Mycologia Iranica 6(1): 9 – 20, 2019 Original Article DOI: 10.22043/mi.2020.121030

Molecular identification of *MPaB* and *MPaE* genes from MPA gene cluster in new strain of *Penicillium brevicompactum*

N. Vatani

F. Rahmani ✉

Department of Biology, Faculty of Sciences, Urmia University, Urmia, Iran
&
Institute of Biotechnology, Urmia University, Urmia, Iran

Y. Ghosta

Department of Plant Protection, Faculty of Agriculture, Urmia University, Urmia, Iran

Abstract: Mycophenolic acid (MPA) is a fungal metabolite possessing antiviral, antifungal, antibacterial, antitumor and anti-psoriasis activities. It is being used as an immunosuppressive agent in kidney, heart and liver transplantation patients. In the presence of MPA, the proliferation of the B and T lymphocytes is inhibited. The *MPaB* and *MPaE* genes reside in a 25 kb gene cluster in the genome of *Penicillium brevicompactum*. In this study, the genomic DNA was extracted from *P. brevicompactum* grown on potato dextrose (PD) medium. To amplify the *MPaB* and *MPaE* fragments, the specific primers were designed using Gene Runner software according to *P. brevicompactum* IBT23078 sequence database under HQ731031.1 accession number. The amplified *MPaB* and *MPaE* genes were cloned in the PTG19-T PCR cloning vector and transformed to *Escherichia coli* (*E. coli*) top 10 competent cells. The insertion of *MPaB* and *MPaE* in the PTG19-T cloning vector was further confirmed by PCR. The *MPaB* and *MPaE* amplification produced amplicons of 1477 and 780 (nt), respectively, with the same length according to the *MPaB* and *MpaE* genes deposited in the GenBank. However, the alignment results showed some differences at nucleotide and amino acid levels, implying a new strain of *P. brevicompactum*.

Key words: Mycophenolic acid, sequence, cloning, transplantation, alignment

INTRODUCTION

Mycophenolic acid (MPA) is a fungal metabolite that was early introduced by Bartolomeo Gosio in 1893 as an antibiotic against *Bacillus anthracis*. Moreover, MPA possess antiviral (Barroto et al. 2004), antifungal (Nicoletti et al. 2004), antibacterial (Kavanagh 1947), antitumor (Tressler et al. 1994) and antipsoriasis activities (Muth et al. 1975). Most significantly, MPA is being prescribed as an immunosuppressant in kidney, heart and liver (Geissler & Schlitt 2019) transplantation patients (Geissler & Schlitt 2019) and commercialized under the brands entitled CellCept (mycophenolate mofetil, Roche) and myfortic (mycophenolate sodium, Novartis) (Patil et al. 2012). Mycophenolate, the main combination in both drugs, inhibits IMP dehydrogenase (IMPDH) (Chang et al. 2018). MPA is an uncompetitive and reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH) and therefore inhibits the *de novo* pathway of guanosine nucleotide synthesis without incorporation to DNA (Danafar & Hamidi 2015, Farazi et al. 1997, Regueira et al. 2011). The T and B lymphocytes are associated with their proliferation on *de novo* synthesis of purines, whereas other cell types can utilize salvage pathways (Regueira et al. 20119). The MPA has potent cytostatic effects on lymphocytes (Guzara et al. 2016) and accordingly, is the definite cause that why MPA are applied widely as an immunosuppressive pharmaceutical (Danafar & Hamidi 2015, Asfari et al. 2005).

The molecular basis of MPA biosynthesis was unknown until recently. The genomic cluster that may be responsible for MPA biosynthesis has been identified in *P. Brevicompactum.* In this fungus, the cluster consists of eight genes including *MPaA* (encoding Prenyltransferase), *MPaB* (encoding a protein with unknown function), *MPaC* (encoding Polyketide synthase), *MPaD* (encoding P450 monooxygenase), *MPaE* (encoding Zn-dependent hydrolase), *MPaF* (encoding IMP dehydrogenase), *MPaG* (encoding an O-methyltransferase) and *MPaH*

Submitted 18 Jan. 2019, accepted for publication 16 May 2019
✉ Corresponding Author E-mail: f.rahmani@urmia.ac.ir

http://mij.areeo.ac.ir

(encoding an oxidative cleavage enzyme) (Del Cid et al. 2016).

By using various techniques, only three out of the eight *MPa* genes from *P. brevicompactum* have been experimentally shown to be involved in the MPA biosynthesis. The *MPaC* is the main gene in this cluster and consequently, mutant strain missing this gene loses its ability of MPA synthesis. The *MpaC* gene catalyzes the formation of 5-methylorsellinic acid (5-MOA), which is the first step in the MPA biosynthesis (Regueira et al. 2011, Dubus et al. 2002). The *MPaD* and *MPaE* genes were biochemically characterized *in vivo* by heterologous expression in a strain of *Aspergillus nidulans* which expresses MPaC and produces 5-MOA (Hansen et al. 2012). This biochemical characterization provides the second step in the MPA biosynthesis. The MpaG gene, the putative O-methyl transferase, was biochemically characterized *in vitro*. The results indicated that MpaG catalyzes the methylation of demethyl mycophenolic acid (DMMPA) (Brennan & Brakeman. 2019) to produce MPA which is the final step in the biosynthesis of MPA (Zhang et al. 2015).

Several species of *Penicillium* specially *P. brevicompactum, P. stoloniferum* and *P. requeforty* can produce MPA as a secondary metabolite (Ismaiel & Papenbrock, 2015). The purpose of this investigation was cloning and sequencing of *MPaB* and *MPaE* genes from the new strain of *P. brevicompactum*. Finally, these sequences were aligned with *MPaB* and *MPaE* genes, registered in NCBI GenBank.

MATERIALS AND METHODS

Strains and plasmids

The *P. brevicompactum* fungus was obtained from the strain collection at the Agriculture Faculty of Urmia University and used as the source of genomic DNA. The plasmid PTG19_T harboring the ampicillin resistance cassette, under the control of the Lac promoter, was used as a template for constructing the gene-targeting manipulation of plasmid DNA and introduction of plasmids into *E. coli* BL21 by chemical transformation, according to the standard procedures.

