Five new species of *Penicillium* and *Talaromyces* for mycobiota of Iran

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Abstract

During study on biodiversity of *Penicillium* and *Talaromyces* species occurring on grape berries and raisin in vineyards of East- & West Azarbaijan and Gazvin provinces of Iran, five species of *Penicillium* and *Talaromyces*, namely, *Penicillium crocicola*, *P. olsonii*, *P. sumatrense*, *Talaromyces atroroseus* and *T. minioluteus* were characterized based on combination of morphological data and *β-tubulin* gene sequence. A phylogeny inferred based on sequence data of *β-tubulin* gene clustered our isolates together with representative type strains for each species from GenBank. All five species are new for the mycobiota of Iran. This work presents the first study on biodiversity of *Penicillium* and *Talaromyces* species on grape and raisin in this region of Iran.

Keywords: *Beta-tubulin* gene, biodiversity, bunch rot, post-harvest diseases, taxonomy

معرفی پنج گونه جدید از جنس‌های *Penicillium* و *Talaromyces* برای مایکوبیوتای ایران*

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خلاصه

طی مطالعه تنوع زیستی گونه‌های جنس *Penicillium* و *Talaromyces* روی میوه انگور و کشمش در باغ‌های انگور استان‌های آذربایجان‌های شرقی و غربی و قزوین، پنج گونه از جنس‌های *Penicillium* و *Talaromyces* شامل: *Penicillium crocicola*، *P. olsonii*، *P. sumatrense*، *Talaromyces atroroseus* و *T. minioluteus* براساس ترکیب ویژگی‌های ریخت‌شناختی و داده‌های توالی ژن بتاتوبولین شناسایی شدند. تجزیه و تحلیل فیلوژنتیک براساس داده‌های توالی ژن بتاتوبولین گونه‌های شناسایی شده در این تحقیق را همراه با نمونه‌های تیپ برای هر یک از گونه‌ها، در یک گروه قرار داد. تمامی گونه‌های شناسایی شده برای مایکوبیوتای ایران جدید می‌باشند. تحقیق حاضر نخستین مطالعه در زمینه تنوع زیستی گونه‌های *Penicillium* روی انگور و کشمش در این منطقه از ایران می‌باشد.

واژه‌های کلیدی: بیماری‌های بعد از برداشت، پوسیدگی خوشه، تاکسونومی، تنوع‌زیستی، ژن بتاتوبولین

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Introduction

Penicillium species are one of the most common fungi occurring in a diverse range of habitats, from soil to vegetation, air, indoor environments and various food products (Visagie *et al*. 2014). Currently, the genus *Penicillium* comprises more than 255 species (28 Aug. 2015; www.mycobank.org). Species of *Penicillium* are economically, ecologically and medically important micro-organisms playing vital roles in natural ecosystems, agriculture and biotechnology (Asan 2004, Visagie 2008). Some *Penicillium* species serve as important source for production of antibiotics (e.g. *P. chrysogenum* and *P. griseofulvum*) and novel enzymes (e.g. *P. citrinum*); while, others are known for their negative impact as degraders of agricultural products, production of mycotoxins in food commodity and pathogenicity on human (Dutta *et al.* 2008, Visagie 2008, Houbraken & Samson 2011). *Penicillium roqueforti* and *P. camemberti* are commercially used in cheese industry for production of high quality Roquefort and Camembert cheeses, respectively.

Many species of the *Penicillium* are among the common postharvest pathogens on a wide range of fruits and vegetables. *P. expansum*, the main cause of blue mold of apple, pear and fruits of other deciduous trees, is an example of destructive pathogens that causes much of post-harvest economic losses on food during storage and marketing stages (Barkai-Golan 2008). *Penicillium* species may produce harmful toxins (e.g. patulin and ochratoxin) to humans and animals in their life cycle (Natskoulis 2009).

The genus *Penicillium* was originally described by Link and *P. expansum* was designated as type (Frisvad & Samson 2004). Since the description of this genus, its taxonomy has been troublesome and several taxonomic schemes have been proposed for species delineation within this genus (Frisvad & Samson 2004). Pitt (1980) in his monograph divided *Penicillium* into four subgenera, viz., *Aspergilloides*, *Biverticillium*, *Furcatum* and *Penicillium* based on conidiophore morphology and branching pattern. The morphological features for species identification within subgenera include structures and branching patterns of conidiophores, shape, color and decoration of conidia, as well as cultural characteristics such as growth rate and colony color (Frisvad & Samson 2004). However, identification of *Penicillium* species solely based on morphological criteria have proven a difficult task, which is mainly due to high similarity and overlap in micromorphology of different species. With the recent advents in molecular biology, taxonomy of *Penicillium* and allied genera has largely been influenced by DNA sequence data. Sequence data from different genomic regions have been used to resolve taxonomy and phylogeny of this genus at different levels of interest. Sequence data from ITS-rDNA region, β-tubulin (β-tub), Cytochrome C oxidase I (COX1), calmodulin (CAL) and Translation elongation factor 1-alpha (EF-1a) genes have been widely used for species identification in *Penicillium* (Peterson 2000, Seifert *et al.* 2007).

The genus *Talaromyces* was introduced in 1955 by Benjamin for teleomorphic phase of *Penicillium* species and *T. vermiculatus* was designated as the type species. This genus also was associated with the anamorph genera such as *Geosmithia* and *Paecilomyces* (Pitt *et al.* 2000). Members of this genus are characterized by a cleistothecial wall of interwoven hyphae and typically yellow ascomata with soft ascocarps and ovate to globose asci containing mostly spiny ascospores (Benjamin 1955). The genus *Hamigera* was later introduced for *Talaromyces* species with single asci (Stolk & Samson 1971).

Recent phylogenetic studies have shown *Penicillium* subgenus *Biverticillium* being a polyphyletic assemblage (Seifert *et al.* 2004, Wang & Zhuang 2007). Thus, members of *Penicillium* subgenus *Beverticillium* along with *Talaromyces* species form a monophyletic clade, distinct from other subgenera of *Penicillium* (Houbraken & Samson 2011). In this regard, towards moving into a single and stable nomenclature for fungi,

Penicillium subgenus *Biverticillium* was transferred to *Talaromyces* (Yilmaz *et al.* 2014).

