



Production of Mycophenolic Acid by a Newly Isolated Indigenous *Penicillium glabrum*

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Abstract

Soil-occupant fungi produce a variety of mycotoxins as secondary metabolites, one of which is mycophenolic acid (MPA), an antibiotic and immunosuppressive agent. MPA is mainly produced by several species of *Penicillium*, especially *Penicillium brevicompactum*. Here, we present the first report of MPA production by a local strain belonging to *Penicillium glabrum* species. We screened ascomycete cultures isolated from moldy food and fruits, as well as soils, collected from different parts of Iran. MPA production of one hundred and forty *Penicillium* isolates was analyzed using HPLC. Three MPA producer isolates were identified, among which the most producer was subjected to further characterization, based on morphological and microscopic analysis, as well as molecular approach (ITS, rDNA and beta-tubulin gene sequences). The results revealed that the best MPA producer belongs to *P. glabrum* IBRC-M 30518, and can produce 1079 mg/L MPA in Czapek-Dox medium.

Introduction

Mycophenolic acid (MPA) is an antibiotic and immunosuppressant drug which is commercialized as mycophenolate mofetil (MMF) and mycophenolate sodium for the prevention of organ transplant rejection [1, 2]. MPA and its derivatives have diverse biological properties and have been extensively studied for their antineoplastic, anti-inflammatory, anti-psoriasis, antifungal and antiviral activities [3, 4]. Recent reports indicate anti-SARS-CoV-2 activity of MPA [5]. It is a selective and non-competitive inhibitor of the enzyme Inosine Monophosphate DeHydrogenases (IMPDH) involving in de novo biosynthesis of guanosine nucleotide, the only supporting pathway of purines synthesis in both B and T lymphocytes [4]. Inhibition of IMPDH arrests cell

proliferation as a result of DNA and RNA biosynthesis blocking [2, 6].

MPA is a meroterpenoid consisting of a polyketide nucleus connected to farnesyl pyrophosphate; a mevalonate pathway C-15 intermediate [7]. This metabolite is produced by several *Penicillium* spp, among which *P. brevicompactum* and *P. stoloniferum* indicate a relatively high ability of production. MPA producing strains of this genus are very common in forest soil, greenhouse and farmland all over the world [8]. Furthermore, these species could be found in moldy food, fruit and dairy products [9–12]. *Penicillium glabrum* is a filamentous fungus, belonging to the section *Aspergilloides*. It has a worldwide distribution, frequently encountered in the food manufacturing industry. It is one of the main causes of post-harvest fruit and vegetable rots and food contamination [13]. It has been previously isolated from a wide range of food products such as cheese, maize, rice, jam and bottled water [14]. There is no report on MPA production by *P. glabrum*.

The aim of this study was to isolate a *Penicillium* MPA producer in the hope to be suitable for large scale production of MPA.

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Materials and Methods

Isolation of *Penicillium* Species

Samples were collected mostly from garden, greenhouse and farmland soils of various provinces of Iran, as well as moldy food, fruit and dairy products. Soil samples were taken from 10 cm depths with a sterile spatula [15]. 5 g of each sample were mixed with 50 mL peptone water (1%) containing 0.01% tween 80 [16]. A serial dilutions up to 10^{-5} of supernatant of each sample were prepared [15]. Finally, 100 μ L of each dilution was spread on two potato dextrose agar (PDA) plates containing 30 μ g/mL of Chloramphenicol and Kanamycin, to prevent bacterial growth. The plates were incubated in a dark place at 25–28 °C for 5–7 days [17].

Single Spore Isolation

Macroscopic observation of cluster structures and broom handles of each *Penicillium* was performed using a stereomicroscope. Then a suspension of *Penicillium* spores in sterile distilled water was prepared. The number of conidia in each water droplet was adjusted between one and 10 after spore count with a Neobar slide. A droplet of suspension was spread on PDA plates and incubated at 25–28 °C for 24 h. Purified colonies were used for further studies [18].

Penicillium Cultivation and Sample Preparation

MPA production was analyzed in Czapek-Dox (Cz) medium comprised (g/L): Sucrose, 30; NaNO₃, 3; K₂HPO₄·3H₂O, 1.3; MgSO₄·7H₂O, 0.5; KCl, 0.5; FeSO₄·7H₂O, 0.01; CuSO₄·5H₂O, 0.005; ZnSO₄·7H₂O, 0.01 [19, 20]. A spore suspension from fresh colonies in sterile distilled water containing 2.5×10^7 spores was used for inoculation of 50 mL Cz medium which incubated at 25–28 °C for 12 days at 180 rpm. MPA biosynthesis started after approximately 120 h (5 days) and was reached the maximum concentration at 300 h (12 days) [4]. 1 mL of each culture was centrifuged in 5000 \times g at 4 °C for 5 min and the supernatant was filtered through 0.2 μ m filter (Millipore, USA). The filtered samples were stored at – 20 °C for subsequent analysis.

The MPA production was also investigated in Xu medium consisted of (g/L): glucose, 100; glycine, 14; l-methionine, 0.5; KH₂PO₄, 2.0; MgSO₄·7H₂O, 1.0; FeSO₄·7H₂O, 2.2; CuSO₄·5H₂O, 0.3; ZnSO₄·7H₂O, 2.4; MnSO₄·4H₂O, 0.16; and KMoO₄, 0.2. Before autoclave, pH was adjusted to 6.0 with 1 N HCl or NaOH solutions [4]. The cultivation condition was as the Cz medium.

