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Short Communication

Phylogenetic characterization and ochratoxin A – Fumonisin profile of black *Aspergillus* isolated from grapes in Argentina

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ABSTRACT

Aspergillus section *Nigri* populations isolated from seven growing regions from Argentina were characterized by sequencing in order to identify species responsible for production of ochratoxin A (OTA) and fumonisins (FB_s). Sequences of genes encoding calmodulin, β-tubulin, the second largest subunit of RNA polymerase II and translation elongation factor 1 alpha were analysed. The phylogenetic analysis showed the presence of six lineages: *A. carbonarius*, *A. tubingensis*, *A. niger*, *A. japonicus*, *A. homomorphus* and *A. foetidus* grouped in four major clusters. The molecular tools used allowed the identification for the first time of *A. homomorphus* from vineyards. OTA production confirmed the importance of *A. carbonarius* as the main ochratoxigenic species isolated and, to a variable degree, of *A. niger* and *A. tubingensis*, which were by far the most commonly occurring species on grapes in Argentina. The only strains able to produce OTA and fumonisins (B_2–B_4) belong to the *A. niger* cluster.

1. Introduction

Aspergillus section *Nigri* are commonly isolated in vineyards and include species, mainly *A. carbonarius* and *A. niger* aggregate, responsible for ochratoxin A (OTA) contamination of grapes and wines (Visconti et al., 2008). The percentage of *A. carbonarius* strains able to produce OTA is higher than that found among members of the *A. niger* aggregate. However, in vineyards the higher incidence of species belonging to the *A. niger* aggregate can also contribute to OTA contamination to a varying degree (Battilani et al., 2006; Leong, 2007; Ponsone et al., 2007). Molecular studies have shown that within the *A. niger* aggregate, *A. tubingensis* and *A. niger* are the most frequent potentially ochratoxigenic species isolated in European vineyards (Bau et al., 2006; Perrone et al., 2006a). Besides OTA production, fumonisin production by *A. niger* strains and the natural occurrence of fumonisin in must have been demonstrated recently (Frisvad et al., 2007; Pel et al., 2007; Logrieco et al., 2010; Mansson et al., 2010). Fumonisins have been associated with leukoencephalomalacia in equine species, pulmonary edema in pigs, and esophageal cancer in humans (FAO, 2001). The European Commission Scientific Committee for Food (2003) has established a provisional maximum tolerable daily intake of 2 μg/kg of body weight per day for FB_1, FB_2, and FB_3, either alone or in combination. Therefore, due to the widespread occurrence of *A. niger* strains on a wide range of food, including grapes, this new potential risk of contamination with fumonisins should be taken in consideration.

The characterization of *Aspergillus* section *Nigri* species has been revised by Samson et al. (2004). Later studies have added new species such as *A. ibericus*, *A. uvarum*, *A. sclerotiicarbonarius* and *A. aculeatinus* (Serra et al., 2006; Noonim et al., 2008; Perrone et al., 2008). The use of a polyphasic approach, which includes DNA sequences, physiological and ecological data as well as extrolite analyses for identification at species level within section *Nigri*, has been also proposed in other current studies (Geiser et al., 2007; Perrone et al., 2007; Samson et al., 2007).

Studies on the grape mycobiota using morphological criteria carried out in Argentina showed that species included within the *A. niger* "aggregate" were the dominant species isolated from the Mendoza grape-growing region (Magnoli et al., 2003; Ponsone et al., 2007). In contrast, *A. carbonarius* and "uniseriate" *Aspergillus* species were mainly isolated from other regions such as La Rioja and San Juan (Chiotta et al., 2009). Using Amplified Fragment Length Polymorphism (AFLP) these species were also identified and *A. tubingensis* was separated from the *A. niger* group (Chiotta et al., 2011). Based on these previous findings, it was considered relevant to confirm the biodiversity of *Aspergillus* section *Nigri* populations isolated from Argentinean vineyards using a multilocus genealogical approach; so calmodulin, β-tubulin, the second largest subunit of RNA polymerase II and translation elongation factor 1 alpha were considered. The ochratoxin A and fumonisin production ability of the strains was evaluated in order to understand the potential toxicological risk in grapes.