There are very limited number of studies on biodiversity of *Penicillium* species on grape and raisin worldwide. Five species of *Penicillium* including *P. brevicompactum*, *P. citrinum*, *P. echinulatum*, *P. expansum* and *P. solitum* have been reported from grapes in Korea (Won *et al.* 2007). Serra & Peterson (2007) have described two new species of *Penicillium*, namely, *P. astrolabium* and *P. neocrassum* from contaminated grapes in Portugal. The reports on *Penicillium* species occurring on grape and raisin from Iran are very rare (Rahmani *et al.* 2012, Maulani *et al.* 2012). So far, some species of *Penicillium* including *P. expansum* (Zakii *et al.* 1995), *P. brevicompactum* (Doulaty Baneh *et al.* 2000) and *P. glabrum* (Houbraken *et al.* 2014) have been reported from Iran (Ershad 2009). Accordingly, the aim of this study was to characterize *Penicillium* and *Talaromyces* species occurring on grape and raisin in East- & West Azarbaijan and Gazvin provinces using morphological and molecular data.

Materials and Methods

- Sampling, isolation and morphology

Berry samples were collected from vineyards of East, West Azarbaijan and Gazvin provinces during Sept.-Nov. 2011–13. At the same time, raisin samples were collected from various grape-processing factories in Bonab, Maragheh and Malekan cities of East Azarbaijan (Iran).

Isolations were made by direct plating on culture media and wetted filter paper techniques. Pure cultures were established using a single spore technique and cultures were deposited in Culture Collection of Tabriz University (CCTU) and CBS Fungal Biodiversity Centre, Utrecht, The Netherlands. Cultural and microscopic characteristics were studied on CYA at 25 and 37º C and MEA, CY20S and CZ at 25º C according to Klich & Pitt (1988) and Klich (2002). Microscopic slides were prepared from MEA plates and 60% lactic acid was used as a mounting medium. Thirty measurements were made for each microscopic structure where possible and 95th percentiles were determined for the measurements with the extremes given in parentheses. Photographs were captured using a light Olympus-BX41 microscope with an Olympus digital camera system (DP 25) and software to analyze photographs.

Primary grouping of *Penicillium* isolates was based on morphological characteristics and performed following the key provided by Frisvad & Samson (2004).

- DNA extraction, sequencing and phylogenetic analysis

Genomic DNA was extracted from fresh mycelia of 8-d-old fungal colonies grown on 2% MEA according to Moller *et al.* (1992).

The sequence data from *β-tubulin* gene was used for phylogenetic analysis. Part of β-tubulin gene was amplified using primer sets T1 (5´AAC ATG CGT GAG ATT GTA AGT) (O'Donnell & Cigelnik 1997) and Bt2b (5´ACC CTC AGT GTA GTG ACC CTT GGC) (Glass and Donaldson 1995). PCR was carried out in a final volume of 12.5 µl containing 1 µl (10-15 ng) of genomic DNA, 1.25 µl (1X) of 10X reaction buffer, 0.5 µl of 1 mM (0.04 mM) dNTPs, 0.375 µl of 50 mM (1.5 mM) MgCl2, 1.25 µl of 10 pM/µl (1 pM/µl) of each primer, 0.5 µl of DSMO and 0.125 µl of 5 U/µL (0.625 U/µl) *Taq* DNA polymerase. The reaction was performed on a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA) with cycling conditions consisting of 5 min at 96° C for primary denaturation, followed by 40 cycles of denaturation at 94º C for 30s, annealing at 56º C and 52º C for 30s, extension at 72º C for 60s, with a final extension at 72º C for 7 min. PCR products were purified using a 'GFX™ PCR DNA and gel band purification kit' (Amersham Pharmacia Biotech., Inc.). The same primer set was used to sequence amplicons. PCR products were sequenced were sequenced in both directions using the PCR primers and ABI Prism BigDye® Terminator Cycle Sequencing Reaction Kit Ver. 3.1 (Applied Biosystems, Foster City, CA), Cycle Sequencing Kit according to the recommendation of the vendor and analyzed on an ABI Prism 3700 (Applied Biosystems, Foster City, CA). Amplicons were purified through Sephadex G-50

Superfine columns (Sigma Aldrich, St. Louis, MO) in 96-well MultiScreen HV plates (Millipore, Billerica, MA). Raw sequence files were edited manually by using SeqMan™II (DNASTAR, Madison, Wisconsin, USA) and a consensus sequence was generated for each of the sequences. Sequences were subjected to Megablast search analysis at NCBI's GenBank nucleotide database for sequence similarity and sequence with high similarity were downloaded from GenBank (Table 1). The sequences were aligned by using ClustalW algorithm implemented in MEGA 5 (Tamura *et al*. 2011). The automatically aligned file was further checked manually. Phylogenetic trees were constructed using neighbor-joining method (Saitou & Nei 1987) with following criteria: substitution model as Jukes-Cantor; gaps treatment as pairwise deletion and transitions and transversions (with the equal ratio). The support for the internal nodes of the tree was evaluated by the bootstrap method (Efron 1979) with 1000 replicates. *Fusarium solani* (CBS 122429) served as out-group.

Results and Discussion

Based on of morphological characteristics *Penicillium* isolates recovered from grape and raisin in this study, belonged to three subgenera, namely, *Aspergilloides*, *Biverticillum* (*Talaromyces*) and *Penicillium*.

The identity of the isolates was further ascertained using sequence data of by *β-tubulin* gene. Approximately 430 bp was obtained for *β-tubulin* gene. The alignment file included 54 ingroup sequences (including 28 taxa from this study and 26 taxa from NCBI) (Table 1). *Fusarium solani* was used as an out-group.

The phylogeny inferred using the sequence data obtained in this study, together with the sequence data from GenBank, clustered our isolates in three subgenera: *Aspergilloides*, *Penicillium* and *Biverticillium* (*Talaromyces*) in four sections, namely, *Aspergilloides*, *Coronata*, *Citrina* and *Trachyspermi*. Five species, viz., *Penicillium crocicola* (subgenus *Aspergilloides* section *Aspergilloides*), *P. olsonii* (subgenus *Penicillium* section *Coronata*), *P. sumatrense* (subgenus *Aspergilloides* section *Citrina*), *Talaromyces atroroseus* (section *Trachyspermi*) and *T. minioluteus* (section *Trachyspermi*) are reported in this study (Fig. 1).