HPLC Analysis for MPA Determination

MPA concentration was evaluated by a KNAUER HPLC (Germany) consisting of pump: LC-20 AD, auto sampler: SIL-20A, detector: SPD-20 A, degasser: DGU-20 A. The analysis was carried out with a C₁₈ high persil column, at 40 °C and 250 nm. The injection volume of 20 μ L with mobile phase consisted of 0.1 M KH₂PO₄ and acetonitrile at a ratio of 50:50, pH 3.0 at a flow rate of 0.5 mL/min was used [21, 22]. HPLC grade MPA (AppliChem) was used to prepare the calibration standard curve. Working standard solutions (10–400 μ g/mL) were prepared from 1 mg/mL stock solution in HPLC grade methanol.

Characterization of MPA Producing Strain

Characterization of the selected MPA producer was performed based on morphological and microscopic properties as well as molecular analysis. After 7 days, some principles of the colony features including diameter, texture and color of conidia describing the morphology of *Penicillium* species, and degree of sporulation, the abundance of color and texture of mycelia, the presence and colors of soluble pigments and exudates were considered [23].

Colony characteristics on specific media are prominent features broadly used for species identification. MEA (Malt Extract Autolysate agar) [20] and CYA (Czapek Yeast Autolysate agar) [24] are recommended as standard media for *Penicillium* species isolation [23].

Penicillium strains with the highest MPA production were inoculated on the mentioned media in a three-point position and incubated at 25 °C for 7 days. Colonies characteristics were recorded and compared with available identification keys [24].

A piece of agar (1 cm²) was placed on a sterile glass microscope slide. Then the slide was placed in a sterile Petrie dish to prevent contamination and drying during incubation. Some conidia were transferred to every four sides of the agar block and incubated at 25 °C for 5 days. Intact fruiting body structure, including phialides, hyphae and arrangement of conidia were observed after staining with lactophenol cotton blue [25].

Molecular Analysis

Genomic DNA was extracted using CTAB buffer containing: Tris, 10 mM; EDTA, 20 mM; CTAB, 0.02 M; NaCl, 0.8 M; Sorbitol, 0.13 M; PVP (Polyvinylpyrrolidone), 1%.

For molecular characterization, both 18 s and ITS (internal transcribed spacer) regions of rRNA genes, as well as beta-tubulin gene were amplified using universal related primers, ITS1 (5'-TCCGTAGGTGAACCTGCGG-3'), ITS4

(5'-TCCTCCGCTTATTGATATGC-3'), 18 s F (5'-CCTGGT TGATCCTGCCAGTA-3'), 18 s R (5'-GCTTGATCCTTC TGCAGGTT-3'), Bt2a (5'-GGTAACCAAATCGGTGCT GCTTTC-3') and Bt2b (5'-ACCCTCAGTGTAGTGACC CTTGGC-3'), respectively.

PCR products were cloned in pTZ57R/T (Thermo scientific), sequenced and subjected to nBLAST against their fungal homologs.

The ITS sequence was subjected to phylogenetic analysis which was performed by molecular evolutionary genetics analysis (MEGA) software, version 7.0 using UPGMA method, with a bootstrap of 500 replicates.

Results

Isolation of MPA Producing *Penicillium* Species

To isolate a native MPA producing fungus, 140 *Penicillium* isolates were obtained from 50 samples of soil from forest, greenhouse, and agricultural lands of the various provinces of Iran, as well as 24 samples of moldy food, fruit and dairy products, as shown in Table 1.

Evaluation of MPA Production

HPLC method was used for the evaluation of MPA production by *Penicillium* isolates. Three MPA producing isolates were obtained as shown in Table 2. Twenty microliters of samples prepared from culture supernatants were injected into the HPLC column. Chromatograms of standard solution and GGST1 isolate with a retention time of 12 min is shown in Fig. 1. A comparison between two chromatograms confirmed that the GGST1 isolate has the ability of MPA production. Strains cultivation and MPA determination were repeated three times.

Table 2 MPA producing strains

MPA producing isolates	Average MPA production ($\mu\text{g}/\text{mL}$)	
	Cz medium	Xu medium
GGST1: <i>P. glabrum</i> IBRC-M 30518	1079	371
MP4	522	53
FSM2	268	88

MPA production by selected strains in Xu medium was also evaluated. The results showed that 371, 53 and 88 $\mu\text{g}/\text{mL}$ MPA were produced by GGST1, MP4 and FSM2 strains, respectively. Unexpectedly, all three strains were produced less quantities of MPA in Xu medium compared to the Cz medium as shown in Table 2 and Fig. 2.

