nephrotoxic, hepatotoxic, neurotoxic, teratogenic and immunotoxic in various animals *in vitro*, with renal toxicity and carcinogenesis being the key adverse effects (Heussner and Bingle, 2015). Cereals, pulses, coffee, beer, wine and grape juice as well as dried vine fruits, nuts, cacao products and spices have been found to be contaminated frequently with OTA (EFSA, 2006). To date the European Union has established maximum OTA levels for different food products (Commission of the European Communities, 2006, 2010, 2012, 2015).

OTA is produced by some *Penicillium* and *Aspergillus* spp. In *Aspergillus* section *Nigri*, different studies have shown that *Aspergillus carbonarius* and *Aspergillus niger* are an important source of OTA in food commodities such as wine, grapes and dried vine fruits (Abarca et al., 2004; Cabañes and Bragulat, 2018; Frisvad et al., 2007; Visconti et al., 2012). The taxonomy of strains in the *Aspergillus* section *Nigri* has been studied and debated for decades. Recently, the taxon *Aspergillus niger sensu stricto* has been split into *A. niger* and *A. welwitschiae* (Hong et al., 2013; Perrone et al., 2011). Both species can not be distinguished by phenotypic or ecological data including extrolite profiles (Perrone et al., 2011).

Temperature and water activity (a_w) are the key environmental factors that influence both the rate of fungal spoilage and the production of mycotoxins (Magan and Aldred, 2007). To date, there is no ecophysiological information of *A. welwitschiae* and there are only some studies on the impact of both environmental factors on the growth and OTA production by *A. niger* isolates on semisynthetic media (Astoreca et al., 2007; Barberis et al., 2009a, 2009b; Lasram et al., 2016), on simulated grape juice medium (Leong et al., 2006; Passamani et al.,

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2014; Selouane et al., 2009; Zouhair et al., 2017) or on natural substrates (Alborch et al., 2011; Astoreca et al., 2009a, 2009b). In most of those studies, only one or two *A. niger* strains were included.

In ecophysiological studies, growth is usually assessed by radial growth measurement of fungal colonies developed at each culture media and incubation conditions. The use of replicate plates for each condition studied and the subsequent OTA extraction and quantification make these studies time-and labor intensive and not suitable when a large number of isolates has to be evaluated. In our laboratory we developed a new screening method to detect growth and OTA production by some *Aspergillus* spp. and *Penicillium* spp. growing in a small quantity of culture media, using microtiter plates (Abarca et al., 2014). The aim of this study was to adapt this method to determine simultaneously the effects of three culture media at three water activity levels and three incubation temperatures on the growth and OTA production by *A. niger* and *A. welwitschiae*.

2. Materials and methods

2.1. Strains and molecular identification

Four *A. niger* strains and six *A. welwitschiae* strains, mainly isolated from grapes and raisins were studied (Table 1). All the strains were previously detected as OTA-producers in our laboratory and had been initially identified as *A. niger*.

All the strains were confirmed for identity by sequencing of the calmodulin gene. Briefly, DNA was extracted and purified from 48 h old cultures in malt extract broth according to the FastDNA Spin kit protocol with the FastPrep FP-24 instrument (MP Biomedicals, Biolink, Barcelona, Spain). The DNA was kept at -20 °C until used as template for PCR amplification. Following the DNA extraction, the calmodulin gene was amplified and sequenced by using the fungal primers CL1/CL2A (O'Donnell et al., 2000). For the phylogenetic analyses, sequences obtained were aligned using Clustal X v2.0.12 (Larkin et al., 2007) and analyzed to generate a phylogenetic tree in Mega 6 software (Tamura et al., 2013). The Neighbor-Joining method based on the Tamura- Nei model (Tamura and Nei, 1993) with 1,000 bootstrap replicates was used. *Aspergillus flavus* CBS 569.65^T was used as outgroup in this analysis.