- Descriptions and Illustrations

Subgenus *Aspergilloides*

Species in subgenus *Aspegilloides* are characterized by monoverticillate penicilli. Pitt (1980) introduced sections *Aspergilloides* and *Exilicaulis* in subgenus *Aspergilloides* based on the presence or absence of a swelling at the stipe apex. Peterson (2000) was among the first researchers that divided *Penicillium* genus using nrDNA sequence dada into six groups with group two containing species mainly belonging to section *Aspergilloides* (*P. glabrum*, *P. purpurescens*, *P. spinulosum*, *P. fuscum* (syn. *E. pinetorum*), *P. thomii*, *P. lividum*, *P. lapidosum* (syn. *E. lapidosum*) and *P. asperosporum*) (Houbraken *et al*. 2014). Currently, subgenus *Aspergilloides* includes some sections such as *Aspergilloides* and *Citrina*.

Table 1. *Penicillium* and *Talaromyces* species used for phylogenetic analysis in this study

Taxon	Collection No.	GenBank accession No. for β-tub	Taxon	Collection No.	GenBank accession No. for β-tub
P. brevicompactum	ATHUM 5087	FJ004389	*P. crocicola*	DTO 266-A4	KM089019
P. chrysogenum	NRRL 824	AY371600	*P. crocicola*	DTO 265-H7	KM089018
P. citrinum	KAS 2608	JN637994	*P. crustosum*	DTO 266-B3	KU516391
P. commune	ATHUM 5042	FJ004397	*P. expansum*	DTO 266-A1	KU507293
P. crocicola	CBS 745.70	KJ834445	*P. expansum*	DTO 266-A7	KU507294
P. crustosum	ATHUM 5080	FJ004401	*P. expansum*	DTO 266-A9	KU507295
P. expansum	ATHUM 5098	FJ004407	*P. expansum*	DTO 266-B4	KU507296
P. expansum	ATHUM 5086	FJ004406	*P. expansum*	DTO 265-I3	KU507290
P. flavigenum	CBS 110411	JX996830	*P. expansum*	DTO 265-I4	KU507291
P. galliacum	CBS 41869	JN606845	*P. expansum*	DTO 265-I5	KU507292
P. glabrum	ATHUM 5126	FJ004411	*P. expansum*	DTO 265-H6	KU507289
P. glabrum	CBS 125543	GU981619	*P. glabrum*	DTO 266-A8	KM089020
P. griseofulvum	NRRL 2300	FJ004414	*P. glabrum*	DTO 265-I6	KU507287
P. italicum	ATHUM 3004	KJ004417	*P. olsonii*	DTO 266-B5	KU507286
P. mirabile	CBS 624.72	KF114797	*P. sumatrense*	DTO 266-A2	KU507284
P. olsonii	ATHUM 5184	FJ004424	*P. sumatrense*	DTO 266-B1	KU507285
P. patens	CBS 260.87	KJ834481	*P. sumatrense*	DTO 265-I8	KU507283
P. roqueforti	ATHUM 3007	FJ004430	*T. atroroseus*	DTO 266-A6	KU516398
P. sclerotiorum	NRRL 2074	JN626001	*T. atroroseus*	DTO 266-B2	KU516399
P. sumatrense	CBS 127362	JN606644	*T. atroroseus*	DTO 265-H9	KU516396
P. thomii	CV 905	JX271577	*T. atroroseus*	DTO 265-I7	KU516395
P. verrucosum	ATHUM 5079	FJ004438	*T. atroroseus*	DTO 265-I9	KU516397
P. viridicatum	ATHUM 5435	FJ004440	*T. minioluteus*	DTO 266-A3	KU516400
T. atroroseus	CBS 133447	KF114795	*T. minioluteus*	DTO 266-A5	KU516404
T. minioluteus	CBS 270.35	KM066129	*T. minioluteus*	DTO 265-H8	KU516401
T. udagawae	CBS 579.72	KF114796	*T. minioluteus*	DTO 265-I1	KU516402
P. brevicompactum	DTO 266-B6	KU516394	*T. minioluteus*	DTO 265-I2	KU516403