Genomic DNA isolation

Genomic DNA was extracted from culture using Plant Genomic DNA Extraction Kit (IBRC cat no. MBK0011) according to the manufacturer's guideline. The quality and quantity of the purified genomic DNA were assessed by 1% agarose gel electrophoresis in 1x TBE buffer containing 0.5 µg/mL ethidium bromide (Lee et al. 2012) and spectrophotometer (260/280 nm) Biophotometer (Eppendorf, Germany), respectively.

Gene amplification

The *MPa* genes were amplified by PCR using genomic DNA as template. The specific primers were designed using Gene Runner software according to the *P. brevicompactum* IBT23078 sequence database under HQ731031.1 accession number, according to the available nucleotide sequences on the NCBI GenBank. The specific primers for *MPaB* and *MPaE* genes amplification were as follows:

MPaBFWD: 5′-ATG TCTTTGCCTTTGCCTCCAG-3′,
MPaBREV: 5′-CTAATGGAAGGGACATTTCCCCGT–3
MPaEFWD: 5′-ATGATCAAATCGCAAACGGTCATC-3′
MPaEREV: 5′-TTACTTCTGTCCTTCTATG GAATTCTC AATATC-3′

PCR amplification was performed in a 25 µL total volume reaction containing 100 ng of template DNA, 10 µM for each primer, 2 mM Mg^{2+}, 200 µM of each dNTP, 1x PCR buffer and 2.5 unit of *Taq* DNA polymerase. The following conditions were used for the amplification: hot start at 94 °C (5 min), followed by 30 cycles of denaturation at 94 °C (1 min), annealing at 58 °C (1 min) and extension at 72 °C (2 min). The PCR products were analyzed by electrophoresis in 1% agarose gel in 0.5 X TBE buffer and visualized by ethidium bromide staining on UV transilluminator. The PCR product was purified from agarose gel by high pure PCR product Gel Purification Kit (Yekta Tajhiz cat no.YT9027) according to the manufacturer's guideline.

MPaB and *MPaE* genes cloning

DNA bands were sliced under the long-wave ultraviolet (UV) light and recovered by purification kit, subsequently, the purified PCR products were ligated into PTG19_T cloning vector by T/A cloning strategy according to the manufacturer's instructions (Vivantis, USA). This reaction was performed in a single tube contained: PTG19-T, ligation buffer, PCR product, T4-DNA ligase and sterile Milli-Q water. The ligation product was kept at 16 °C for 16 h.

Transformation and recombinant plasmid isolation

Escherichia coli BL21 competent cells were prepared and the recombinant vectors were transformed into the competent *E. coli* BL21 cells. The amount of 100 µl of chemo competent cells were added to 5 µl of ligation products. The cells were transformed by the process of heat-shocking. After recovery at 37 °C for 1 h in 100 µl Luria-Bertani medium (LB) without antibiotics, transformed cells were spread on the LB agar plates containing 100 µg/ml ampicillin (Lapointe et al. 2016). The agar plates were incubated overnight at 37 °C. The bacterial clones harboring recombinant plasmid DNA were screened based on their colony PCR. The PCR was used for fidelity verification of *E. coli* BL21 transformants. In this stage, the number of 5 colonies was selected and individual colonies subjected directly to the PCR master mix using Thermo Scientific reagents. Finally, positive PCR colonies were cultured in LB broth containing ampicillin and used for recombinant DNA extraction. The plasmids were purified using the miniprep, Plasmid Mini Kit (Yekta Tajhiz Cat no. YT9001).

Nucleotide sequences analysis

After the selection of positive screened colonies using colony-PCR, the purified plasmids were subjected to sequencing (Macrogen, South Korea). The obtained nucleotide sequences were analyzed by homology search and alignment with other *MPaB* and *MPaE* genes using Basic Local Alignment Search Tool (BLAST) and Clustal W software, respectively.

RESULTS

Amplification of *MPaB* and *MPaE* genes

The genomic DNA of *P. brevicompactum* was extracted and *MPaB* and *MPaE* genes were amplified with the resulting fragments of 1477 and 780 bp, respectively, compared to the 100 bp DNA ladder (Fig. 1).

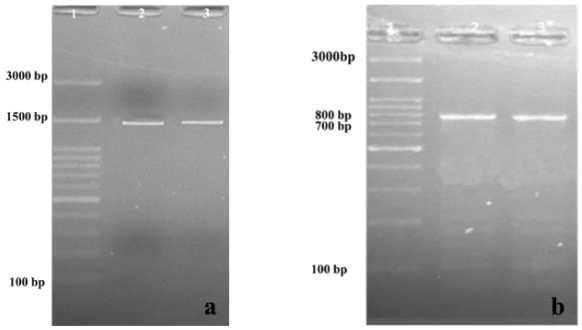

Fig. 1. a. Gel electrophoresis for detection of PCR products of *MPaB*, lane 1 shows 100 bp DNA ladder. Lanes 2 and 3 show amplified fragments by PCR using the specific primers for *MPaB* which correspond to 1477 nt. b. Gel electrophoresis for detection of PCR products of *MPaE*, lane 1 shows 100 bp DNA ladder. Lanes 2 and 3 show amplified fragments by PCR using the specific primers for *MPaE* which correspond to 780 nt.

Confirmation of cloning

Among selected colonies on agar plates, some colonies showed amplified fragments of *MPaB* and *MPaE* genes (Fig. 2) on 1% agarose gel electrophoresis.

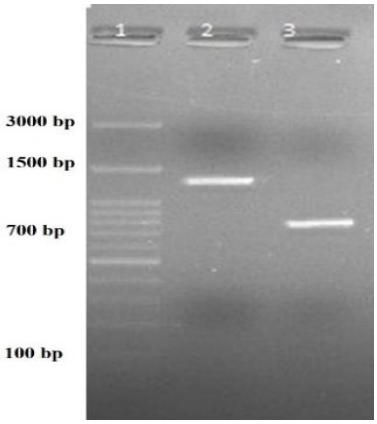

Fig. 2. Agarose gel displaying the results of colony PCRs to check for positive insertion events in transformation assays. Lane 1 shows 100 bp DNA ladder. Lane 2 shows PCR amplification with the MPaB specific primers to check for positive cloning events of MPaB gene (1477 nt). Lane 3 shows PCR amplification with the MPaE specific primers to check for positive cloning events of MPaE gene (780 nt).