Characterization of the Best MPA Producing Isolate

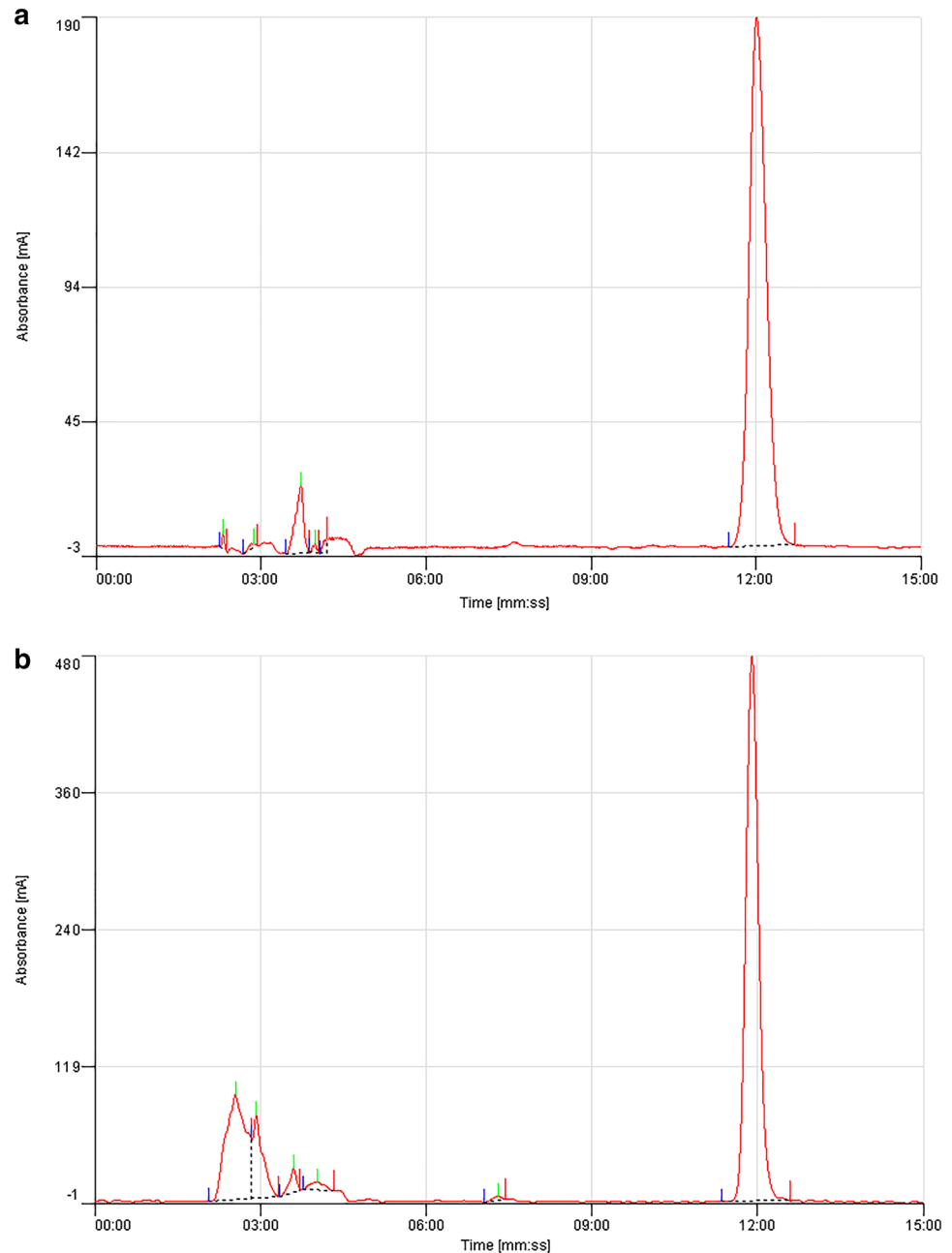
The MPA producer, GGST1 isolate, was identified based on its morphological (macroscopic and microscopic) characteristics as well as ITS, 18 s rDNA and beta-tubulin gene sequences.

The GGST1 strain colonies on CYA medium display 35–45 mm diameter, velvet surface, and radial white mycelium and produce grey-green conidia. The reverse color was in shades of grey to brown. On the other hand, on MEA medium, colonies with 40–50 mm diameter, velvet surface, radial grooves, white mycelium only in the center of the colony, high production of dark green conidia and sometimes secrete yellow exudates droplets were observed. Reverse color was yellow to yellow-orange. Furthermore, the stipes with 50–100 μm length and smooth walls with slight roughness, 10–12 flask-shaped phialides in a row with 8–12 μm length and the smooth to finely roughness conidia with 3–5 μm diameter were produced in long columns. These

Table 1 Number of *Penicillium* species isolated from various sources

Samples	Sampling sources	Number of <i>Penicillium</i> isolates	Number of MPA producing isolates
Soil	Fruit garden	27	1
	Forest and agricultural land	33	1
	Alfalfa farm	8	–
	Greenhouse	13	–
	Areas around the pharmaceutical company	17	–
Moldy materials	Fruits	22	–
	Grain	4	–
	Decayed cooked food	16	1
	Dairy product	–	–
Total		140	3

Fig. 1 HPLC chromatograms of **a** standard solution of HPLC grade MPA; **b** MPA produced by strain GGST1



characteristics confirmed that the isolate belongs to *P. glabrum* species [26] (Fig. 3).

The sequences of amplicons from GGST1 isolate with sizes of 519, 1798 and 466 nucleotides were deposited in NCBI Genbank nucleotide sequence under accession numbers of MK611771 MN044770 and MW002460 for ITS, 18S rDNA and beta-tubulin gene, respectively. The GGST1 isolate ITS sequence has 100% identity with many *P. glabrum* isolates ITS sequences, such as HLJ_16, Y56, ND70 and MS31-1. The 18S rDNA sequence has 99% identity with *P. glabrum* strain KCTC16099 18S rDNA sequence. Furthermore, housekeeping beta-tubulin gene sequence of

this isolate has > 99% identity with a number of *P. glabrum* strains such as 207P, KAS7776, KAS 7774 and many others. Therefore, the isolate was recognized as *P. glabrum* IBRC-M 30518 and kept for further studies and probable applications. The sequence was subsequently deposited in the GenBank (Accession Number MW002460).