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2. Materials and methods

2.1. Fungal samples

Seventy strains randomly selected during the 2008/09 vintage, together with 29 strains previously isolated and identified by AFLP (Chiotta et al., 2011), were included in the present analysis (Table 1). The morphological identification followed the methodology described by Klich (2002).

2.2. Sequences analysis

For mycelium production, a suspension of spores from each fungal strain was grown in Wickerham medium, containing 40 g glucose, 5 g peptone, 3 g yeast extract, 3 g malt extract and water up to 1 L. Mycelia were filtered and lyophilized for total DNA isolation. The fungal DNA extraction was done with the Wizard Magnetic DNA purification Kit (Promega) with some modifications, starting from 10 mg of lyophilized mycelium. The quality of genomic DNA was determined by electrophoresis and the quantification using a Spectophotometer ND-1000 (Nano Drop).

Amplification of part of the β-tubulin (*benA*), calmodulin (*caM*), the second largest subunit of RNA polymerase II (*rpb2*) and translation elongation factor 1α (*ef-1α*) genes, was performed using Bt2a/Bt2b, CL1/CL2a, E1/E2 and A-EF_F/A-EF_R primers, respectively (Glass and Donaldson, 1995; O'Donnell et al., 2000). PCR reactions were carried out on a thermal cycler 9700 (Applied Biosystems, Ontario, Canada) and the reaction conditions were: denaturation at 94 °C for 5 min; 35 cycles of the denaturation at 94 °C for 50 s, annealing at 57 °C, 60 °C, 59 °C and 56.5 °C for 50 s for *caM*, *benA*, *ef-1α* and *rpb2*, respectively, extension at 72 °C for 1 min; final extension at 72 °C for 7 min, followed by cooling at 4 °C to develop the next step. After amplification, the products were purified with the enzymatic mixture EXO/SAP (Exonuclease I, *E. coli*/Shrimp Alkaline Phosphatase).

Sequence analysis using the Big Dye Terminator Cycle Sequencing Ready reaction Kit for both strands was evaluated. The PCR program for the amplification was: one cycle of the denaturation 96 °C for 10 s; 35 cycles of annealing to 50 °C for 5 s, extension at 60 °C for 4 min and then one cycle of held at 4 °C.

All the sequencing reactions were purified by gel filtration through Sephadex G-50 (Amersham Pharmacia Biotech) equilibrated in double-distilled water and analyzed on an ABI PRISM 3730 Genetic Analyzer (Applied Biosystems).

2.3. OTA production by Aspergillus section Nigri strains

Ochratoxin A production was evaluated in CYA medium (K_2HPO_4, 1 g; Czapek concentrate with trace metals, 10 ml; powdered yeast extract, 5 g; sucrose, 30 g; agar, 15 g and water up to 1 L) according to Bragulat et al. (2001). The strains identified as *A. carbonarius* that did not produce detectable OTA levels in CYA medium were further analyzed using different culture media: Yeast Extract-sucrose broth (YES) containing 2% yeast extract, 15% sucrose, and Potato-dextrose broth (PDB) (Oxoid, Basingstoke, UK). After the incubation period, the cultures were filtered and the extraction of OTA was performed by mixing 100 μl of culture with 900 μl of mobile phase. The extract was filtered again through a nylon membrane filter (0.45 Pm) prior to analysis by HPLC.

The HPLC apparatus used for determination of OTA was a Hewlett-Packard 1100 (Hewlett-Packard company, Palo Alto, CA, USA) chromatograph with a loop of 50 μl, equipped with a fluorescence detector ($\lambda_{exc} = 330$ nm and $\lambda_{em} = 460$) and a C18 column (150 × 4.6 mm, 5 μm particle size; Supelcosil LCABZ, Supelco, Bellefonte, PA, USA). The mobile phase was pumped at 1.0 ml/min, and consisted of an isocratic system comprising: acetonitrile, water, acetic acid (99:99:2).