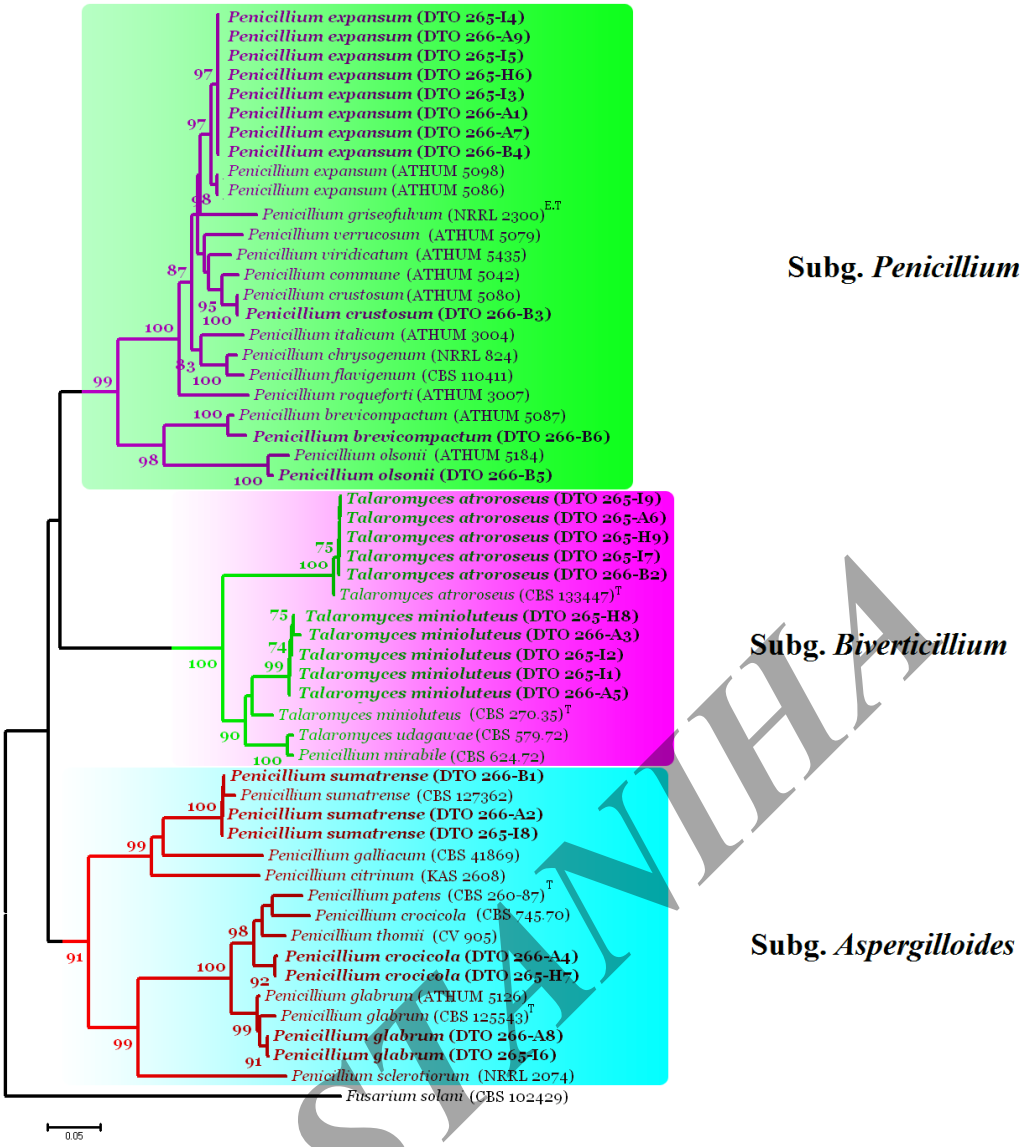

Fig. 1. Phylogenetic tree constructed based on *β-tubulin* gene sequence for 54 isolates of *Penicillium* and *Talaromyces* species by neighbor joining method, using MEGA5. Bootstrap values > 65% shown on the nodes. The phylogram was rooted to *Fusarium solani* (CBS 122429). The scale bar shows 0.05 substitutions per site. Isolates with bold face and DTO prefix sequenced in this study. E.T stands for ex-type culture; T stands for type strain.

Section *Aspergilloides*

Most species in the section *Aspergilloides* have phenotypic characters such as vesiculate, monoverticillate conidiophores and a moderate to fast growth rate on CYA and/or MEA. Additionally, many species of section *Aspergilloides* produce crusts of conidia on MEA that either shift or fall off in mass, similar to the characteristic colonies of *P. crustosum* (sect. *Penicillium*) (Houbraken *et al.* 2014). In this study, we characterized *Penicillium crocicola* from subgenus *Aspergilloides* section *Aspergilloides*.

Penicillium crocicola T. Yamam., in Yamamoto, Maeda & Oyasu, Sci. Rep. Hyogo Univ. Agric., Ser. 2, Agr. Biol. 2(2): 28 (1956), *Penicillium* subgenus *Aspergilloides* section *Aspergilloides*

Macroscopic characteristics: Colony diameter at 25° C after one week in dark condition: on CYA, 42.0–45.0 mm; on MEA, 33.0–37.0 mm; on YES, 43.0–45.0 mm, on CYA at 30° C, 37.0–39.0 mm, no growth occurred at 37° C on CYA. Colonies on CMA, MEA and YES are dark green, dark green and grass green, respectively. Colonies with colorless exudates, pale yellow diffusible pigments on YES. Without sclerotia and synnemata.

Microscopic characteristics: Conidiophores: (2.4–)2.8–3.0(–3.2) µm in width, monoveticillate, smooth. Phialides (2.8–)3.1–3.2(–4) × (10.0–)10.5–10.7(–10.8) µm, lageniform. Conidia (2.0–) 2.0–2.2(–2.5) × (2.0–) 2.4–2.6(–3.0) µm, globose to oval, in chain, smooth (Fig. 2).

Penicillium crocicola was originally described from corm of *Crocus sativus* in Japan by Yamamoto *et al.* (1956). Later, Pitt (1980) reduced this species as synonym of *P. thomii*. However, now it is phylogenetically distinct from *P. thomii* as seen in Figure 1 and has been accepted as separate species (Houbraken *et al.* 2014).