Phylogeny

The phylogenetic position of the *P. glabrum* IBRC-M30518 was inferred from a comparison of its ITS sequence with the strains of *P. verhagenii*, *P. saturniforme*, *P. bussumense*, *P.*

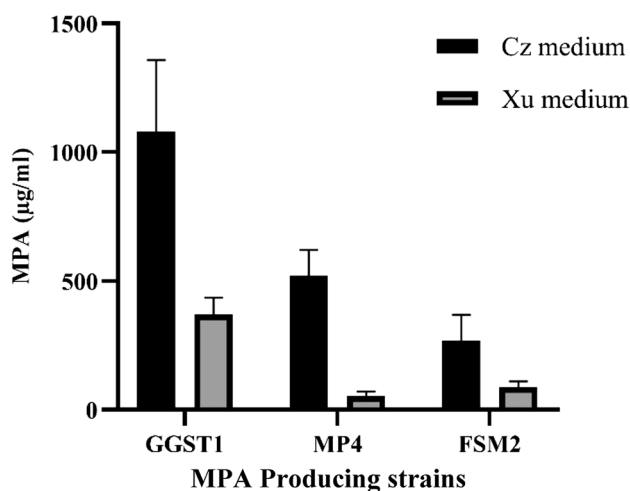


Fig. 2 Comparison of MPA production in two different media. Cz and Xu media are displayed in black and gray, respectively

pulvis, *P. rudallense*, *P. armarii*, *P. purpurascense*, *P. spinulosum*, *P. vagum*, *P. tsitsikamaense*, *P. fuscum*, *P. sublectaticum*, *P. adametzii*, *P. camemberti*, *P. glabrum*, *P. thomii*, *P. commune*, *P. solitum*, *P. chrysogenum*, *P. kewense*, *P. frei*, *P. expansum*, *P. roqueforti*, and *P. brevicompactum* (Fig. 4). Like the type strain of *P. glabrum*, the newly isolated strain belonged to the *Aspergilloides* clade, which is defined by characteristics such as possessing monoverticillate conidiophores and growing moderately or quickly on agar media. According to Houbraken et al. the most important feature distinguishing members of this section are colony size on agar media, growth at 30 °C, ornamentation and shape of conidia, sclerotium production and stipe roughness [26].

Discussion

Filamentous fungi are widely associated with the ability to produce bioactive secondary metabolites that have important pharmaceutical applications [27]. One of the most extensive genera of filamentous fungi is *Penicillium*, with more than 354 accepted species [23], many of which are in high importance due to their ability to the production of pharmaceuticals and industrial agents as secondary metabolites. One of such prominent secondary metabolites is MPA, produced by several *Penicillium* species such as *P. brevicompactum*, *P. roqueforti*, *P. puberulum*, and *P. stoloniferum* as well as other fungi *Byssoschlamys nivea* and *Aspergillus* strains [28–31].

There are plenty of reports implying the isolation of various strains of *Penicillia* from soil and moldy foods and fruits, with the aim of MPA production (Table 3). Despite the large implication of the *P. glabrum* in food contamination, some studies have been conducted to investigate its

ability to enzyme production [32, 33]. Meanwhile, there is no report on MPA production by *P. glabrum*.

According to Landeweert et al. $\geq 99\%$ of sequence identity was identified to species level, 95–99% to genus level, and $\leq 95\%$ to family level [34]. The ITS sequence (partial sequence of ITS 1, complete sequence of 18S ribosomal RNA gene and ITS 2, and partial sequence of large subunit ribosomal RNA gene), were analyzed using nBLAST in NCBI search tool and demonstrated that this isolate could be assigned to series of species including *P. glabrum*, *P. spinulosum*, *P. aragonense*, *P. expansum*, *P. spinuloramigenum* and many others. Regarding some studies, ITS sequence is not sufficient for identification at the species level, therefore housekeeping beta-tubulin gene was sequenced and compared with their fungal homologs. The result demonstrates that this isolate could be assigned to *P. glabrum* (GenBank Accession Number EU128574.1) with an identity of 99.57%. A phylogenetic analysis using the ITS region revealed that the newly isolated strain is belonged to the species *P. glabrum*, which is considered as one of the top three well characterized members of the section *Aspergilloides*, along with *P. spinulosum* and *P. thomii*. These worldwide distributed species, constituting 3 out of 12 clades of the section, could be isolated from many substrates including soil, food, bark and indoor environments [26]. The majority of members of the section *Aspergilloides* are predominantly monoverticillate and most grow quickly on agar media [35]. According to phenotypical features, the species from *P. glabrum*-clade represent a remarkable relation to those of the *P. spinulosum*-clade. Yet, they could be distinct based on colony texture, as the colony surface is defined “strictly velvety” for *P. glabrum*, and be “loose textured” for *P. spinulosum* [26].

According to some studies, the highest reported MPA production is related to *P. brevicompactum* ATCC 16024 strain [21], producing 5900 mg/L MPA in an Xu medium in 250 mL shake flask [4].