Alignment of *MPaB* and *MPaE* sequences

Various sequences of the *MPaB* and *MPaE* genes have been recorded in the GenBank, obtained by different sequencing methods. The names, accession numbers and other information are given (Table 1). Furthermore, the alignment of these recorded genes was performed (Fig. 3, Fig 4).

Table 1. Various sequences of the *MPaB* and *MPaE* gene recorded in the GenBank.

Source	Strain	Accession number	nt	Identity (%)
***MPaB* gene**				
MPaB, Penicillium Brevicompactum	IBT23078	HQ731031	1477	
MPaB', Penicillium Brevicompactum	NRRL864	KM595305	1443	79
hypothetical protein. *Penicillium brasilianum*	PMG11_03546	CDHK01000003	1284	55
conserved hypothetical protein, *Aspergillus fumigatus*	Af293	XM_747362	1309	55
conserved hypothetical protein, *Aspergillus fumigat*	A1163	DS499594	1309	55
hypothetical protein, *Neosartorya udagawae*	AUD_7781	BBXM01000137	1137	55
hypothetical protein. *Aspergillus fumigatus Z5*	Y699_08182	KQ087361	1287	55
Domain of unknown function DUF2236	FM164	HG792019	897	70
Permease, cytosine/purine, uracil, thiamine, allantoin, *Penicillium italicum*		JQGA01000866	2958	58
similar to An08g03170, *Aspergillus kawachii*	IFO 4308	DF126468	1226	55
unnamed protein product, *Aspergillus niger*		AM270165	1341	55
hypothetical protein ANI_1_1818074, *Aspergillus niger*	CBS 513.88	XM_001392397	1293	55
***MPaE* gene**				
putative metallo-beta-lactamase superfamily II enzyme, *Penicillium brevicompactum*	IBT23o78	HQ731031.1,	786	
MpaD/MPaE fusion protein, *Penicillium brevicompactum*		BK008023.1	2562	100
MpaDE', *Penicillium brevicompactum*	NRRL864	KM595305.1	2562	98
Cytochrome P450, *Penicillium roqueforti*	FM164	HG792019.1	2799	80
pisatin demethylase, *Neosartorya udagawae*	IFM 46973	BBXM01000144.1	2520	58

```
DF126461.1          ------------------------------------------------------------
KQ087361.1          -----MEQLAAGLKNGPLAILTTDVSHVGFGRLGPYILAGLAAYPVLCSFLRFRKMRWLH
AM270165.1          ------------------------------------------------------------
XM001392397.2       ------------------------------------------------------------
HG792019.1          ------------------------------------------------------------
HQ731031.1          ------------------------------------------------------------
KM595305.1          ------------------------------------------------------------
CDHK01000003.1      ------------------------------------------------------------
JQGA0100086601      MKPDDVEKAHSSPTLGSPSQFHLD-------KSASNSSSEVSDFAKPDPVLQSRCHRWIA


DF126461.1          ------------------------------------------------------------
KQ087361.1          RKYNFPTRESLARMTDDEAWEIQR-VLLQQEFPFF----------------------FIK
AM270165.1          ------------------------------------------------------------
XM001392397.2       ------------------------------------------------------------
HG792019.1          ------------------------------------------------------------
HQ731031.1          ------------------------------------------------------------
KM595305.1          ------------------------------------------------------------
CDHK01000003.1      ------------------------------------------------------------
JQGA0100086601      SLKNVESCGI-EPIPIEEREPITSSTILHMLLMWFSMTLATNNIVVGSMGTLVLGLSFRD

DF126461.1          ------------------------------------------------------------
KQ087361.1          ALQFALFRTYGIPTISGLLTATSQFSNPETSLKRYTDTSVLIQEFMGNAPSSERACIALA
AM270165.1          ------------------------------------------------------------
XM001392397.2       ------------------------------------------------------------
HG792019.1          ------------------------------------------------------------
HQ731031.1          ------------------------------------------------------------
KM595305.1          ------------------------------------------------------------
CDHK01000003.1      ------------------------------------------------------------
JQGA0100086601      AALCAVFGCLAGNCIVGFISIW----GPRS----GNRTLIVARYFMGYYPSK—VCIILN

DF126461.1          ------------------------------------------------------------
KQ087361.1          RTRFLHTGYRAAGKIQED----------------------DMLYTLGLFAIQPVRFIEKFE
AM270165.1          ------------------------------------------------------------
XM001392397.2       ------------------------------------------------------------
HG792019.1          ------------------------------------------------------------
HQ731031.1          ------------------------------------------------------------
KM595305.1          ------------------------------------------------------------
CDHK01000003.1      ------------------------------------------------------------
JQGA0100086601      --LFTNIGYSMVNSVVGGQILSVVSGGHLSVIVGIIIIAVSSWAMALFGMKIFQLYERVA

DF126461.1          ------------------------------------------------------------
KQ087361.1          WRTLSDMEKCALGTFWKSIGDGLDISYENLPSS-----------KTGFRDGLHWLEEIMA