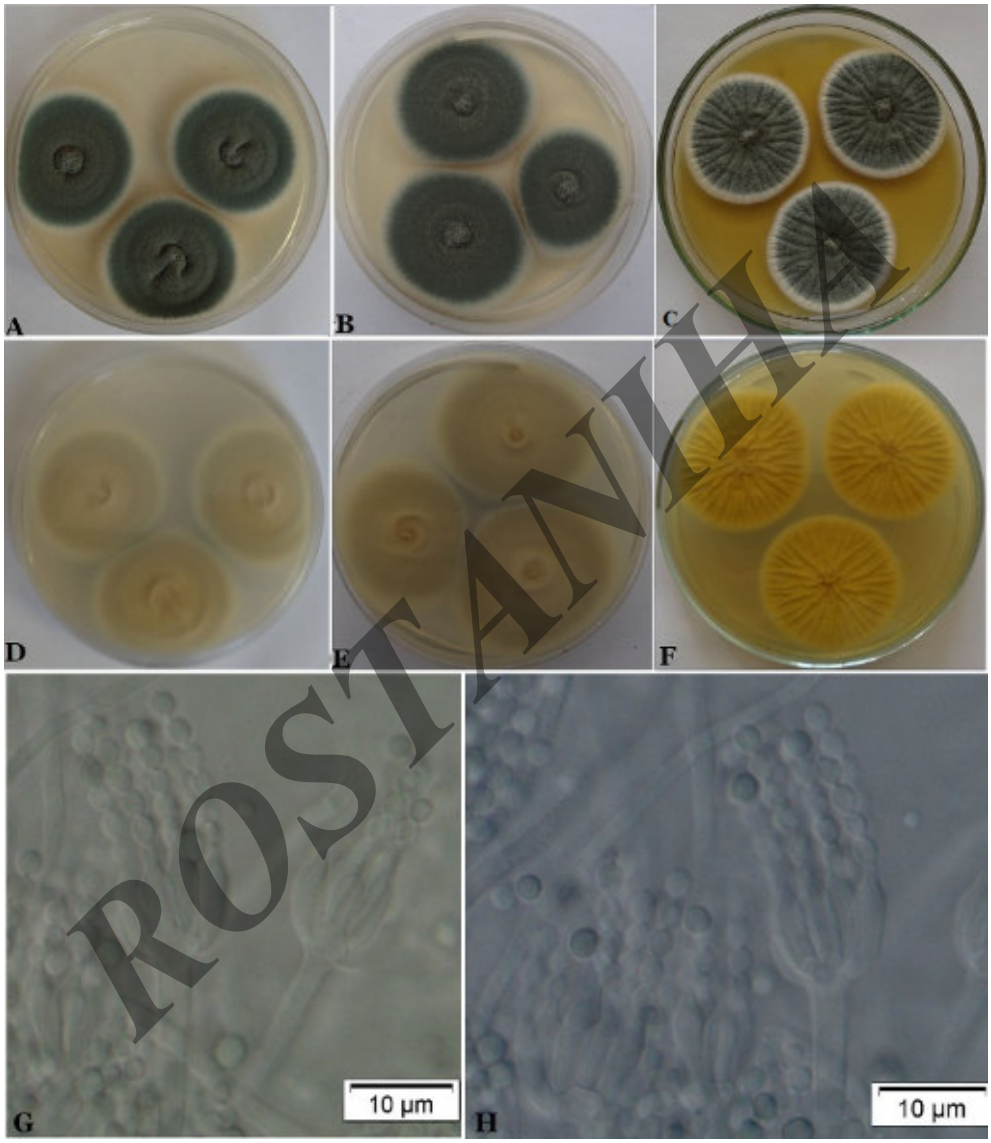

Fig. 2. *Penicillium crocicola*: 7-d-old colonies on CYA (A. Upper side, D. Backside), MEA (B. Upper side, E. Backside) and YES (C. Upper side, F. Backside), G–H. Conidiophores & Conidia.

Section *Citrina*

Currently, section *Citrina* is being treated in subgenus *Aspergilloides*. Species of this section have a worldwide distribution and occur commonly in soils.

They are characterized by production of symmetrically biverticillate conidiophores, flask-shaped phialides and relatively small conidia. Some species may produce grayish-brown colored cleistothecia containing flanged ascospores.

The most important phenotypic characters to distinguish members of section *Citrina* are growth rate

and colony reverse color on the agar media CYA, MEA and YES; shape, size and ornamentation of conidia (Houbraken *et al*. 2011). In this study, we identified one species from subgen. *Aspergilloides* section *Citrina.*

Penicillium sumatrense von Szilvinyi [as 'sumatrense'], Archiv für Hydrobiologie 14(Suppl. 3): 535 (1936), *Penicillium* subgenus *Aspergilloides* section *Citrina*

Macroscopic characteristics: Colony diameter on 25° C after one week in dark condition: on CYA, 28.0–30.0 mm; on MEA, 30.0–33.0 mm; on YES, 43.0–45.0 mm, on CYA at 30° C, 28.0–32.0 mm, without growth at 37° C on CYA. Colonies on CYA, MEA and YES are grass green, grass green and yellowish green, respectively. Colonies with colorless to pale yellow exudates on CYA and MEA, with yellowish orange diffusible pigments on YES, without sclerotia and synnemata.

Microscopic characteristics: Conidiophores (2.1–)2.4–2.6(–2.9) µm in width, mono- or biverticillate, smooth. Phialides (8.8–)9.2–9.5(–10.0) × (2.3–)2.35–2.45(–2.5) µm, lageniform. Conidia (1.9–)2.2–2.4(–2.6) µm, globose, smooth (Fig. 3).

Penicillium sumatrense, was originally described from the rhizosphere of *Lumnitzera racemosa*. This species produces sumalarin A, sumalarin B and sumalarin C (Meng *et al.* 2013).

In this study, *P. sumatrense* and *P. crocicola* (other member of *Aspergilloides* subgenus), produced exudates in culture media but the third member of subgen. *Aspergilloides* (*P. glabrum*) did not produce exudates.

Subgenus *Penicillium*

Species of subgenus *Penicillium* have terverticillate penicilli and are related to ascomycete genus *Eupenicillium* series *Ctustacea*. Many species of this subgenus are very common on stored foods. This subgenus comprises several sections such as *Coronata, Roqueforti*, *Expansa*, *Digitata* and *Viridicata* (Frisvad & Samson 2004).

Section *Coronata*

Species of section *Coronata* are charterised by their compact, often tervertcillate penicilli with long stipes. *Coronata* is most similar to section *Requeforti*. Species of this section grow both at very low water activity and low temperatures, but do not tolerate high growth temperature. The species of *Coronata* section have worldwide distribution form the tropical to Arctic regions (Frisvad & Samson 2004). We identified one species from this section.