The local *P. glabrum* IBRC-M 30518 strain isolated in this study is the first report of MPA production by this species which can produce 1079 mg/L MPA in Cz medium. Although its production yield is less than *P. brevicompactum* ATCC 16024 strain, but it is more than most of the MPA producing species previously reported [21]. Two other *Penicillium* strains, MP4 and FSM2 produced 522 and 268 mg/L MPA in Cz medium, respectively. Meanwhile, MPA production of these three *Penicillium* isolates in Xu medium (the optimum medium for *P. brevicompactum* ATCC 16024) was much lower than in Cz medium as shown in Table 2 [4].

Therefore, it seems necessary to develop a process for the improvement of MPA production by the local *P. glabrum* IBRC-M 30518. To enhance MPA production with *P. glabrum* IBRC-M 30518, medium and process optimization are the next steps in our research which is in the way. Genetic manipulation and mutation-selection approaches can also be

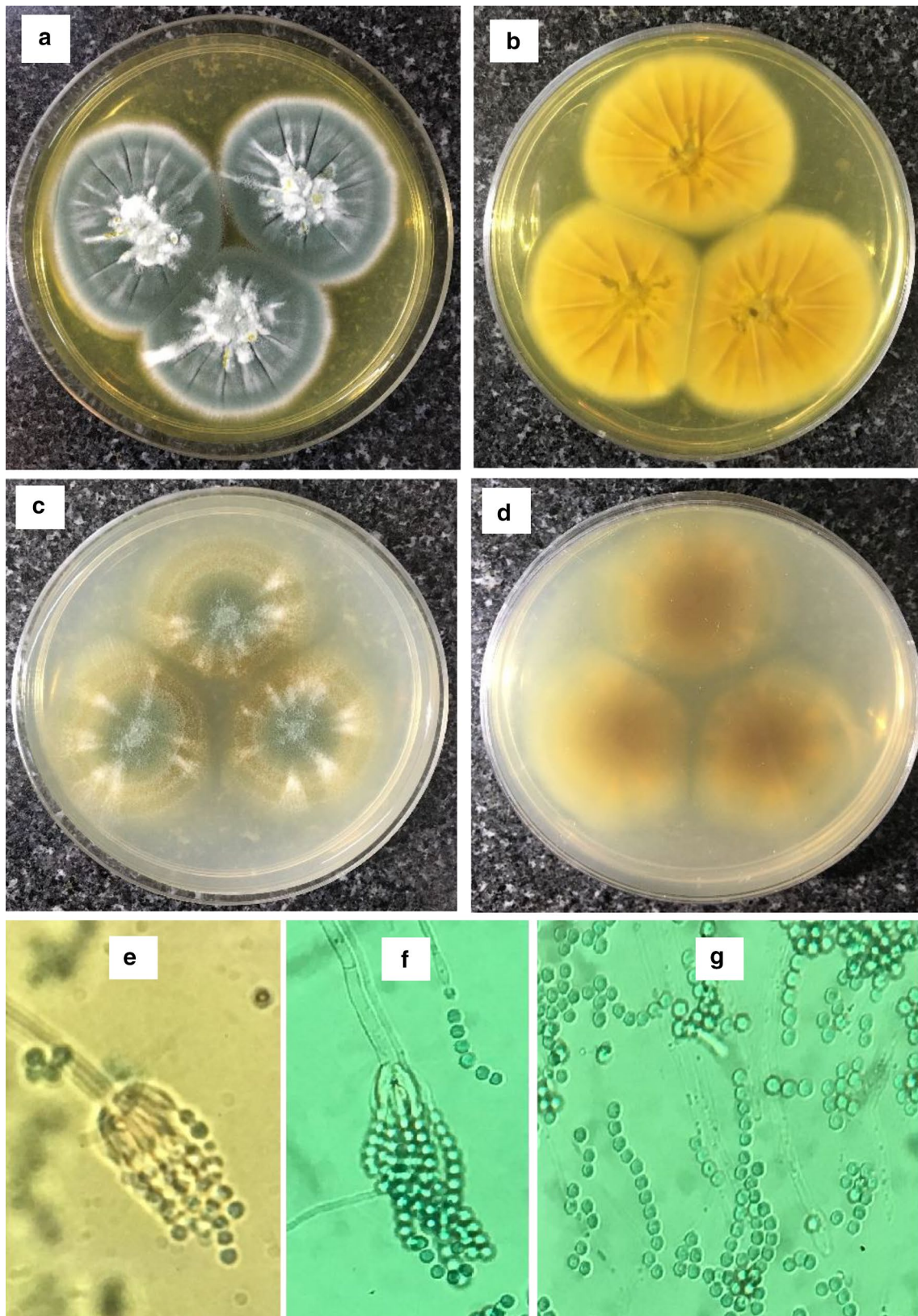


Fig. 3 *Penicillium GGST1*, cultures incubated for 7 days at 25 °C. **a** Obverse MEA, **b** reverse MEA, **c** obverse CYA, **d** reverse CYA. **e–g** Conidiophores, phialides and conidia. Scale bar = 10 μ m

Fig. 4 Cladogram based on the ITS sequences which is obtained using MEGA. Pg-ITS refers to the sequence from newly isolated strain. The other sequences are belonged to the TYPE strains of related species, classified in distinct sections