2.2. Inoculum preparation and verification

Spore concentration was adjusted to around 10^6 conidia/ml. Briefly, the inoculum suspensions were prepared in sterile saline (0.85%) containing 0.05% Tween 80 from 7-day-old cultures on malt extract agar at 25 °C. After heavy particles were allowed to settle for 10–15 min, the upper homogenous suspensions were transferred to sterile tubes and adjusted to 0.6 McFarland turbidity standard (Abarca et al., 2014) by using a photometric method (Densimat, BioMérieux). The inoculum size was confirmed by haemocytometer counting and quantitative colony counts.

Table 1
Strains, identification, source, location and calmodulin sequence type.

Strain number[a]	Identification	Source, location	Calmodulin sequence type (GenBank acc. no.)
A-75	*A. niger*	Feedstuffs, Spain	I (MH614646)
A-136	*A. niger*	Soya beans, Spain	I
A-942	*A. welwitschiae*	Raisins, Spain	III (MH614648)
A-943	*A. welwitschiae*	Grapes, Portugal	IV (MH614649)
A-1609	*A. niger*	Grapes, Spain	I
A-1899	*A. welwitschiae*	Grapes, Italy	V(MH614650)
A-1944	*A. welwitschiae*	Grapes, Portugal	IV
A-3204	*A. welwitschiae*	Popcorn kernel, Spain	IV
A-3694	*A. welwitschiae*	Grapes, Spain	IV
A-3919	*A. niger*	Raisins, Iran	II (MH614647)

[a] Culture collection of the veterinary mycology group, Universitat Autònoma de Barcelona, Spain.

2.3. Culture media and microtiter inoculation

Sterile 96-well flat-bottom microtiter plates were used. Three liquid culture media were assayed: Yeast Extract Sucrose broth (YESB), used as a control, synthetic grape juice medium (SGM) representative of grape composition at mid-veraison (Mitchell et al., 2004), and white grape juice (WGJ).

YESB contained per liter: yeast extract, 20 g; sucrose, 150 g; FeSO4·7 H2O, 0.5 g; pH adjusted to 6.5 (Samson et al., 2000). SGM consisted of D(+) glucose, 70 g; D(−) fructose, 30 g; L(−) tartaric acid, 7 g; L(−) malic acid, 10 g; (NH4)2HPO4, 0.67 g; KH2PO4, 0.67 g; MgSO4·7 H2O, 1.5 g; NaCl, 0.15 g; CaCl2, 0.15 g; CuCl2, 0.0015 g; FeSO4 7 H2O, 0.021 g; ZnSO4·7 H2O, 0.0075 g; (+) Catechin hydrate, 0.05 g; distilled water, 1 l; pH adjusted with 10 M NaOH to pH 4.0–4.2 (Mitchell et al., 2004). WGJ was prepared with 200 ml of commercially sold white grape juice made from ecological grapes and 800 ml of distilled water; pH adjusted at 4.0.

The initial a_w was 0.98 for YESB and SGM media, and 0.99 for WGJ. These initial values were modified to 0.95 a_w and 0.90 a_w by the addition of different amounts of glycerol. Media were autoclaved and the final a_w values were checked with LabMASTER-a_w (Novasina. Switzerland).

For each a_w level, the adjusted fungal suspensions were diluted 1:100 in the culture medium assayed (YESB, SGM, WGJ). In each microplate column, five wells were inoculated with 200 µl of the diluted suspension of each strain and one well, used as a blank, was filled with 200 µl of un-inoculated culture media (YESB, SGM,WGJ).

Growth assessment and OTA production at each a_w level were determined after 1, 2, 4, and 10 days of incubation at three different temperatures (15, 25, and 35 °C). Thus, each strain-$a_w$level-temperature combination was repeated in 4 microplates, one for each reading day. For each sampling occasion and temperature assayed, microtiter plates with the same water activity level were enclosed in sealed polyethylene bags. The entire experiment was repeated twice on different days.

2.4. Growth measurement and OTA extraction procedure

For each culture media, a_w level and temperature assayed, growth was monitored by absorbance measurements at 530 nm using the Multilabel-Reader Mithras LB 940 (Berthold Technologies, Bad Wildbad, Germany) after 1, 2, 4 and 10 days of incubation. The absorbance of the corresponding uninoculated medium, used as blank was subtracted to the absorbance values of the inoculated media. After each reading, microplates were sealed and stored at -80 °C until they were analyzed for OTA content.