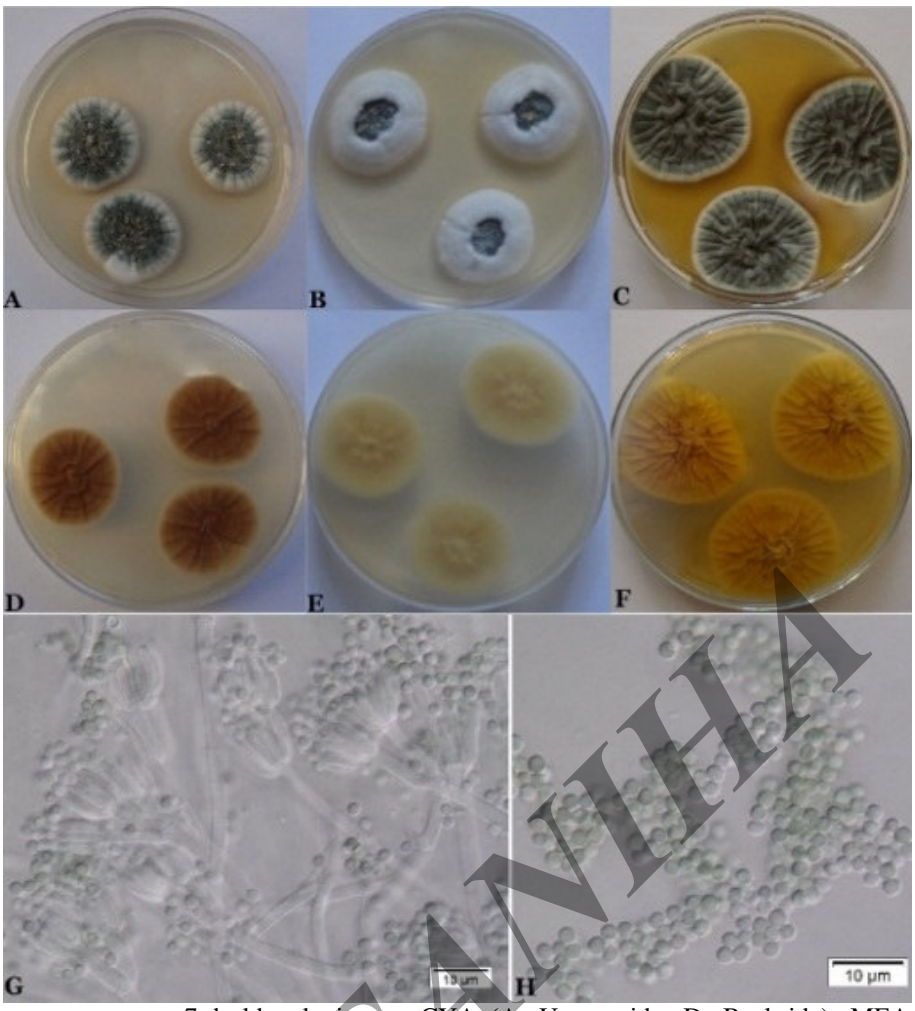

Fig. 3. *Penicillium sumatrense*: 7-d-old colonies on CYA (A. Upper side, D. Backside), MEA (B. Upper side, E. Backside) and YES (C. Upper side, F. Backside), G–H. Conidiophores & Conidia.

Penicillium olsonii Bain. & Sartory [as 'olsoni'], Annls mycol. 10(4): 398 (1912), *Penicillium* subgenus *Penicillium* section *Coronata*

Macroscopic characteristics: Colony diameter at 25° C after one week in dark condition: on CYA, 20.0–22.0 mm; on MEA, 26.0–29.0 mm; on YES, 25.0–27.0 mm, on CYA at 30° C, 25.0–28.0 mm, no growth at 37° C on CYA. Colony texture: velutinous. Colonies on CMA, MEA and YES are Grayish green to light green, grass green and grayish green to light green, respectively. Colonies with colorless to pale yellow exudates on CYA, without diffusible pigments on media, without sclerotia and synnemata.

Microscopic characteristics: Conidiophores (3.8–)4.1–4.2(–4.5) µm in width, terverticillate, smooth. Rami: (6.3–)11.0–13.2(–16.3) × (2.8–)3.5–3.8(–4.5) µm. Metulae (8.8–)10.5–11.0(–13.8) × (3.0–)3.6–4.0(–4.8) µm. Phialides (8.8–)9.5–9.8(–10.0) × (2.5–)2.7–2.8(–3.0) µm, lageniform. Conidia (2.8–)3.3–3.5(–3.8) × (2.3–)2.5–2.6(–2.8) µm, oval to globose, smooth (Fig. 4).

Penicillium olsonii is regarded as closely related to *Penicillium herquei* Bainier and Sartory, but is distinguishable from *P. herquei* primarily upon the basis of its longer and very coarse conidiophores (www.mycobank.org). Based on morphological characteristics *P. olsonii* is similar to *P. brevicompactum* and *P. bialowiezense*, however, it has faster growth rate on CMA and YES in comparison to the other two species (Frisvad & Samson 2004). *Penicillium olsonii* is phylogenetically distinct from *P. brevicompactum* and *P. bialowiezense* (Fig. 1). This species has been reported from a wide range of substrates such as greenhouse, peat soli, tomatoes, rarely on barely and cod roe and in tropical soils from Denmark, Netherlands, Russia, Costa Rica, Canada and other countries (Frisvad & Samson 2004).