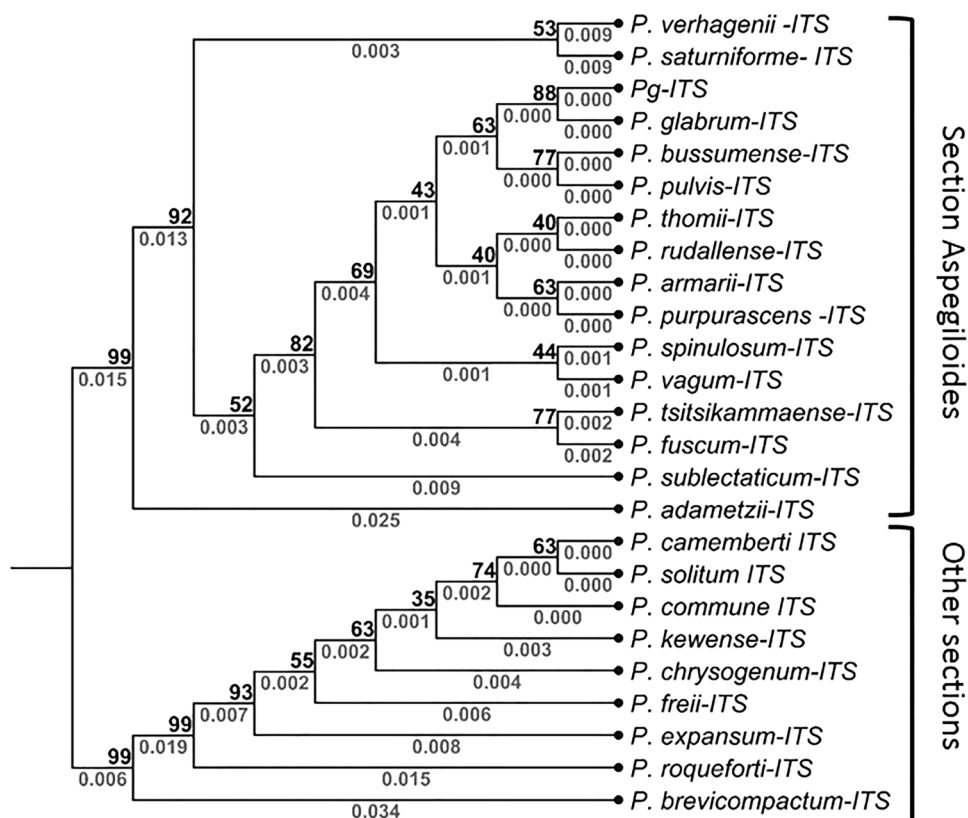


Table 3 The strains that produce MPA

Source	Isolated strain(s)	MPA titer	References
Mouldy maize	<i>P. brevicompactum</i>	–	[37]
Blue-molded cheeses ^a	<i>P. roqueforti</i>	800–4000 mg/kg	[10]
Silage ^a	<i>P. roqueforti</i>	0.02–35 mg/kg	[38]
Food wastes:	<i>P. brevicompactum</i>	0.075–19 mg/kg	[39]
Cereals, fruits, meat, vegetables	<i>P. roqueforti</i>		
Fruits, cheese, bread	<i>p. carneum</i>		
Cereals, bread, meat ^a			
Infected ginger	<i>P. brevicompactum</i>	–	[9]
Tap water	<i>P. brevicompactum</i>	–	[40]
Air intake, Insect ^a	<i>Aspergillus</i> strains	1–3 mg/kg	[31]
Oilseed cakes and indoor air ^a	<i>Aspergillus pseudoglaucus</i>	1–17 mg/kg	[30]
Mould-ripened cheese ^a	<i>Penicillium roqueforti</i>	0.1–23.1 mg/kg	[41]
Grapevine garden soil	<i>P. glabrum</i>	1079 mg/L	This study

^aProduction of MPA on solid media

used for future improvement of the local *P. glabrum* IBRC-M 30518 and possibly low-cost production of MPA [36].

The growth rate of isolated strains was higher and produced more biomass on MEA compared to CYA, in the same time and conditions. This result suggested that, the MEA medium could be a base for medium optimization.

In conclusion, this research was conducted to isolate native MPA producer strains and then identification of the

highest producer by colony morphology combined with molecular analysis. This is the first report showing that *P. glabrum* IBRC-M 30518 can produce this antibiotic which is worthy of further study. However, this assay needs further investigation for large scale production of MPA.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