OTA production was detected using a previously described high-pressure liquid chromatography (HPLC) screening method developed in our laboratory for fungi growing in microtiter wells (Abarca et al., 2014). On each sampling occasion, one of the five replicate wells inoculated for each strain, culture media, a_w level and incubation temperature, were randomly selected and their content was removed and extracted with 0.5 ml of methanol. The extracts were filtered and injected into the HPLC. The limit of quantification was 0.045 µg/ml for this mycotoxin.

2.5. Statistical analysis

Data obtained from the different conditions tested were statistically analyzed by means of one-way analysis of variance test and Student's test. The Pearson's coefficient (r) was used to quantify the relationship between haemocytometer counts and colony counts of inocula. All

statistical analyses were performed using Minitab 17 statistical software (Minitab Inc., State College, Pennsylvania, USA).

3. Results

3.1. Molecular species identification

Based on the calmodulin sequences, six strains were identified as *A. welwitschiae* and four as *A. niger*. The phylogenetic tree was reconstructed showing that the isolates split into two distinct clades: one grouping with *A. niger* CBS 554.65^T and the other with the sibling species *A. welwitschiae* CBS 139.54^T (Fig. 1). The nucleotide sequences of the calmodulin gene determined in this study have been deposited in the GenBank and their accession numbers are given in Table 1. Sequence analysis revealed the existence of 2 sequence types in *A. niger* and 3 sequence types in *A. welwitschiae*. Sequence positions and differences of *A. welwitschiae* compared to *A. niger* JX500080 in calmodulin gene were: 146 (T), 169 (C), 190–191 (CT), 197–198 (TT), 221 (−), and 505 (T) (Fig. S1).

3.2. Inoculum standardization

Mean colony counts of *A. niger* and *A. welwitschiae* suspensions adjusted to 0.6 McFarland turbidity standard were $0.8 \pm 0.2 \times 10^6$ cfu/ml and $0.7 \pm 0.2 \times 10^6$ cfu/ml respectively. The inocula enumerated with a cell-counting haemocytometer provided suspensions of $1.8 \pm 0.1 \times 10^6$ conidia/ml for *A. niger*, and $1.9 \pm 0.2 \times 10^6$ conidia/ml for *A. welwitschiae*. Pearson's coefficient between both systems of measurements was 0.912.