Table 1
Origin of *Aspergillus* section *Nigri* strains included in this study.

Strain	Species	Origin	Vintage
ITEM 11790	*A. tubingensis*	Mendoza – Noreste	2006/07
ITEM 11796	*A. tubingensis*	San Juan – Valle del Tulum	2006/07
ITEM 11795	*A. tubingensis*	Mendoza – Valle de Uco	2007/08
ITEM 11780	*A. tubingensis*	San Juan – Valle del Tulum	2006/07
ITEM 11818	*A. tubingensis*	San Juan – Valle del Tulum	2008/09
ITEM 11821	*A. tubingensis*	San Juan – Valle del Tulum	2008/09
ITEM 11779	*A. tubingensis*	Neuquén – Río Negro	2006/07
ITEM 11782	*A. tubingensis*	Mendoza – Noreste	2007/08
ITEM 11819	*A. tubingensis*	San Juan – Chilecito	2008/09
ITEM 11813	*A. tubingensis*	Neuquén – Río Negro	2008/09
ITEM 11832	*A. tubingensis*	Mendoza – ZARM	2008/09
ITEM 11838	*A. tubingensis*	La Rioja – Chilecito	2008/09
ITEM 11856	*A. tubingensis*	Mendoza – Sur	2008/09
ITEM 11781	*A. tubingensis*	Mendoza – Noreste	2007/08
ITEM 11868	*A. tubingensis*	Mendoza – Valle de Uco	2008/09
ITEM 11785	*A. tubingensis*	Neuquén – Río Negro	2007/08
ITEM 11847	*A. tubingensis*	Mendoza – Noreste	2008/09
ITEM 11857	*A. tubingensis*	Mendoza – Sur	2008/09
ITEM 11775	*A. tubingensis*	La Rioja – Chilecito	2006/07
ITEM 11845	*A. tubingensis*	Mendoza – Noreste	2008/09
ITEM 11792	*A. tubingensis*	Neuquén – Río Negro	2006/07
ITEM 11797	*A. tubingensis*	La Rioja – Chilecito	2007/08
ITEM 11773	*A. tubingensis*	La Rioja – Chilecito	2007/08
ITEM 11860	*A. tubingensis*	Mendoza – Valle de Uco	2008/09
ITEM 11799	*A. tubingensis*	Neuquén – Río Negro	2006/07
ITEM 11787	*A. tubingensis*	Neuquén – Río Negro	2007/08
ITEM 11808	*A. tubingensis*	Neuquén – Río Negro	2008/09
ITEM 11803	*A. tubingensis*	La Rioja – Chilecito	2008/09
ITEM 11823	*A. tubingensis*	San Juan – Chilecito	2008/09
ITEM 11851	*A. tubingensis*	Mendoza – Sur	2008/09
ITEM 11801	*A. tubingensis*	Mendoza – ZARM	2007/08
ITEM 11871	*A. tubingensis*	La Rioja – Chilecito	2008/09
ITEM 11811	*A. tubingensis*	Neuquén – Río Negro	2008/09
ITEM 11853	*A. tubingensis*	Mendoza – Sur	2008/09
ITEM 11863	*A. tubingensis*	Mendoza – Valle de Uco	2008/09
ITEM 11824	*A. tubingensis*	San Juan – Chilecito	2008/09
ITEM 11840	*A. tubingensis*	Mendoza – Noreste	2008/09
ITEM 11866	*A. tubingensis*	Mendoza – Valle de Uco	2008/09
ITEM 11833	*A. tubingensis*	Mendoza – ZARM	2008/09
ITEM 11834	*A. tubingensis*	Neuquén – ZARM	2008/09
ITEM 11806	*A. tubingensis*	Neuquén – Río Negro	2008/09
ITEM 11800	*A. tubingensis*	Mendoza – Valle de Uco	2007/08
ITEM 11783	*A. tubingensis*	Mendoza – Noreste	2007/08
ITEM 11798	*A. tubingensis*	San Juan – Chilecito	2006/07
ITEM 11850	*A. tubingensis*	Mendoza – Sur	2008/09
ITEM 11788	*A. tubingensis*	Mendoza – Noreste	2007/08
ITEM 11861	*A. tubingensis*	Mendoza – Valle de Uco	2008/09
ITEM 11835	*A. tubingensis*	Mendoza – ZARM	2008/09
ITEM 11784	*A. tubingensis*	Neuquén – Río Negro	2007/08
ITEM 11826	*A. tubingensis*	Mendoza – ZARM	2008/09
ITEM 11812	*A. tubingensis*	Neuquén – Río Negro	2008/09
ITEM 11849	*A. tubingensis*	Mendoza – Noreste	2008/09
ITEM 11841	*A. tubingensis*	Mendoza – Noreste	2008/09
ITEM 11794	*A. tubingensis*	Mendoza – ZARM	2007/08
ITEM 11848	*A. tubingensis*	Mendoza – Noreste	2008/09
ITEM 11814	*A. tubingensis*	Neuquén – Río Negro	2008/09
ITEM 11829	*A. tubingensis*	Mendoza – ZARM	2008/09
ITEM 11831	*A. tubingensis*	Mendoza – ZARM	2008/09
ITEM 11807	*A. tubingensis*	Neuquén – Río Negro	2008/09
ITEM 11809	*A. tubingensis*	Neuquén – Río Negro	2008/09
ITEM 11802	*A. tubingensis*	San Juan – Valle del Tulum	2007/08
ITEM 11810	*A. tubingensis*	Neuquén – Río Negro	2008/09
ITEM 11846	*A. tubingensis*	Mendoza – Noreste	2008/09
ITEM 11776	*A. tubingensis*	San Juan – Valle del Tulum	2007/08
ITEM 11852	*A. tubingensis*	Mendoza – Sur	2008/09
ITEM 11791	*A. tubingensis*	La Rioja – Chilecito	2006/07
ITEM 11865	*A. tubingensis*	Mendoza – Valle de Uco	2008/09
ITEM 11777	*A. tubingensis*	Mendoza – Noreste	2007/08
ITEM 11837	*A. tubingensis*	La Rioja – Chilecito	2008/09
ITEM 11793	*A. tubingensis*	Mendoza – Noreste	2007/08
ITEM 11864	*A. tubingensis*	Mendoza – Valle de Uco	2008/09
ITEM 11843	*A. foetidus*	Mendoza – Noreste	2008/09
ITEM 11844	*A. niger*	Mendoza – Noreste	2008/09
ITEM 11867	*A. niger*	Mendoza – Valle de Uco	2008/09
ITEM 11786	*A. niger*	Mendoza – ZARM	2006/07
ITEM 11854	*A. niger*	Mendoza – Sur	2008/09