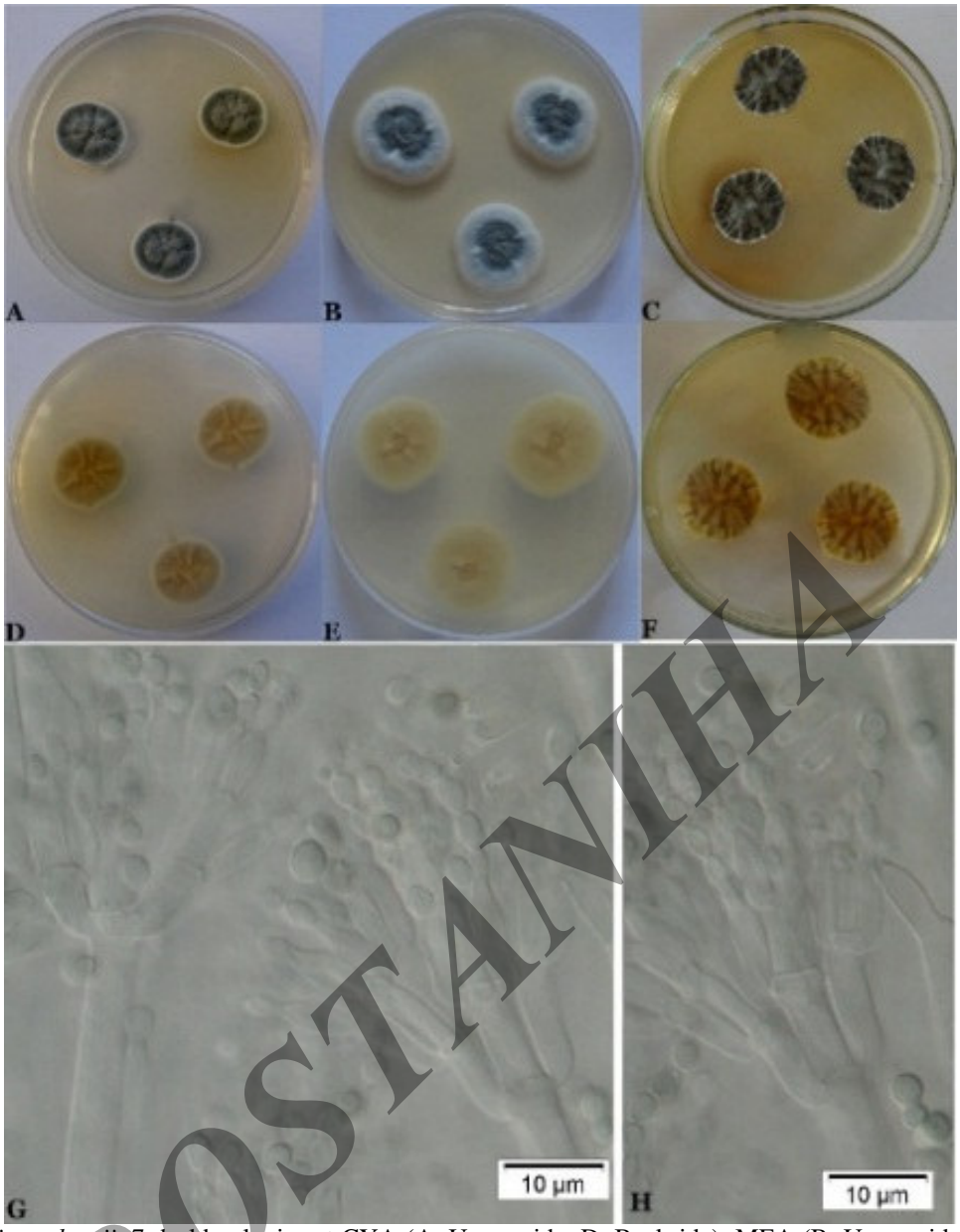

Fig. 4. *Penicillium olsonii*: 7-d-old colonies at CYA (A. Upper side, D. Backside), MEA (B. Upper side, E. Backside) and YES (C. Upper side, F. Backside), G–H. Conidiophores & Conidia.

Talaromyces* section *Trachyspermi

Species in section *Trachyspermi* are charterised by their restricted growth on CYA and YES, growing slightly faster on MEA. Some species of this section produce abundant red pigments. Conidiophores are generally biverticillate and ascomata, when produced, have a creamish white or yellow color. This section encompasses species such as *Talaromyces atroroseus*, *T. minioluteus* and *T. albobiverticillius* that are biotechnologically important, with their pigments used as colorants in the food industry (Frisvad *et al*. 2013). In this study, we identified two species from this section.

Talaromyces atroroseus N. Yilmaz, Frisvad, Houbraken & Samson, in Frisvad, Yilmaz, Thrane, Rasmussen, Houbraken & Samson, PLoS ONE 8(12): e84102, 8 (2013), *Talaromyces* section *Trachyspermi*

Macroscopic characteristics: Colony diameter at 25^{o} C after one week in dark condition: on CYA, 30.0–36.0 mm; on MEA, 32.0–33.0 mm; on YES, 36.0–37.0 mm, on CYA at 30 and 37^{o} C on CYA, 35.0 and 17.0–19.0 mm, respectively. Colonies on CYA, MEA and YES are Grayish green, dark green and grass green, respectively. Colonies without exudates in culture, with red diffusible pigments on CYA and YES, without sclerotia and synnemata.

Microscopic characteristics: Conidiophores (2.0–)2.5–2.9(–3.2) µm in width, biverticillate, smooth to finely roughened, umbrella is opened. Metulae (10.0–)11.0–11.4(–12.0) × (2.0–)2.1–2.3(–2.4) µm. Phialides (10.0–)11.0–11.6(–13.0) × (1.6–)2.3–2.9(–3.6) µm, acerose. Conidia (2.4–)2.5–2.7(–2.8) × (2.8–)3.0–3.4 (–3.6) µm. globose to oval, finely roughened (Fig. 5). Ascomata not observed.

Talaromyces atroroseus produced soluble red pigment and very dark green conidia on CYA and MEA (Fig. 5). Because of the production large amounts of red pigments, it can be potentially used for coloring foods, as it does not produce any known mycotoxins (Frisvad *et al.* 2013).

At present, ascomata was not observed for *T. atroroseus*; however, the ascomata in members of *Talaromyces* section *Trachyspermi* has a creamish white or yellow color if produced. This species along with *T. minioluteus* are biotechnologically important, because their pigments are used as colorants in the food industry. *Talaromyces atroroseus* secretes large amounts of Monascus red pigments, without the production of any known mycotoxins (Frisvad *et al.* 2013).