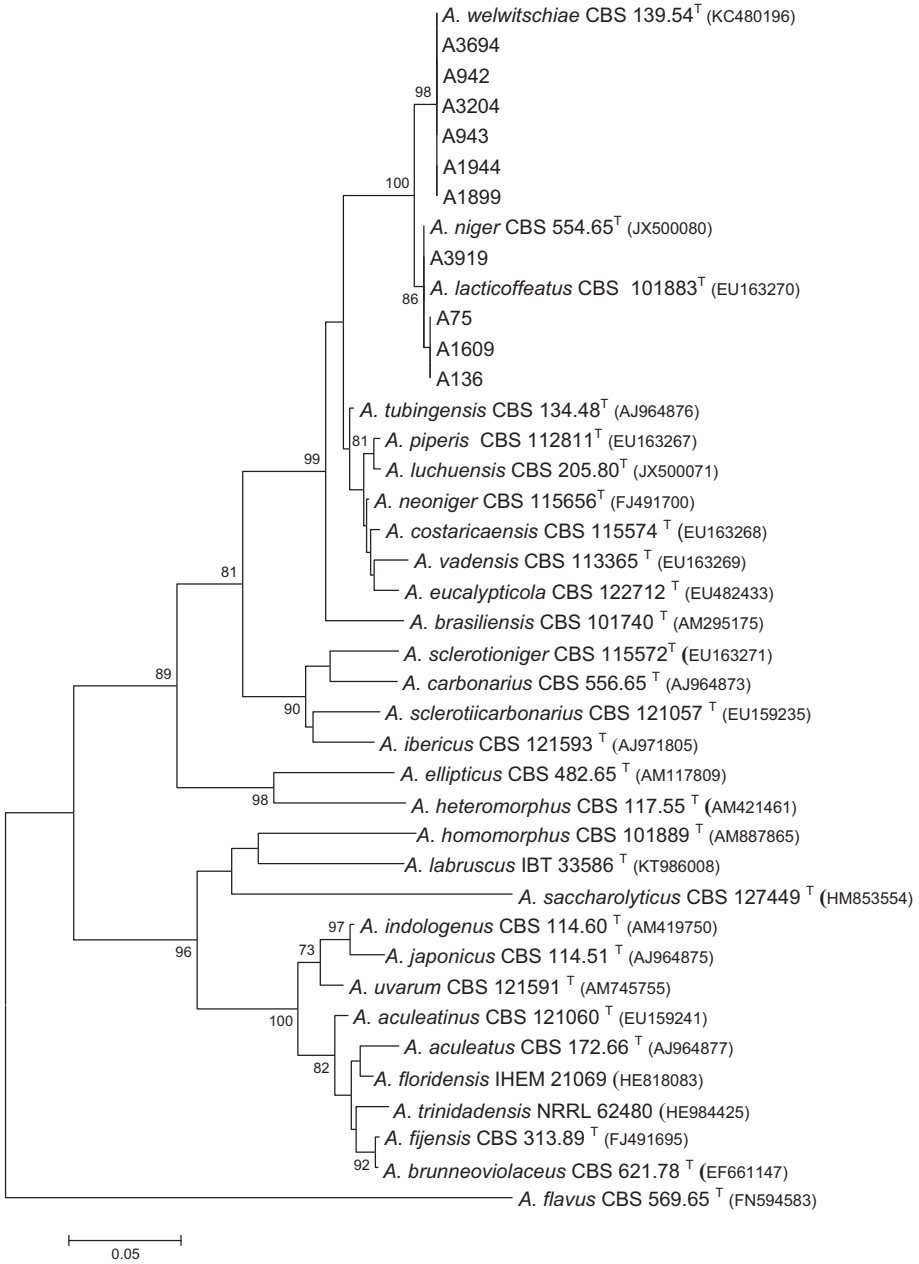

Fig. 1. Phylogenetic tree of *Aspergillus* section *Nigri* inferred from Neighbor-Joining analysis of partial calmodulin gene. Bootstrap values > 70% in 1000 replications are shown at nodes. Sequence of *Aspergillus flavus* CBS 569.65^T was selected as outgroup for the tree construction.

Table 2
One-way analysis of variance of Absorbance (ABS) and Ochratoxin A (OTA) values *versus* (*vs.*) each of the variables assayed.

Strains		p value					
		vs. EXP	*vs.* a_w	*vs.* T (°C)	*vs.* culture media	*vs.* spp.	*vs.* strain
All (n = 10)	ABS	0.463	0.000 ($0.99^a > 0.95^b > 0.90^c$)	0.000 ($35^{\circ\,a} > 25^{\circ\,b} > 15^{\circ\,c}$)	0.000 ($YESB^a > SGM^b > WGJ^c$)	0.373	0.974
	OTA	0.422	0.000 ($0.99^a > 0.95^b > 0.90^b$)	0.000 ($25^{\circ\,a} > 15^{\circ\,a,b} > 35^{\circ\,b}$)	0.000 ($YESB^a > WGJ^b > SGM^b$)	0.676	0.300
A. niger (n = 4)	ABS	0.779	0.04 ($0.99^a > 0.95^{a,b} > 0.90^b$)	0.000 ($35^{\circ\,a} > 25^{\circ\,b} > 15^{\circ\,c}$)	0.000 ($YESB^a > SGM^b > WGJ^c$)	–	0.787
	OTA	0.468	0.018 ($0.99^a > 0.95^b > 0.90^b$)	0.011 ($25^{\circ\,a} > 15^{\circ\,b} > 35^{\circ\,b}$)	0.000 ($YESB^a > WGJ^b > SGM^b$)	–	0.634
A. welwitschiae (n = 6)	ABS	0.478	0.000 ($0.99^a > 0.95^b > 0.90^b$)	0.000 ($35^{\circ\,a} > 25^{\circ\,b} > 15^{\circ\,c}$)	0.000 ($YESB^a > SGM^b > WGJ^c$)	–	0.968
	OTA	0.691	0.018 ($0.99^a > 0.95^{a,b} > 0.90^b$)	0.010 ($15^{\circ\,a} > 25^{\circ\,a} > 35^{\circ\,b}$)	0.000 ($YESB^a > WGJ^b > SGM^b$)	–	0.058