Table 1 (*continued*)

Strain	Species	Origin	Vintage
ITEM 11830	*A. niger*	Mendoza – ZARM	2008/09
ITEM 11842	*A. niger*	Mendoza – Noreste	2008/09
ITEM 11789	*A. niger*	Mendoza – Noreste	2006/07
ITEM 11872	*A. niger*	La Rioja – Chilecito	2008/09
ITEM 11855	*A. niger*	Mendoza – Sur	2008/09
ITEM 11836	*A. niger*	La Rioja – Chilecito	2008/09
ITEM 11820	*A. niger*	San Juan – Chilecito	2008/09
ITEM 11828	*A. niger*	Mendoza – ZARM	2008/09
ITEM 11774	*A. niger*	San Juan – Valle del Tulum	2006/07
ITEM 11778	*A. niger*	Mendoza – ZARM	2006/07
ITEM 11825	*A. carbonarius*	San Juan – Chilecito	2008/09
ITEM 11804	*A. carbonarius*	La Rioja – Chilecito	2008/09
ITEM 11822	*A. carbonarius*	San Juan – Valle del Tulum	2008/09
ITEM 11816	*A. carbonarius*	San Juan – Valle del Tulum	2008/09
ITEM 11817	*A. carbonarius*	San Juan – Valle del Tulum	2008/09
ITEM 11839	*A. carbonarius*	La Rioja – Chilecito	2008/09
ITEM 11869	*A. carbonarius*	Mendoza – Valle de Uco	2008/09
ITEM 11870	*A. homomorphus*	La Rioja – Chilecito	2008/09
ITEM 11805	*A. japonicus*	La Rioja – Chilecito	2008/09
ITEM 11815	*A. japonicus*	Neuquén – Río Negro	2008/09
ITEM 11858	*A. japonicus*	Mendoza – Sur	2008/09
ITEM 11859	*A. japonicus*	Mendoza – Sur	2008/09
ITEM 11862	*A. japonicus*	Mendoza – Valle de Uco	2008/09