AM270165.1          ------------------------------------------------------------
XM001392397.2       ------------------------------------------------------------
HG792019.1          ------------------------------------------------------------
HQ731031.1          ------------------------------------------------------------
KM595305.1          ------------------------------------------------------------
CDHK01000003.1      ------------------------------------------------------------
JQGA0100086601      WLPQLLVI-CV---MVGSAGPNMDFNIQTPMSTEQLIAKRLTFFSLALSIGLAWAPLAAD

DF126461.1          ------------------------------------------------------------
KQ087361.1          WSDE-----YEVRSMLPDVKNRETADQTTAVLLYMIPG--PL-------------QPIGL
AM270165.1          ------------------------------------------------------------
XM001392397.2       ------------------------------------------------------------
HG792019.1          ------------------------------------------------------------
HQ731031.1          ------------------------------------------------------------
KM595305.1          ------------------------------------------------------------
CDHK01000003.1      ------------------------------------------------------------
JQGA0100086601      YYVYLPPQMKSSRTFLATVSAATT---AMSIVLLMGIGLGTVIASSAHFTAKYGSSPGGV

DF126461.1          ------------------------------------------------------------
KQ087361.1          KFVSFMM--------DDRLRKAMLYDAPPAAYTKVFSFILRVRRFVMRYLALPRPYFLRY
AM270165.1          ------------------------------------------------------------
XM001392397.2       ------------------------------------------------------------
HG792019.1          ------------------------------------------------------------
HQ731031.1          ------------------------------------------------------------
KM595305.1          ------------------------------------------------------------
CDHK01000003.1      ------------------------------------------------------------
JQGA0100086601      LMTAYDGLGGFGKFCAVINVLALVANNTPGAYSMGMNFQMIG----GVFGKVPQPVFTTL

DF126461.1          ------------------------------------------------------------
KQ087361.1          QPY--T----ERPDEHNRIFVTQWDAAPYY---------VKPTFWNRWGPTAWLTWALGK
AM270165.1          ------------------------------------------------------------
XM001392397.2       ------------------------------------------------------------
HG792019.1          ------------------------------------------------------------
HQ731031.1          ------------------------------------------------------------
```

```
KM595305.1          ------------------------------------------------------------
CDHK01000003.1      ------------------------------------------------------------
JQGA0100086601      ATVIYAACAMGGRDQLYQIFKSFLPLIGYWVMMFVVIVFEEHTIFRWRKGYDWSQWNCRD

DF126461.1          ------------------------------------------------------------
KQ087361.1          PLPGDVGD-KYYPQGYYTADVG--PKYFEGKGQQTVEKITEELKVSRAGKC-----PFMM
AM270165.1          ------------------------------------------------------------M
XM001392397.2       ------------------------------------------------------------
HG792019.1          ------------------------------------------------------------
HQ731031.1          ------------------------------------------------------------
KM595305.1          ------------------------------------------------------------
CDHK01000003.1      ------------------------------------------------------------
JQGA0100086601      KLPLGIAAGAAFLIGWAGAVVGMSQAYYIGPIAQMAAEADLGLWL-GAGFTGMFFPPLRA

DF126461.1          ---------------MD-----NSTQLTPGKF-----LGFV--------------------
KQ087361.1          LQLRSYLRAITEDHHHH-----QTHPLTPGKF-----LGFV--------------------
AM270165.1          LQLRSYLRAITEDHHHH-----QTHPLTPGKF-----LGFV--------------------
XM001392397.2       ---------------MD-----NTTQLTPGKF-----LGFV--------------------
HG792019.1          --------------------------------MDKGTSFFTTPSF-----------------
HQ731031.1          -----------------------------------MSLPLPPAL-----------------
KM595305.1          -----------------------------------MSLSLPPAL-----------------
CDHK01000003.1      ------------------------------MDNATSLLASQSL-----------------
JQGA0100086601      LEL-KFLGLLTRHDRVHASHTTENAIVGPGNVDSHAHFLFPEARTPSAEVPIPRHHVNMN
                                                    :
DF126461.1          ---PNTDPRVAGLGFQQLVTCVCLLLPAYAFLVSQLRFRYLRRLHQKYPYTTRESFSRMT
KQ087361.1          ---PNTDPR-AGLGFQQLVTYACLLLPAYAFLVSHLRFRYLQRLHQKYPYNTRESFSRMT
AM270165.1          ---PNTDPR-AGLGFQQLVTYACLLLPAYAFLVSHLRFRYLQRLHQKYPYNTRESFSRMT
XM001392397.2       ---PNTDPR-AGLGFQQLVTYACLLLPAYAFLVSHLRFRYLQRLHQKYPYNTRESFSRMT
HG792019.1          ---SAT-TRAIFNTMPQWFSFAVGLLIAYPLLINSLRYRRLKQLQKKFYFPTRESMAKMT
HQ731031.1          ---SEL-ARALPYSRTQWLPIFVGFLIGYPILIRALRYK----------------------
KM595305.1          ---SEL-ARALPYSRTQWLPILVGFLIGYPLLIKALRYKRLGEMKKKFYFPTRESMAEMT