[a,b,c] values of variables with the same superscript are not significantly different ($p > 0.05$).
Abbreviations: EXP, experiment; a_w, water activity; T, temperature; spp., species; YESB, Yeast extract sucrose broth; SGM, Synthetic grape juice medium; WGJ, White grape juice.

3.3. Growth measurement

Results of one-way analysis of variance of absorbance values *versus* each of the variables assayed are shown in Table 2. No significant differences were observed in absorbance values neither between the species, nor between the assays, nor between the replicates ($p > 0.05$). Temperature, culture media and water activity significantly affected ($p < 0.01$) growth of all the strains studied. The higher the a_w and temperature, the higher absorbance values. The highest significant absorbance values ($p < 0.001$) were obtained at 35 °C and 0.99 a_w. Regarding culture media, the highest significant absorbance values ($p < 0.001$) were recorded in YESB, followed by SGM and WGJ.

Table 3 shows mean absorbance values of all the studied strains at each condition assayed and incubation time. As no statistically significant differences were observed between species, replicates or experiment, the results are expressed as a mean value. The highest absorbance values were obtained at 35 °C in all culture media and a_w. At this high temperature a statistically significant growth increase was recorded after 2 days (0.98–0.99 a_w and 0.95 a_w) or 4 days (0.90 a_w) of incubation, while at 25 °C this increase was observed after 2–4 days (0.98–0.99 a_w and 0.95 a_w) or 10 days (0.90 a_w) of incubation. At 15 °C, none of the strains grew in any culture media adjusted at 0.90 a_w during the experimental period of this study. At this low temperature, a significant increase in absorbance values was observed at 10 days of incubation at 0.95 a_w and 0.98–0.99 a_w, although a slight increase was observed after four days of incubation in YESB and WGJ. In all culture media, initial growth in the microtiter wells could be visually detected at the naked eye, when absorbance value was > 0.1.

3.4. OTA production

No significant differences were observed in OTA concentration neither between the species, nor between the assays ($p > 0.05$) (Table 2). Temperature, culture media and water activity significantly affected ($p < 0.01$) OTA production of all the strains studied. The highest significant OTA values ($p < 0.001$) were obtained at 0.98–0.99 a_w. The best culture media ($p < 0.001$) for OTA production was YESB, followed by WGJ and SGM, although no statistically significant differences were observed between both last culture media.

In order to see the individual behavior of strains, OTA concentration produced by *A. niger* and *A. welwitschiae* at each condition assayed and incubation time are shown in Tables 4 and 5 respectively. Results are expressed as mean value of both experiments as no statistically significant differences were observed ($p > 0.05$). Interval Plot of OTA mean values in *A. niger* and *A. welwitschiae* strains at each a_w and temperature assayed is shown in Fig. 2. The studied strains of *A. niger* produced the highest mean OTA level at 25 °C whereas *A. welwitschiae* strains produced the highest mean OTA concentration at 15 °C, although not differing significantly from concentration produced at 25 °C. For both species, YESB was in general the most favorable medium for OTA production. In the remaining media, mean OTA levels were higher in WGJ than in SGM, although this difference was not statistically significant. In relation to the a_w, the greatest production of OTA was observed at the highest a_w. None of the strains produced detectable levels of OTA in any culture media adjusted at 0.90 a_w.