2.4. Fumonisin production by Aspergillus strains

Testing for fumonisin production was performed according to the methodology proposed by Frisvad et al. (2007) with some modifications. Czapek Yeast Autolysate with 20% sucrose (CY20S) agar containing 1 g of K_2HPO_4, 10 ml of Czapek concentrate with trace metals, 5 g of powdered yeast extract, 200 g of sucrose, 15 g of agar and water up to 1 L, was used to inoculate black *Aspergillus* strains. The cultures were incubated for 7 days in darkness at 25 °C. Five agar plugs (0.5–0.7 g) were cut from the colony and extracted with a solution of methanol/water (70:30, v/v). Samples were placed for 50 min in an ultrasonic bath, and filtered (RC 0.2 μm, Phenomenex, CA, USA). The extracts were completely dried using a centrifuge evaporator (Savant Instrument, Farmingdale, NY, USA), dissolved in the extracting solution and resuspended at an equivalent agar concentration of 1 g of agar ml^{-1} for LC/MS/MS analysis.

Fumonisins were determined by HPLC Series 200 (Perkin Elmer, Ontario, Canada) with a Gemini 5u C_{18} 110 A column (150 × 2 mm) (Phenomenex, Torrance, CA, USA). The mobile phase consisted of a gradient system comprising: A, water 0.1% formic acid; and B, CH_3CN/MeOH (80:20, v/v), 0.1% formic acid. The gradient programme was the following: 30% to 100% B (8 min), 100% B (3 min), and 100% to 30% B (3 min), at a constant flow of 0.2 ml min^{-1}. The injection volume was 20 μl. MS/MS analyses of FB_2 and FB_4 were performed on an API 3000 triple quadrupole mass spectrometer (Applied Biosystems) equipped with a turbo-ion-spray source.

3. Results

The nucleotide sequence data obtained by the alignment of the four loci were 536 (*caM*), 430 (*benA*), 694 (*ef-1α*) and 1083 bp (*rpb2*), respectively. Considering the 2743 nucleotides sequenced, 32.2% were variable characters, and 28.1% were parsimony informative in the different strains of black Aspergilli. The lowest number was 101 parsimony informative sites/characters in *EF-1α* region, and the highest was 268 in *rpb2* region.