- Holt CD (2017) Overview of immunosuppressive therapy in solid organ transplantation. *Anesthesiol Clin* 35(3):365–380. <https://doi.org/10.1016/j.anclin.2017.04.001>
- Takahashi K, Go P, Stone CH, Safwan M, Putchakayala KG, Kane WJ, Malinzak LE, Kim DY, Denny JE (2017) Mycophenolate mofetil and pulmonary fibrosis after kidney transplantation: a case report. *Am J Case Rep* 18:399–404. <https://doi.org/10.12659/AJCR.902380>
- Wang Y, Zhou X, Debing Y, Chen K, Van Der Laan LJ, Neyts J, Janssen HL, Metselaar HJ, Peppelenbosch MP, Pan Q (2014) Calcineurin inhibitors stimulate and mycophenolic acid inhibits replication of hepatitis E virus. *Gastroenterology* 146(7):1775–1783. <https://doi.org/10.1053/j.gastro.2014.02.036>
- Xu Z-N, Yang S-T (2007) Production of mycophenolic acid by *Penicillium brevicompactum* immobilized in a rotating fibrous-bed bioreactor. *Enzyme Microb Technol* 40(4):623–628. <https://doi.org/10.1016/j.enzymictec.2006.05.025>
- Kato F, Matsuyama S, Kawase M, Hishiki T, Katoh H, Takeda M (2020) Antiviral activities of mycophenolic acid and IMD-0354 against SARS-CoV-2. *Microbiol Immunol* 64:635–639. <https://doi.org/10.1111/1348-0421.12828>
- Jonsson CA, Carlsten H (2003) Mycophenolic acid inhibits inosine 5'-monophosphate dehydrogenase and suppresses immunoglobulin and cytokine production of B cells. *Int Immunopharmacol* 3(1):31–37. [https://doi.org/10.1016/S1567-5769\(02\)00210-2](https://doi.org/10.1016/S1567-5769(02)00210-2)
- García-Estrada C, Martín J-F (2016) Biosynthetic gene clusters for relevant secondary metabolites produced by *Penicillium roqueforti* in blue cheeses. *Appl Microbiol Biotechnol* 100(19):8303–8313. <https://doi.org/10.1007/s00253-016-7788-x>
- Vinokurova N, Ivanushkina N, Kochkina G, Arinbasarov M, Ozer-skaya S (2005) Production of mycophenolic acid by fungi of the genus *Penicillium* link. *Appl Biochem Microbiol* 41(1):83–86. <https://doi.org/10.1007/s10438-005-0015-7>
- Overy DP, Frisvad JC (2005) Mycotoxin production and post-harvest storage rot of ginger (*Zingiber officinale*) by *Penicillium brevicompactum*. *J Food Prot* 68(3):607–609. <https://doi.org/10.4315/0362-028X-68.3.607>
- Lafont P, Debeaupuis J-P, Gaillardin M, Payen J (1979) Production of mycophenolic acid by *Penicillium roqueforti* strains. *Appl Environ Microbiol* 37(3):365–368. <https://doi.org/10.1128/AEM.37.3.365-368.1979>
- Kure CF, Skaar I, Brendehaug J (2004) Mould contamination in production of semi-hard cheese. *Int J Food Microbiol* 93(1):41–49. <https://doi.org/10.1016/j.ijfoodmicro.2003.10.005>
- Ndagijimana M, Chaves-López C, Corsetti A, Tofalo R, Sergi M, Paparella A, Guerzoni M, Suzzi G (2008) Growth and metabolites production by *Penicillium brevicompactum* in yoghurt. *Int J Food Microbiol* 127(3):276–283. <https://doi.org/10.1016/j.ijfoodmicro.2008.07.019>
- Knob A, Beitel SM, Fortkamp D, Terrasan CRF, Almeida AFd (2013) Production, purification, and characterization of a major *Penicillium glabrum* xylanase using brewer's spent grain as substrate. *Biomed Res Int*. <https://doi.org/10.1155/2013/728735>
- Nevarez L, Vasseur V, Le Madec A, Le Bras M, Coroller L, Leguérinel I, Barbier G (2009) Physiological traits of *Penicillium glabrum* strain LCP 085568 a filamentous fungus isolated from bottled aromatised mineral water. *Int J Food Microbiol* 130(3):166–171. <https://doi.org/10.1016/j.ijfoodmicro.2009.01.013>
- Azaz AD (2003) Isolation and identification of soilborne fungi in fields irrigated by GAP in Harran plain using two isolation methods. *Turk J Bot* 27(2):83–92
- Fraga M, Zonta E, Balieiro F (2011) Isolation and selection of filamentous fungi from petroleum contaminated soil. *Biores Bull* 4:227–235
- Scudamore K, Clarke J, Hetmanski M (1993) Isolation of *Penicillium* strains producing ochratoxin A, citrinin, xanthomegnin, viomellein and vioxanthin from stored cereal grains. *Lett Appl Microbiol* 17(2):82–87. <https://doi.org/10.1111/j.1472-765X.1993.tb00377.x>
- Ghiasian SA, Rezayat SM, Kord-Bacheh P, Maghsood AH, Yazdanpanah H, Shephard GS, Van Der Westhuizen L, Vismer HF, Marasas WF (2005) Fumonisin production by *Fusarium* species isolated from freshly harvested corn in Iran. *Mycopathologia* 159(1):31–40. <https://doi.org/10.1007/s11046-004-3899-5>
- Bartman C, Doerfler D, Bird B, Remaley A, Peace J, Campbell I (1981) Mycophenolic acid production by *Penicillium brevicompactum* on solid media. *Appl Environ Microbiol* 41(3):729–736. <https://doi.org/10.1128/AEM.41.3.729-736.1981>
- Raper KB, Thom C (1949) A manual of the *Penicillia*. Baillière, Tindall & Cox, London
- Ardestani F, Fatemi SS, Yakhchali B, Hosseini SM, Najafpour G (2010) Evaluation of mycophenolic acid production by *Penicillium brevicompactum* MUCL 19011 in batch and continuous submerged cultures. *Biochem Eng J* 50(3):99–103. <https://doi.org/10.1016/j.bej.2010.03.008>
- Elbarbry FA, Shoker AS (2007) Therapeutic drug measurement of mycophenolic acid derivatives in transplant patients. *Clin Biochem* 40(11):752–764. <https://doi.org/10.1016/j.clinbiochem.2007.03.006>
- Visagie C, Houbraken J, Frisvad JC, Hong S-B, Klaassen C, Perrone G, Seifert K, Varga J, Yaguchi T, Samson R (2014) Identification and nomenclature of the genus *Penicillium*. *Stud Mycol* 78:343–371. <https://doi.org/10.1016/j.simyco.2014.09.001>
- Pitt JI (1988) A laboratory guide to common *Penicillium* species. *CSI Res Org Div Food Processing, North Ryde*
- Johnston PR, Jones D (1997) Relationships among *Colletotrichum* isolates from fruit-rots assessed using rDNA sequences. *Mycologia* 89(3):420–430. <https://doi.org/10.1080/00275514.1997.12026801>
- Houbraken J, Visagie C, Meijer M, Frisvad JC, Busby P, Pitt J, Seifert K, Louis-Seize G, Demirel K, Yilmaz N (2014) A taxonomic and phylogenetic revision of *Penicillium* section aspergilloides. *Stud Mycol* 78:373–451. <https://doi.org/10.1016/j.simyco.2014.09.002>
- Nielsen JC, Grijseels S, Prigent S, Ji B, Dainat J, Nielsen KF, Frisvad JC, Workman M, Nielsen J (2017) Global analysis of biosynthetic gene clusters reveals vast potential of secondary metabolite production in *Penicillium* species. *Nat Microbiol* 2(6):17044. <https://doi.org/10.1038/nmicrobiol.2017.44>
- Patel G, Biswas K, Patil MD, Chisti Y, Banerjee UC (2018) Bioreactor studies of production of mycophenolic acid by *Penicillium*