In *A. niger* strains, the highest OTA concentration was recorded after 4 days of incubation at 25 °C in YESB-0.98a_w (strains A-75, A-136 and A-1609) and in SGM-0.98a_w (strain A-3919). In three of the four strains studied, OTA production could be detected after only two days in WGJ-0.99 a_w-25 °C and in YESB-0.98 a_w-35 °C.

In *A. welwitschiae* strains, the highest OTA concentration was recorded also after 4 or 10 days of incubation at 25 °C in YESB-0.98a_w (strains A-942, A-943, A-3204 and A-3694). Strains A-1899 and A-1944 instead, achieved their maximum OTA concentration at 15 °C in YESB-

Table 3
Mean absorbance values recorded in both experiments by all the studied strains (*A. niger* and *A. welwitschiae*) at each condition and incubation time tested.

Culture media/days	15 °C			25 °C			35 °C		
	0.98–0.99	0.95	0.90	0.98–0.99	0.95	0.90	0.98–0.99	0.95	0.90
YESB/1	0.0080^a	0.0068^a	0.0113^a	0.0276^a	0.0023^a	0.0044^a	0.0545^a	0.0298^a	0.0111^a
/2	0.0081^a	0.0057^a	0.0041^b	0.3862^b	0.0242^a	0.0066^a	1.6657^b	1.1940^b	0.0289^a
/4	0.0662^b	0.0089^a	0.0040^b	1.7813^c	1.4915^b	0.0310^b	2.1753^c	2.0634^c	1.3995^b
/10	1.7138^c	0.3164^b	0.0054^b	2.4674^d	2.3731^c	1.7550^c	2.8329^d	2.6408^d	2.3439^c
SGM/1	0.0003^a	0.0006^a	0.0228^a	0.0057^a	0.0023^a	0.0134^a	0.0445^a	0.0258^a	0.0155^a
/2	0.0013^a	0.0010^a	0.0103^b	0.0413^a	0.0177^a	0.0111^a	0.6125^b	0.2068^b	0.0251^a
/4	0.0280^a	0.0023^a	0.0110^b	0.8444^b	0.3203^b	0.0217^a	1.1583^c	0.5651^c	0.2336^b
/10	0.6844^b	0.2215^b	0.0084^b	1.2521^c	0.7638^c	0.3105^b	1.3923^d	0.7060^d	0.5238^c
WGJ/1	-0.0007^a	-0.0005^a	0.0074^a	0.0358^a	0.0060^a	0.0051^a	0.1386^a	0.0494^a	0.0202^a
/2	0.0093^b	-0.0005^a	0.0046^a	0.1937^b	0.0492^b	0.0101^a	0.4262^b	0.3041^b	0.0713^b
/4	0.1422^c	0.0252^b	-0.0051^b	0.3291^c	0.2175^c	0.0394^b	0.5508^c	0.3793^c	0.1876^c
/10	0.3365^d	0.2447^c	0.0191^c	0.4302^d	0.3152^d	0.1472^c	0.6890^d	0.5209^d	0.3166^d

[a,b,c,d] In columns, values with the same superscript within each culture medium are not significantly different ($p > 0.05$).

Table 4
OTA concentration in μg/ml produced by *A. niger* strains at each condition assayed and incubation time.

Ref. strain	Days	15 °C		25 °C						35 °C
		0.98–0.99		0.98–0.99			0.95			0.98
		YESB	WGJ	YESB	SGM	WGJ	YESB	SGM	WGJ	YESB
A-75	2	–[a]	–	–	–	0.055	–	–	–	0.16
	4	–	–	4.68	0.057	0.05	0.18	–	0.052	0.14
	10	0.73	0.052	2.59	–	0.068	0.14	–	0.052	–
A-136	2	–	–	–	–	0.1	–	–	–	0.12
	4	–	–	8.83	0.052	0.094	0.15	0.095	0.055	0.091
	10	0.055	0.055	0.82	–	0.11	0.12	–	0.055	–
A-1609	2	–	–	–	–	0.06	–	–	–	0.14
	4	–	–	10.1	–	0.06	0.2	–	0.062	–
	10	0.15	0.052	1.15	–	0.068	0.075	–	0.055	–
A-3919	2	–	–	–	–	–	–	–	–	–
	4	–	–	–	1.65	–	–	–	–	–
	10	0.16	–	0.16	0.61	–	–	–	–	–

[a] –, denotes not detected.