The evolutionary history of the strains was determined by constructing a phylogenetic tree using the distance method Neighbor-Joining (Saitou and Nei, 1987). The phylogenetic tree obtained with *rpb2* gene showed higher intraspecific variability than in the *benA*, *caM* and *EF-1α* trees. However, sequences of the four genes were analyzed phylogenetically as combined data sets since the trees reflected a similar topology (Fig. 1). Six lineages grouped in four major clusters were identified within the section *Nigri*. Cluster I included the largest number of strains tested ($n = 72$) which were grouped together with *A. tubingensis* CBS 134.48 reference strain with a bootstrap of 97%. The strains ITEM 11773, 11775 and 11776, previously considered as *A. tubingensis* "atypical" by AFLP analysis because of its low homology with the reference strain (Chiotta et al., 2011), belonged in the *A. tubingensis* cluster (Fig. 1). Nine strains included in this cluster were positive for OTA production (Table 2). Cluster II was represented by 14 strains identified as *A. niger* by phylogenetic analysis and grouped with the *A. niger* group species, and in particular with *A. awamori* CBS 557.65 reference strain (bootstrap 99%). Within this group, two strains were OTA-producers while five strains were fumonisin producers (FB_2–FB_4). Production levels of FB_2 and FB_4 ranged from 223 to 17,450 and 69.3 to 6955 ng/ml, respectively (Table 2). Fumonisin production was not detected by strains included in the other clusters. Cluster III grouped seven strains of *A. carbonarius* which were clustered with the *A. carbonarius* CBS 556.65 reference strain with a bootstrap of 100%. The *A. carbonarius* strains that had previously showed no OTA production in CYA were able to produce OTA on PDB and YES media. The last cluster included five strains morphologically identified as uniseriate, and by sequencing as *A. japonicus* (bootstrap 100%). The strains grouped in this cluster did not produce OTA.

Two strains ITEM 11843 and ITEM 11870 were identified as *A. foetidus* and *A. homomorphus* having a bootstrap of 99% and 100% with CBS 564.65 and CBS 101889 reference strains, respectively. *A. homomorphus* strain ITEM 11870 was previously identified morphologically as *A. carbonarius* due to the size of its conidia (7 mm). This is the first time that this rarely occurring species has been found in a vineyard.

4. Discussion

The phylogenetic data corroborate the biodiversity of *Aspergillus* section *Nigri* populations found in a previous study carried out in Argentina using molecular markers (AFLP) (Chiotta et al., 2011). The sequencing results showed that the strains were grouped in four main clusters, *A. carbonarius*, *A. tubingensis*, *A. niger* and *A. japonicus.* Also two biseriate species, *A. homomorphus* and *A. foetidus*, were identified. The presence of *A. foetidus* has been previously observed in grapes in Argentina; although, the identity of this species was never confirmed using molecular methods (Chulze et al., 2006; Ponsone et al., 2007). Only one strain was identified as *A. homomorphus* and its isolation is relevant because it is the first time that it has been found in vineyards and in Argentina. This species is distinguished from the other black Aspergilli by its extrolite profile and it is unique in the production of dehydrocarolic acid (Samson et al., 2007).

The species belonging to the *A. niger* aggregate were separated in two main clusters, *A. tubingensis* and *A. niger.* Three strains considered atypical by AFLP in a previous study were included in the analysis and were grouped together with the *A. tubingensis* reference strain, confirming their identification. These results are consistent with data obtained in European vineyards by AFLP analysis and sequencing of the β-tubulin, calmodulin and rRNA genes (Perrone et al., 2006b). Similar data were obtained using other molecular markers such as rep-PCR (Palencia et al., 2009), ITS-RFLP (Martinez-Culebras and Ramon, 2007) and RFLP (Oliveri et al., 2008), together with the sequencing of genes. The ability to differentiate *A. tubingensis* from *A. niger* through molecular analysis offers the possibility of assessing the ecophysiological characteristics of these species in order to determine if the strains have some particular ecological feature and/or toxin production. The high frequency of isolation of *A. tubingensis* in this study suggests a major adaptation to the grape agro-ecosystem of this species; this result is also in agreement with European data (Perrone et al., 2006a; Martínez-Culebras et al., 2009). All 14 strains grouped in the *A. niger* cluster