- brevicompactum*. *Biochem Eng J* 140:77–84. <https://doi.org/10.1016/j.bej.2018.09.007>
29. Burkin A, Kononenko G (2010) Producers of mycophenolic acid in ensiled and grain feeds. *Appl Biochem Microbiol* 46(5):545–550. <https://doi.org/10.1134/S0003683810050145>
 30. Séguin V, Gente S, Heutte N, Vêrité P, Kientz-Bouchart V, Sage L, Goux D, Garon D (2014) First report of mycophenolic acid production by *Eurotium repens* isolated from agricultural and indoor environments. *World Mycotoxin J* 7(3):321–328. <https://doi.org/10.3920/WMJ2013.1619>
 31. Mouhamadou B, Sage L, Pérignon S, Séguin V, Bouchart V, Legendre P, Caillat M, Yamouni H, Garon D (2017) Molecular screening of xerophilic *Aspergillus* strains producing mycophenolic acid. *Fungal Biol* 121(2):103–111. <https://doi.org/10.1016/j.funbio.2016.10.001>
 32. Van de Lagemaat J, Pyle D (2005) Modelling the uptake and growth kinetics of *Penicillium glabrum* in a tannic acid-containing solid-state fermentation for tannase production. *Process Biochem* 40(5):1773–1782. <https://doi.org/10.1016/j.procbio.2004.06.044>
 33. Barreto M, Houbraken J, Samson R, Frisvad JC, San-Romão M (2011) Taxonomic studies of the *Penicillium glabrum* complex and the description of a new species *P. subericola*. *Fungal Divers* 49(1):23–33. <https://doi.org/10.1007/s13225-011-0090-4>
 34. Landeweert R, Leeftang P, Kuyper TW, Hoffland E, Rosling A, Wernars K, Smit E (2003) Molecular identification of ectomycorrhizal mycelium in soil horizons. *Appl Environ Microbiol* 69(1):327–333. <https://doi.org/10.1128/AEM.69.1.327-333.2003>
 35. Houbraken J, Samson R (2011) Phylogeny of *Penicillium* and the segregation of trichocomaceae into three families. *Stud Mycol* 70:1–51. <https://doi.org/10.3114/sim.2011.70.01>
 36. Dong Y, Zhang J, Xu R, Lv X, Wang L, Sun A, Wei D (2016) Insertion mutation in HMG-CoA lyase increases the production yield of MPA through agrobacterium tumefaciens-mediated transformation. *J Microbiol Biotechnol* 26(11):1924–1932. <https://doi.org/10.4014/jmb.1603.03038>
 37. Clutterbuck PW, Oxford AE, Raistrick H, Smith G (1932) Studies in the biochemistry of micro-organisms: the metabolic products of the *Penicillium brevicompactum* series. *Biochem J* 26(5):1441. <https://doi.org/10.1042/bj0261441>
 38. Schneweis I, Meyer K, Hörmansdorfer S, Bauer J (2000) Mycophenolic acid in silage. *Appl Environ Microbiol* 66(8):3639–3641. <https://doi.org/10.1128/AEM.66.8.3639-3641.2000>
 39. Rundberget T, Skaar I, Flåøyen A (2004) The presence of *Penicillium* and *Penicillium* mycotoxins in food wastes. *Int J Food Microbiol* 90(2):181–188. [https://doi.org/10.1016/S0168-1605\(03\)00291-5](https://doi.org/10.1016/S0168-1605(03)00291-5)
 40. Gonçalves AB, Paterson RRM, Lima N (2006) Survey and significance of filamentous fungi from tap water. *Int J Hyg Environ Health* 209(3):257–264. <https://doi.org/10.1016/j.ijheh.2005.12.001>
 41. Cakmakci S, Gurses M, Hayaloglu AA, Cetin B, Sekerci P, Dagdemir E (2015) Mycotoxin production capability of *Penicillium roqueforti* in strains isolated from mould-ripened traditional Turkish civil cheese. *Food Addit Contam* 32(2):245–249. <https://doi.org/10.1080/19440049.2014.997808>

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