0.98a_w. Quantifiable levels of OTA were detected after only 2 days in WGJ in some conditions.

4. Discussion

For each strain and reading day (1, 2, 4 and 10 days), the total number of conditions studied were 27 (3 culture media at 3 water activities and 3 incubation temperatures). Only few studies have examined the combined effect of temperature and water activity on growth and OTA production by *A. niger* strains. These studies are usually carried out in only one solid culture medium and with a reduced number of strains. In previous studies on YES agar, the *A. niger* strains A-75 and A-136 produced the highest OTA level at 20–25 °C and 0.98–0.99 a_w (Esteban et al., 2004, 2006). So, similar results have been obtained by using microtiter plates.

As no statistically significant differences were observed in absorbance and OTA values between the experiments, our method are suitable to assess the impact of a great number of environmental conditions on growth and OTA production of a large number of isolates.

In spectrophotometric methods inoculum size is a critical variable. In this paper we have used the initial inoculum size (10^6 conidia/ml) and final inoculum concentration in the wells (10^4 conidia/ml) recommended in the *in vitro* antifungal susceptibility testing of filamentous fungi (CLSI, 2008). Using conidial suspensions adjusted to 0.6 Mc Farland units, good correlation between haemocytometer counting of conidia and colony counts were obtained. So, the adjustment of suspensions using the densitometer Densimat (BioMérieux) can be a good alternative to adjust turbidity of suspensions of *A. niger* and *A. welwitschiae* as we have previously recommended for some *Aspergillus* spp. and *Penicillium* spp. (Abarca et al., 2014; Cabañas et al., 2009).

Aspergillus welwitschiae and *A. niger* cannot be separated from each other using either morphological or extrolite data and only molecular approaches can be used reliably to distinguish them (Hong et al., 2013; Perrone et al., 2011; Varga et al., 2011). We found the fixed nucleotide differences between them suggested for their identification by Hong et al. (2013) in the calmodulin sequences.

Our results show that the effect of water activity, culture media and temperature on growth and ochratoxin A production by *A. niger* and *A. welwitschiae* have been very similar and no significant differences between both species were observed neither in absorbance values nor in OTA concentration. As we have previously reported for *A. niger* strains, the range of a_w and temperature conditions for growth was wider than

Table 5
OTA concentration in μg/ml produced by *A. welwitschiae* strains at each condition assayed and incubation time.

Ref. strain	Days	15 °C					25 °C						35 °C
		0.98–0.99			0.95		0.98–0.99			0.95			0.98
		YESB	SGM	WGJ	SGM	WGJ	YESB	SGM	WGJ	YESB	SGM	WGJ	YESB
A-942	2	–[a]	–	–	–	–	–	–	0.11	–	–	0.052	–
	4	–	–	–	–	–	3.37	0.067	0.10	0.21	0.20	0.08	0.6
	10	1.21	0.084	0.052	0.062	0.062	5.94	0.052	0.075	2.20	0.070	0.063	–
A-943	2	–	–	–	–	–	–	–	–	–	–	–	–
	4	–	–	–	–	–	0.19	–	0.052	–	–	–	–
	10	0.067	0.060	0.052	–	–	0.17	–	0.057	–	–	–	–
A-1899	2	–	–	–	–	–	–	–	0.062	–	–	–	–
	4	–	–	–	–	–	1.05	0.11	0.06	0.12	–	0.052	–
	10	5.76	0.12	0.06	–	–	0.68	–	0.06	1.47	–	0.052	–
A-1944	2	–	–	–	–	–	–	–	0.06	–	–	–	–
	4	–	–	–	–	–	2.28	0.078	0.06	0.44	–	0.055	–
	10	7.30	0.08	0.08	0.055	0.052	0.88	–	0.055	1.08	–	–	–
A-3204	2	–	–	–	–	–	–	–	0.055	–	–	–	–
	4	–	–	–	–	–	1.61	0.080	0.06	0.10	–	–	–
	10	0.37	0.07	0.055	–	–	0.25	–	0.052	0.12	–	–	–
A-3694	2	–	–	–	–	–	–	–	0.057	–	–	–	0.08
	4	–	–	–	–	–	1.62	–	0.074	0.072	–	–	–
	10	0.25	–	0.055	–	–	0.51	–	0.055	0.065	–	–	–