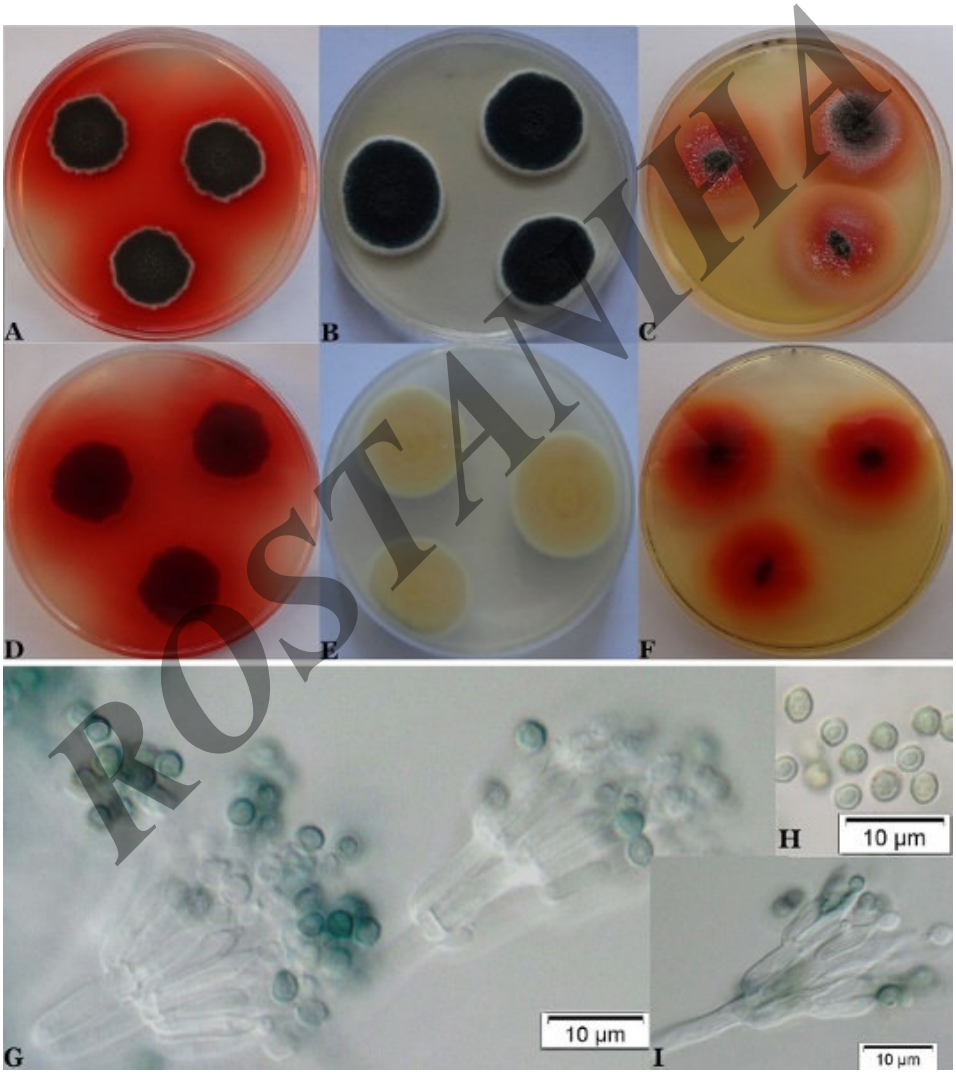

Fig. 5. *Talaromyces atroroseus*: 7-d-old colonies on CYA (A. Upper side, D. Backside), MEA (B. Upper side, E. Backside) and YES (C. Upper side, F. Backside), G–I. Conidiophores & Conidia.

Talaromyces minioluteus (Dierckx) Samson, N. Yilmaz, Frisvad & Seifert in Samson, Yilmaz, Houbraken, Spierenburg, Seifert, Peterson, Varga & Frisvad, Stud. Mycol. 70: 176 (2011), *Talaromyces* section *Trachyspermi*

Macroscopic characteristics: Colony diameter at 25° C after one week in dark condition: on CYA, 23.0–66.0

mm; on MEA, 25.0–26.0 mm; on YES, 26.0–27.0 mm, on CYA at 30° C, 22.0–24.0 mm, without growth at 37° C on CYA. Colonies on CYA, MEA and Yes are grass green, dark green and dark green, respectively. Colonies without exudates on media, with yellowish orange diffusible pigments on MEA and YES, without sclerotia and synnemata.

Microscopic characteristics: Conidiophores (2.0–)2.5–2.7(–3.6) µm in width, biverticillate, finely roughened, umbrella is closed. Metulae (12.0–)12.8–13.2(–16.0) × (2.0–) 2.4–2.8(–3.2) µm. Phialides (12.0–)12.5–13.1 (–14.0) × (1.6–)2.0–2.4(–2.8) µm, acrose. Conidia (2.0–)2.1–2.2(–2.4) × (2.0–)2.4–2.8(–3.2) µm, globose to oval, finely roughened (Fig. 6). Ascomata not observed.

Talaromyces minioluteus has been considered as a member of sub-genus *Biverticillium* until 2011 (Samson *et al.* 2011). The main difference of this species with *T. atroroseus* is that, the umbrella is closed in *T. atroroseus* but it is opened in *T. minioluteus*. In addition, *T. atroroseus* has red diffusible pigments on CYA and YES, but in *T. minioluteus* the diffusible pigments are yellowish-orange and are produced on MEA and YES.

The *Talaromyces minioluteus* can be distinguished from other closely related species by moderate growth rate on general media and production of weak acid on CREA (Yilmaz *et al.* 2014).

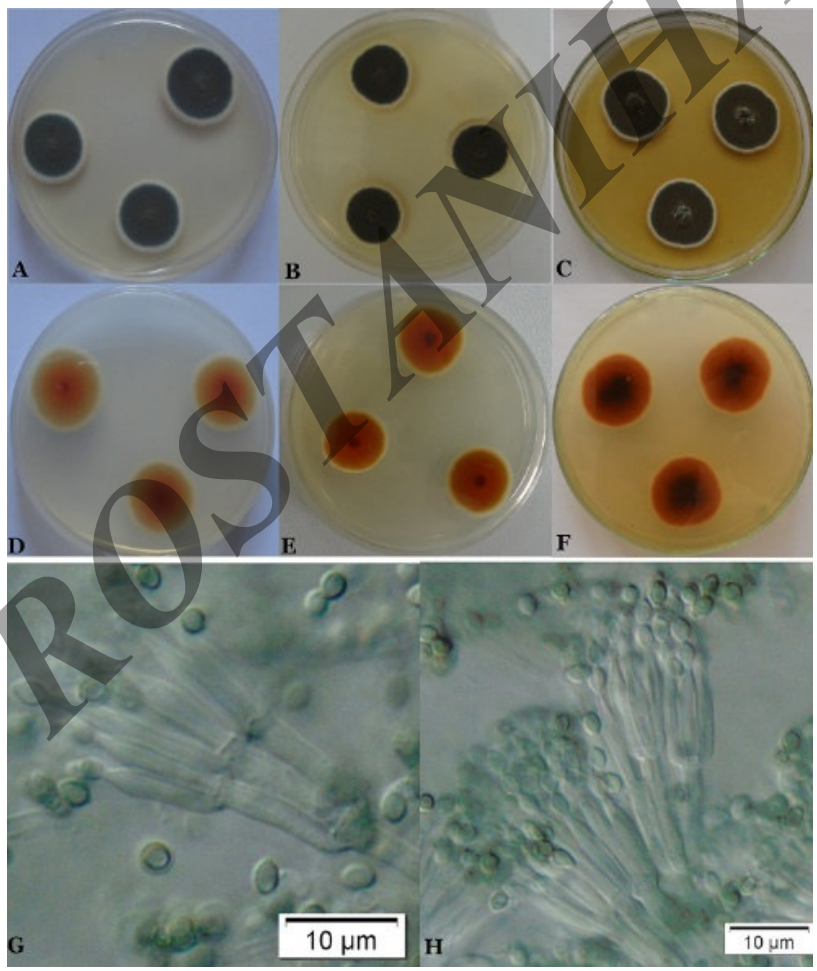

Fig. 6. *Talaromyces minioluteus*: 7-d-old colonies at CYA (A. Upper side, D. Backside), MEA (B. Upper side, E. Backside) and YES (C. Upper side, F. Backside), G–H. Conidiophores & Conidia.

In this study, integration of morphological data together with sequence data enabled us to identify *Penicellium* spp. occurring on grapes and raisins in East- & W Azarbaijan and Gazvin provinces of Iran. Five species were characterized including *Penicillium crocicola*, *P. olsonii*, *P. sumatrense*, *Talaromyces atroroseus* and *T. minioluteus*. All five species represent new records for the mycobiota of Iran.

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