CDHK01000003.1      ---TDAARALLDAAPRQWLPYVLGLLVGYPLLTGSLRYQRMRNMYKKYPYVTRHDMANMT
JQGA0100086601      YTSSWTSPLPAEGMTTQLFPYAVGLLLAWLVVTTTLRFQRLRKLHKQYAYPTRESMSKMT
                                  * .     :* .: .:   **::
DF126461.1          DDEACEIQKGLVQLEFPFLYLKSLQFALFRTYGIPTISTVLTNTAQFSKPETSLKRYADT
KQ087361.1          DDEACEIQKGLVQLEFPFLYLKSLQFALFRTYGIPTISTVLTNTTQFSKPDTALKRYADT
AM270165.1          DDEACEIQKGLVQLEFPFLYLKSLQFALFRTYGIPTISTVLTNTTQFSKPDTALKRYADT
XM001392397.2       DDEACEIQKGLVQLEFPFLYLKSLQFALFRTYGIPTISTVLTNTTQFSKPDTALKRYADT
HG792019.1          DEEAFQIQKETAQLEFPFMFVKAGQFALFRTYGIPTISHLLIKTGQFSKPETSFKRYTDT
HQ731031.1          --------------EFPFMFLTSGQFALFRTYGIPTISHLLTKTGQFSKPETSFKRYTDT
KM595305.1          DEEAFLIQKEMAQLEFPFMFLTSGQFALFRTYGIPTISHLLTKTGQFSKPETSFKRYTDT
CDHK01000003.1      DDHAFEIQKAVAQLEFPFMFIKSLQFALFRTYGIPTISHLLTKTSQFSSPETSLKRYTDT
JQGA0100086601      DEEAFQIQKQVAQLEFPLMFMKSLQFALFRTYGIPSISTLLAKTSQFSSPETSLKRYTDT
                              ***:::::.: ***********:** :* :* ***.*:*::***:**
DF126461.1          VALVQEFVGHAPTSRRACTSIARTRWIHSSYRASGKILEDDMLYTLGLFAIQPVRFVKMY
KQ087361.1          AALIQEFVGHAPTSRRACTSIARTRWIHSNYRASGKILEDDMLYTLGLFAIQPVRFVEMY
AM270165.1          AALIQEFVGHAPTSRRACTSIARTRWIHSNYRASGKILEDDMLYTLGLFAIQPVRFVEMY
XM001392397.2       AALIQEFVGHAPTSRRACTSIARTRWIHSNYRASGKILEDDMLYTLGLFAIQPVRFVEMY
HG792019.1          AALIGEMVENSPTSQRAFLSVARTRFLHSGYQASGKILDTDLLYTLALFAVQPVNFIAAF
HQ731031.1          AALIGEMVENSPTSQRAFISVARTRFLHSGYQASGKILDADLLYTLALFAVQPVRFIENF
KM595305.1          AALIGEMVENSPTSQRAFISVARTRFLHSGYQASGKILDADLLYTLALFAVQPVRFIENF
CDHK01000003.1      SALVQEMVGNSPTSARAYASFARTRFLHSGYRASGKILDDDMLYTLALFALEPIRFISRY
JQGA0100086601      SVLVQEMVGNNPTSARAYLGLARTRYLHSGYRASGKILDDDMLFTLALFALQPIRFINRY
                     .*: *:* . *** **  ..****::** *:******: *:*:**.***::*:.*:  :
DF126461.1          EWRDLSDLERCAIGTFWKSLGDSLNISYDALPSGKTGFRDGLHWLEEIMAWSDDYEVRCM
KQ087361.1          EWRDLSDLERCAIGTFWKSLGDSLDISYDALPSGKTGFRDGLHWLEEMMVWSDDYEVRCM
AM270165.1          EWRDLSDLERCAIGTFWKSLGDSLDISYDALPSGKTGFRDGLHWLEEMMVWSDDYEVRCM
XM001392397.2       EWRDLSDLERCAIGTFWKSLGDSLDISYDALPSGKTGFRDGLHWLEEMMVWSDDYEVRCM
HG792019.1          EWRPLSDLERCAIGTFWKSLGDALGISSDILPSGKTGFRDGIHWLEEVDTWSQEYEVKYM
HQ731031.1          EWRTLSDLELCAIGTFWKSLGDALGISSEILPSGKTGFKDGIQWLEEVDVWSQDYEAKYM
KM595305.1          EWRTLSDLELCAIGTFWKSLGDALGISSEILPSGKTGFKDGIQWLEEVDVWSQDYEAKYM
CDHK01000003.1      EWRELSDLERCAIGTFWKSAGDALGIGYEKLPSGATGFKDGIQWLEELEAWSEAYEAKCM
JQGA0100086601      EWRQLSDLERCAIGTFWKSLGDALDIRYEKLPSGKTGFRDGIQWLEEIDAWSKEYEARCM
                    *** ***** ********* **:* *  : **** ***:**::****: .**. **.: *

DF126461.1          VPHVKNREVAEQTIAVLLYMLPQSLQFIG.MRFVSFMMDDRLRKAMLIDPPPAFDAKLFSL
KQ087361.1          VPHLKNREVADQTIAVLLYMLPQPLHFIG.MRFVSFMMDDRLRKAMLIDPPPAFDAKLFSL
AM270165.1          VPHLKNREVADQTIAVLLYMLPQPLHFIG.MRFVSFMMDDRLRKAMLIDPPPAFDAKLFSL
XM001392397.2       VPHLKNREVADQTIAVLLYMLPQPLHFIG.MRFVSFMMDDRLRKAMLIDPPPAFDAKLFSL
HG792019.1          VPDAQNRESADQATAVLLYNLPKVFHPVGLQFTSFMMDDRLRKAMLYVE-----------
HQ731031.1          VPDPKNRESADQATAMLIS-------------------------NRYEAPTPGWSMVFST
KM595305.1          VPDPKNRESADQATAVLLYNLPKILHPIGLQFTSYMMDDRLRKAMLYEAPSPGWSAVFSS
CDHK01000003.1      VPDVKNRETADQTTAVLLYMLPKVLHPVGLQFVSFMMDERLRKAMLYDAPSSVAVIAFSS
JQGA0100086601      VPHAKNRETADQTTAVLVYMLPKMLHPVGLQVVSYMMDDRLRKAMFYDPPSALCSTLLSA
                    **. :*** *:*: *:*:
DF126461.1          LFSARRFALRYLALPRPYFLRYVSYTDDYNKDGRIYMTNWDAAPYYVEPSFRNRWGPVAW
KQ087361.1          LFSARRFALRYLALPRPYFLRYVSYTDDYNENGRIYMTNWDAAPYYVKPSLRNRWGPVAW
```

```
AM270165.1          LFSARRFALRYLALPRPYFLRYVSYTDDYNENGRIYMTNWDAAPYYVKPSLRNRWGPVAW
XM001392397.2       LFSARRFALRYLALPRPYFLRYVSYTDDYNENGRIYMTNWDAAPYYVKPSLRNRWGPVAW
HG792019.1          ------------------------------------------------------------
HQ731031.1          LLAIRKLILRYLSPPRPAALAVSNIAQKPDKDDRYHRMSWDALPFYIRPTFWNRWGPMAW
KM595305.1          LLATRKFVLRYLSPPRPAALAVSNIAQKPDKDDRYHRMSWDALPFYIRPTFWNRWGPMAW
CDHK01000003.1      LLTVRKLFLRYLSLPRPYFLRSVSFTDEPDEHNRFFLTQWEAAPYYVKPTFWNRWGPTAL
JQGA0100086601      ILTARRLFLRYLALPRPYFLRFASFTEEPDQNNRFFLTEWEAAPYYVKPTFWNRWGPMAW

DF126461.1          LTWVLGRPLPGDEGDKYYPKGYDNLNVGPKYFEGKGRQGMEDIMKELETTRRGQCPFH
KQ087361.1          LTWALGRPLPGDEGDKYYPKGYDNLNVGPKYFEGKGRQGMEDIMKELENTRRGQCPFH
AM270165.1          LTWALGRPLPGDEGDKYYPKGYDNLNVGPKYFEGKGRQGMEDIMKELENTRRGQCPFH
XM001392397.2       LTWALGRPLPGDEGDKYYPKGYDNLNVGPKYFEGKGRQGMEDIMKELENTRRGQCPFH
HG792019.1          ------------------------------------------------------------
HQ731031.1          ISWLMGHPVPGDLGQKKGPQGDPGNDEGIKDLKDGEM-SLPLVWSKYHATTND-----
KM595305.1          ISWLMAHPVPGDHGQKYYPQGYHIQDIGPKYFEGKGHKEIQEMMKELKISRTGKCPFH
CDHK01000003.1      LTRILGRPVPGDERDKYYPQGYSIQDVGPKYFEGKGRKAIEEAMEEFKEYRTGKCPFH
JQGA0100086601      LTWALGRPVPGDDGDKYYPNGYSVPDVGPKYFEGKGKKQLDETILELKGYRTGKCPFH
```