[a] –, denotes not detected.

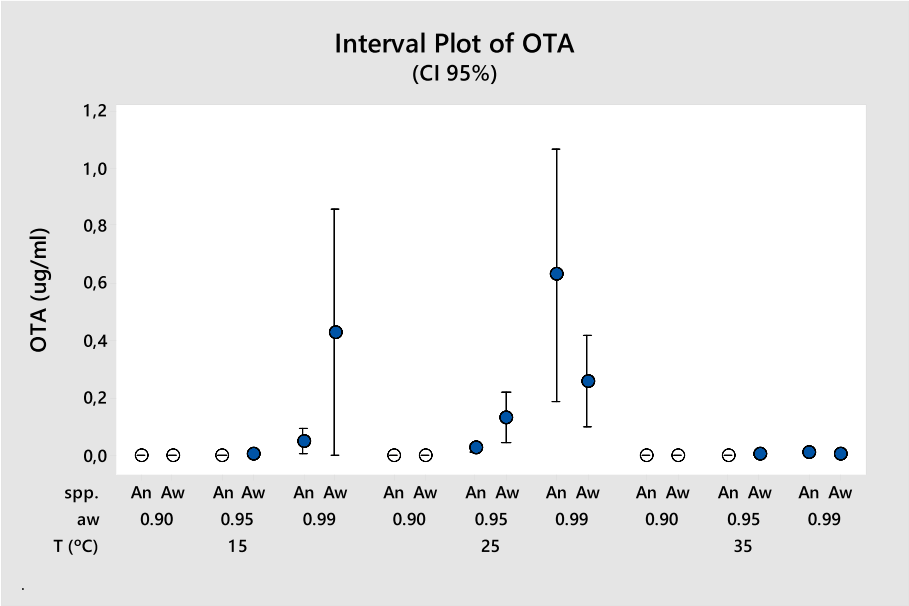

Fig. 2. Interval Plot of mean OTA production/values in *A. niger* (An) and *A. welwitschiae* (Aw) strains at each water activity (a_w) and temperature (T) values assayed.

that for OTA production and in some cases, the amounts of OTA detected decreased when increasing incubation time (Esteban et al., 2004, 2006). Some authors suggested that strains could remove and assimilate the phenylalanine moiety from the OTA molecule, as other nitrogen sources of the culture medium become exhausted (Téren et al., 1996).

Nevertheless, taking into account that *A. niger* and *A. welwitschiae* have been distinguished only recently, the reported ecophysiological data are probably that of both species. Perrone et al. (2011) reported that *A. niger* and *A. welwistchiae* had the same ranges of growth rates in the culture media and temperatures recommended to identify black aspergilli (Samson et al., 2007), but nothing is known about optimal conditions for OTA production. To our knowledge, this is the first report on the impact of some environmental factors on growth and OTA production by *A. welwitschiae*.

In an attempt to find other criteria to distinguish *A. niger* from *A. welwitschiae*, Varga et al. (2011) reported some physiological differences in elastase activities and abilities to utilize 2-deoxy-D-glucose as sole carbon source. In our study we found some differences in the optimum temperature of OTA production: 25 °C for the studied *A. niger* strains and 15–25 °C for *A. welwitschiae*, depending on the strains.

In view of the importance of these species in mycotoxin contamination of various agricultural products, new studies including correctly identified strains are needed. The method used here is simple, technically easy, and appropriate for ecophysiological studies with a large number of isolates and conditions.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2018.11.001.

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