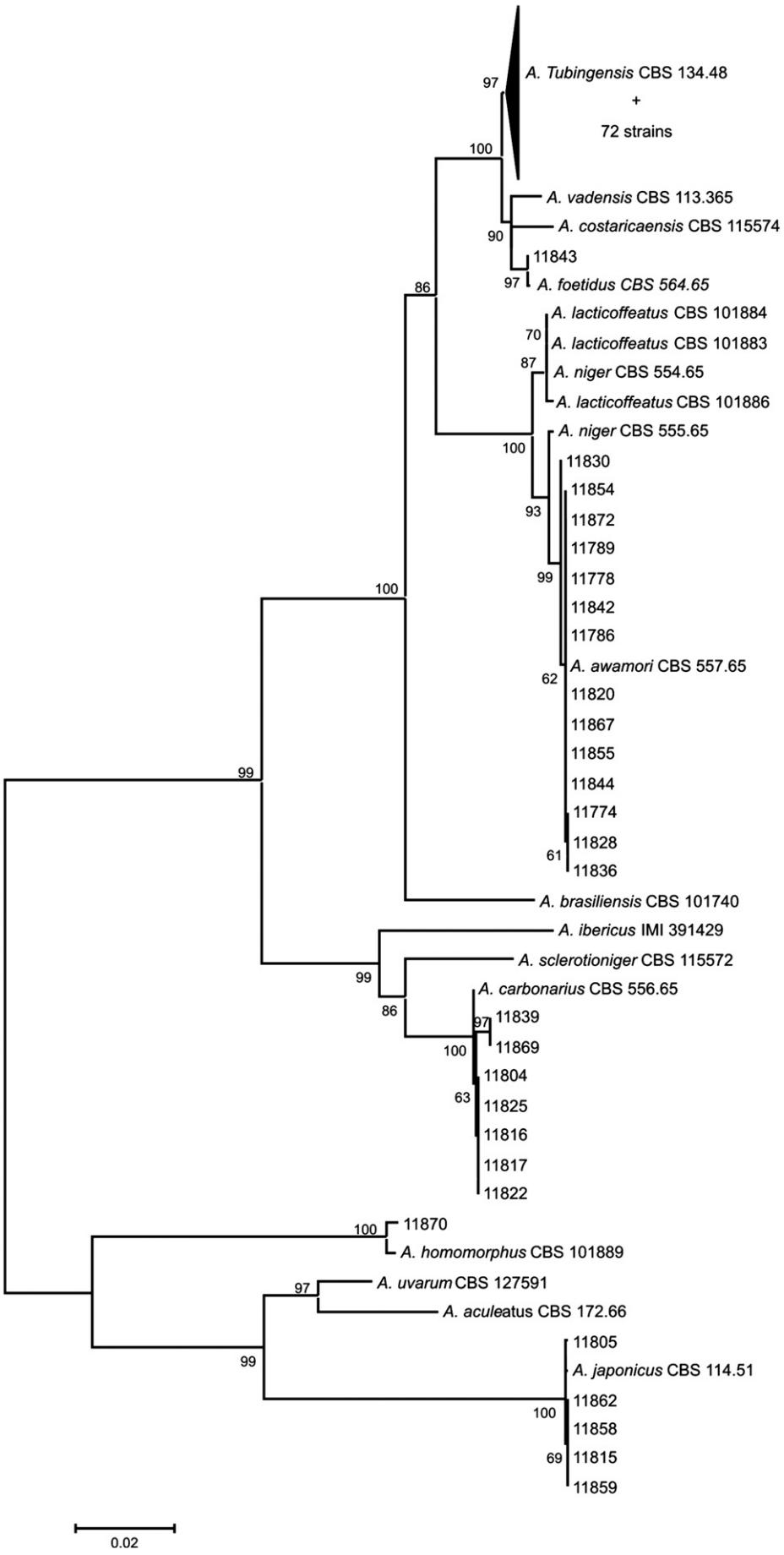
A. Tubingensis CBS 134.48
+
72 strains
A. vadensis CBS 113.365
A. costaricaensis CBS 115574
11843
A. foetidus CBS 564.65
A. lacticoffeatus CBS 101884
A. lacticoffeatus CBS 101883
A. niger CBS 554.65
A. lacticoffeatus CBS 101886
A. niger CBS 555.65
11830
11854
11872
11789
11778
11842
11786
A. awamori CBS 557.65
11820
11867
11855
11844
11774
11828
11836
A. brasiliensis CBS 101740
A. ibericus IMI 391429
A. sclerotioniger CBS 115572
A. carbonarius CBS 556.65
11839
11869
11804
11825
11816
11817
11822
11870
A. homomorphus CBS 101889
A. uvarum CBS 127591
A. aculeatus CBS 172.66
11805
A. japonicus CBS 114.51
11862
11858
11815
11859
97
100
90
97
86
70
87
100
93
99
100
62
99
99
86
97
100
63
100
97
99
100
69
0.02

Table 2
Toxigenic capacity of *Aspergillus* section *Nigri* strains.