Fig. 3. Alignment of amino acid sequences of the most recognized MPaB proteins registered in GenBank which their implications are listed in Table 1.

```
BBXM01000144.1      ------------------------------------------------------------
HG792019.1          MRVEAGGDMRDKLMWIRLYILGNVGQTFGDMKRYIGMWSGMLFPISTKHTRRLISILKVY
KM595305.1          ------------------------------------------------------------
HQ731031.1          ------------------------------------------------------------
BK008023.1          ------------------------------------------------------------

BBXM01000144.1      -----------------MTMLDKIDIPRLAAAAVALYAFYRAFQSFVRLSHVPGPFIAKF
HG792019.1          FIFILNPPAQVYVQDINLYTMDYLIIIRITAVAVVLYLTRYVCCLYLHLQDVPGPLFAKF
KM595305.1          --------------------MESLSLTWITAIAVVLYLVQRYVRSYWRLKDIPGPVLAKL
HQ731031.1          ------------------------------------------------------------
BK008023.1          --------------------MKSLSLTWITAVAVVLYLVQRYVRSYWRLKDIPGPVLAKL

BBXM01000144.1      TNLQRVWWVKSGRAHEYHRQMHERYGKLVRFGPNMVSISDPGAMSIVYPNRPGFQK----
HG792019.1          TNLQRVWWVKSGRAHEYHRRMHAVYGPAVRFGPNMVSISDPRTIPAIYPSRPGFPKSDFY
KM595305.1          TDLQRVWWVKTGRAHEFHRDMHAMYGPIVRFGPNMVSVSDPRVIPTIYPSRPGFPKGDFY
HQ731031.1          ------------------------------------------------------------
BK008023.1          TDLQRVWWVKTGRAHEFHRDMHAMYGPIVRFGPNMVSVSDPRVIPTIYPSRPGFPKGDFY

BBXM01000144.1      ---RPYSPKSGVLPAVFNTQDETLHRQLRKPIASLYSMTSIVGSEPLVDQTLEILFRQLD
HG792019.1          RTQKPYTPNKGAMPAVFNSQDEDLHKRLRSPIAPLYSMTNVVKLESFVDQTLAVLLEQLD
KM595305.1          RTQKPYTRNKGAMPAVFNTQDEDLHKQLRSPIASLYSMTNVVRLEPLVDETLTVLSKQLD
HQ731031.1          ------------------------------------------------------------
BK008023.1          RTQKPYTRNKGAMPAVFNTQDEDLHKQLRSPIASLYSMTNVVRLEPLVDETLTVLSKQLD

BBXM01000144.1      LRFG-ATGRSLDLAEWLQFFAFDVMGMLSFSKRHGFLEQGRDVRGILGGIWAFMKTVAPV
HG792019.1          GRFLGSNDVPFDLGSWLQYFAFDSMGTLTFSRRYGFLEQGRDMNGILGEIWKFMKRVSVM
KM595305.1          ERFVGTNDKPFDLGDWLQYFAFDSMGTLTFSRRYGFLEQGRDMHGILQEIWNFMTRVAVM
HQ731031.1          ------------------------------------------------------------
BK008023.1          ERFVGTNDKPFDLGDWLQYFAFDSMGTLTFSRRYGFLEQGRDMHGILQEIWNFMTRVAVM

BBXM01000144.1      GQIPWFDPVWNKNPIIALFKQTTGLAVLGVVDRFVAERQMSSSQHGAEGKREKRDMLSKF
HG792019.1          GQIPWFDEFCNTNPFIALFRSPTGFGVLKVVDKFILQRLAPRE---KDEVSDEKDMLSQF
KM595305.1          GQIPWFDEIWNKNSFITLFKRPTGFGVLKVVDNFISQRVSSRE---NDEKADEKDMLSQF
HQ731031.1          ------------------------------------------------------------
BK008023.1          GQIPWFDEIWNKNSFITLFKRPTGFGVLKVVDNFISQRVSSRE---NDEKADEKDMLSQF

BBXM01000144.1      LEIQAKDPK-IPAWAPKAWTFSNMLAGSDTTATALTAVMYNLLNCRTSMDTLARELSNAQ
HG792019.1          LNIQASNPD-VMPWAPRAWTFSNIMAGSDSTANVMRTIMYNLLVHRDTLSRLQDELLESE
KM595305.1          LNIQASNPHSIMPWAPRAWTFSNVMAGSDSTANVMRTMMYNLLVDRDTLKSLRAELLEAE
HQ731031.1          ------------------------------------------------------------
BK008023.1          LDIQASNPHSIMPWAPRAWTFSNVMAGSDSTANVMRTMMYNLLVDRDTLRSLRAELLEAE

BBXM01000144.1      RKGRLSRPYPSWHEVRELPYLDACIMEALRLHPPFCLPFERVVPEGGVTVCGTYLAAGTV
HG792019.1          SSNGLSRTCPSWEKVRDLPYLDACVLEALRLHPPFCLPFERVVPGGGLTVCETYLPAGTI
KM595305.1          SSNGLSRSLPSWDGVRSLPYLDACVLEALRLHPPFCLPFERVVPEGGITVCETYLPAGTV
HQ731031.1          ------------------------------------------------------------
BK008023.1          NSNGLSRSLPSWDGVRSLPYLDACVLEALRLHPPFCLPFERVVPEGGITVCETYLPAGTV

BBXM01000144.1      VGMSPYIVNRDRDTYGDDADEWRPERWLNLGEGDRRRLENGILTFGSGRRTCLGRNLAIF
HG792019.1          VGISPYMANRDKETFGNDADEWRPERWLGLSHEDRKRLENSLLTFGAGRRTCLGKNIAIL
KM595305.1          VGISPYLANRDKQTFGDDADKWRPSRWLDLSREDRVKLENSILTFGAGRRTCLGKNIAIL
HQ731031.1          ------------------------------------------------------------
BK008023.1          VGISPYLANRDKQTFGDDADKWRPSRWLDLSREDRVKLENSILTFGAGRRTCLGKNIAIL

BBXM01000144.1      EMKKLLPALLMRYEITAVEPLQLKLENSWLFKQWDLHVHVRLN-----------EALQPP
HG792019.1          EIKKLIPVLLLNYDIQIVNPENYKTENAWFFKQTGLQAVIRKRAKMERGSSNKDKPTLPP
```

```
KM595305.1       EIKKLFPMLLLNYEIEIVNPENYQTTNAWFFRQWGLHAVIRKLPAPERDDTIEQKASIPP
HQ731031.1       ------------------------------------------------------------
BK008023.1       EIKKLFPMLLLNYEIEIVNPENYQTTNAWFFRQWGLQAVIRKLPAPERDDTIEQKASIPP

BBXM01000144.1   PLDVPSSTSTALVRVIDPGTTLDLKPGLFWQPALNGLDKLTVPMYCFLISSGERHILFDL
HG792019.1       VLNIPPSSSTVDVRVIDPGTLLDLRPDLFWQPELPGLRKVTAPTYCFLISVGTRHVLFDL
KM595305.1       ALNIPPSSSTVDVRIIDSGTLLDLRPDLFWTPDLPGLLKVTAPTYCFLISNGSRHVLFDL
HQ731031.1       ------------------------------------------------------------
BK008023.1       ALNIPPSSSTVEVRIIDSGTLLDLRPDLFWTPDLPGLLKVTAPTYCFLISNGTRHVLFDL

BBXM01000144.1   GVRADWENLAPAAAALIRNTTTVYNSRNIADILDTTPIPESSIRTTNIEAIIWSHDHFDH
HG792019.1       GVRQDWERLPPSVVAMIKSQTTIQNPRNISDILDSDA-SSLGIRSTDIEAIIWSHAHFDH
KM595305.1       AVRQDWENLPPSIVAMIKSQTVIQEPRNISDVLDSDE-SSLGIRSKDIEAIIWSHAHFDH
HQ731031.1       ---------------MIKSQTVIQEPRNISDVLDSDE-SSLGVRSKDIEAIIWSHAHFDH
BK008023.1       AVRQDWENLPPSIVAMIKSQTVIQEPRNISDVLDSDE-SSLGVRSKDIEAIIWSHAHFDH
                                 :*:. *.: : ***:*:**:    . .:*:.:******** ****
BBXM01000144.1   IGDPSTFPPSTNLVVGPGVRD-AWPGYPSNPTSRVLDSDIEGRLLREISFGQT---PLKV
HG792019.1       IGDPSTFPLSTELVVGPGIRDSHWPGFPTNPDAINLNSDIQGRKVREISFERTEKEAIKI
KM595305.1       IGDPSTFPPSTELVVGPGIRDTHWPGFPTNPDAINLNTDIQGRNVREISFEKTQKGATKI
HQ731031.1       IGDPSTFPPSTELVVGPGIRDTHWPGFPTNPDAINLNTDIQGRNVREISFEKTQKGATKI
BK008023.1       IGDPSTFPPSTELVVGPGIRDTHWPGFPTNPDAINLNTDIQGRNVREISFEKTQKGATKI
                 ******** **:******:**  ***:*:** :  *::**:** :***** :*     *:
BBXM01000144.1   GPFDAFDYFGDGSFYLLNAPGHSIGHMCGLARVTTSPDTFVFMGADACHHPGVLRPTKYR
HG792019.1       GSFDALDYFGDGSFYLLNAAGHSIGHIGALARVTTSPDSFVFMGGDSCHHAGVLRPSKYL
KM595305.1       GSFDAMDYFGDGSFYLLDAAGHSVGHIGALARVTTSPDSFVFMGGDSCHHAGVLRPTKYL
HQ731031.1       GSFDAVDYFGDGSFYLLDAAGHSVGHIGALARVTTSPVSFVFMGGDSCHHAGVLRPTKYL
BK008023.1       GSFDAVDYFGDGSFYLLDAAGHSVGHIGALARVTTSPVSFVFMGGDSCHHAGVLRPTKYL
                 * ***.***********:* ***:**: .******** :*****.*:*** *****:**
BBXM01000144.1   PLPPGQRSPPGLSPCAACPLTWDESLFKVSPVLASDHARALETVEKIKELDASDDVFVIL
HG792019.1       PCPSHSRHIPL--SS-------ESESVFTLSPVLPSDYDAALKTVDNIKELDAYDNVFLIL
KM595305.1       PCPLDSGDTSL--PC-------KSDSVFTLSPALPTDYTAALRTVENIKELDACEDVFVVL
HQ731031.1       PCPLDSGDTSL--PC-------KSDSVFTLSPALPTDYTAALRTVENIKELDACEDVFVVL
BK008023.1       PCPLDSGDTSL--PC-------KSDSVFTLSPALPTDYTAALRTVENIKELDACEDVFVVL
                 * *  .        .       .:*:*.:**.* :*:  **.**::****** ::**::*
BBXM01000144.1   SHDYTLRGRIRFFPDTINDWQEMGYGSSTRWLFCKDLAAL*-----
HG792019.1       AHDSTLKGNMDFYPLTINDWKAKGYGKQTKWLFYKDLEDAMEGTK*
KM595305.1       AHDATLKGKVDFYPSKINDWKAKEYGKKTKWLFYKDIENAIEGQK*
HQ731031.1       AHDATLKGKVDFYPSKINDWKAKEYGKKTKWLFYKDIENSIEGQk-
BK008023.1       AHDATLKGKVDFYPSKINDWKAKEYGKKTKWLFYKDIENSIEGQK*
                 :** **:*.: *:* .****:  **.*:*** **:
```