	Species	Strain	F B_2/B_4 levels on CY20S (ng/ml)	OTA levels on CYA (ng/g)
Cluster I	*A. tubingensis*	ITEM 11796	–	7.0
	A. tubingensis	ITEM 11868	–	27.5
	A. tubingensis	ITEM 11811	–	28.4
	A. tubingensis	ITEM 11800	–	2.0
	A. tubingensis	ITEM 11861	–	34.2
	A. tubingensis	ITEM 11812	–	27.0
	A. tubingensis	ITEM 11794	–	2.0
	A. tubingensis	ITEM 11802	–	1.2
	A. tubingensis	ITEM 11810	–	26.5
Cluster II	*A. niger*	ITEM 11842	17,450.0–6955.2	–
	A. niger	ITEM 11789	16,600.0–5212.0	–
	A. niger	ITEM 11872	11,350.0–3014.4	–
	A. niger	ITEM 11855	8,060.0–861.1	295.4
	A. niger	ITEM 11820	223.5–69.3	–
	A. niger	ITEM 11774	–	2.0
Cluster III	*A. carbonarius*	ITEM 11825	–	2.4[a]
	A. carbonarius	ITEM 11804	–	515.0
	A. carbonarius	ITEM 11822	–	9.6
	A. carbonarius	ITEM 11816	–	204.3
	A. carbonarius	ITEM 11817	–	1.9[a]
	A. carbonarius	ITEM 11839	–	2.9[a]
	A. carbonarius	ITEM 11869	–	275.3

[a] OTA production in PDB medium (ng/ml).

belonged to *A. awamori*, which was recently considered as a cryptic phylogenetic species within the *A. niger* group (Perrone et al., in press).

The *Aspergillus carbonarius* and *A. japonicus* clusters could be clearly separated forming two homogeneous groups. In addition, the *A. japonicus* cluster showed high association with the *A. homomorphus* strain. This grouping agrees with a previous study where *Aspergillus* section *Nigri* strains were analyzed using the β-tubulin sequence (Samson et al., 2004).

The toxigenic profile of the strains confirmed the important contribution of *A. carbonarius* for OTA contamination in the grape ecosystem. Previous studies carried out in Southern Europe and Israel and in Argentina showed that *A. carbonarius* was the dominant OTA producer, with some *A. niger* aggregate strains producing OTA in lower concentrations (Battilani et al., 2006; Chiotta et al., 2009). These studies also observed an association between the percentage of OTA positive *A. carbonarius* strains, the production levels and the isolation region. Regarding *A. tubingensis*, some strains were OTA producers showing differences in their toxigenic ability. This result agrees with previous studies (Medina et al., 2005; Perrone et al., 2006b), although it is not supported by some other authors (Frisvad et al., 2007; Nielsen et al., 2009). The strains included in the *A. niger* cluster produced both mycotoxins, OTA and FB_s. In this respect they showed a higher production frequency for FB_s (35%) than for OTA (14%). In a study on grapes and raisins, approximately 77% of the strains identified as *A. niger* were able to produce FB_s which was a much greater frequency than OTA production (6-10%) (Battilani et al., 2006; Mogensen et al., 2010). These results are relevant since they demonstrate the presence of fumonisin producing strains in grapes which has not been reported previously in Argentina.

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Fig. 1. Phylogenetic tree of *Aspergillus* section *Nigri* population from Argentina based on the sequencing of β-tubulin, calmodulin, second largest subunit of RNA polymerase II and translation elongation factor 1α genes.

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