Fig. 4. Alignment of amino acid sequences of the most recognized MPaE proteins registered in the GenBank which their implications are listed in table 1.

MPaB and *MPaE* nucleotide sequencing

The partial sequences of *MPaB* and *MPaE* were shown (Fig. 5). The obtained sequence was analyzed using BLAST and Clustal W software programs. Sequence alignment was performed at both the nucleotide (Fig. 6 and 7) and amino acid levels (Fig. 8, and 9). These sequences were compared and aligned with *MPaB* and *MPaE* sequences in *P. brevicompactum* IBT23078. The results showed differences at both nucleotide and amino acid levels. According to the results of the current study, new strain of *P. brevicompactum* has been discovered.

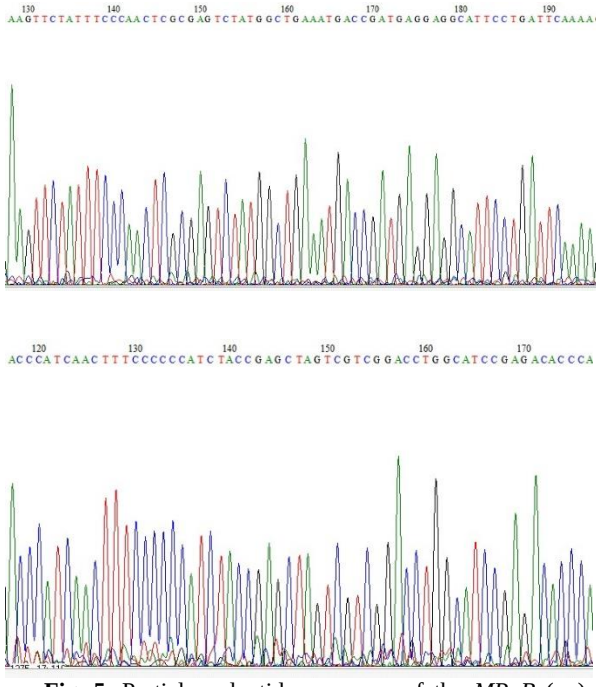

Fig. 5. Partial nucleotide sequence of the *MPaB* (up) and *MPaE* (down) gene sequencing diagram. Peaks represent position of nucleotides.

DISCUSSION

The discovery of the MPA gene cluster has provided new insights into the biosynthesis of MPA, a very powerful important immunosuppressive drug, used in preventing acute rejection in liver transplantation (Hao e al, 2008). To better understand the MPA biosynthesis, identification of the gene cluster responsible for its production has great importance and identification of involved genes in the cluster by sequencing would result in the identification of the complete gene cluster.

```
MPaB(Sequenced)       atgtctttgcctttgcctccagcactttctgagcttgcgagggcgccttcctacagcaga 60
MPaB(HQ731031.1)      atgtctttgcctttgcctccagcactttctgagcttgcgagggcacttccctacagcaga 60
                      ******************************************** * * ***********

MPaB(Sequenced)       actcaatggcttccaatcttggtgggatttctgattgggtacccccctcctcatcaaggca 120
MPaB(HQ731031.1)      actcaatggcttccaatcttcgtgggatttctgattgggtaccccatccttatcagggca 120
                      ******************** ********************** **** **** ****

MPaB(Sequenced)       ttgcgttataagcgacttggagagatgaagaagaagttctatttcccaactcgcgagtct 180
MPaB(HQ731031.1)      ttgcgttataagcgacatggagagatgaagaagaaattctatttcccaactcgcgagtcc 180
                      ***************** ****************** ***********************

MPaB(Sequenced)       atggctgaaatgaccgatgaggaggcattcctgattcaaaaggaaatggcacagctcgag 240
MPaB(HQ731031.1)      atggctgaaatgaccgatgaggaggcattcctgattcaaaaggaaatggcacagctcgag 240
                      ************************************************************

MPaB(Sequenced)       ttcccattcatgttcttgacatctgggcagtttgcactattccgggtatgcagttcaatt 300
MPaB(HQ731031.1)      ttcccattcatgttcttgacatctgggcagtttgcactattccgggtatgcagttcaatt 300
                      ************************************************************

MPaB(Sequenced)       tcattaattatatagttctatacacgaaagccacacgggcacctttgcttgagcttgatt 360
MPaB(HQ731031.1)      tcattaattatatagttctatatatgaaagccacacggacacctttgcttgaacttgatt 360
                      ********************** * ************** ************** *******

MPaB(Sequenced)       tatctttttctaatcatttgcagacatatggcattccaacaatctctcatcttctaacga 420
MPaB(HQ731031.1)      tatctttttctaatcatttacagacatatggcattccgacaatctctcatcttctaacga 420
                      ******************* ****************** ***********************

MPaB(Sequenced)       aaaccgggcaattctccaagccagaaacatccttcaaacgttatacagacacagctgctc 480
MPaB(HQ731031.1)      aaaccgggcaattctccaagccagaaacatccttcaaacgctatacagacacagctgctc 480
                      ****************************************** *******************

MPaB(Sequenced)       tgattggtgaaatggtagagaatagtcctacctcgcagagggcattcatctcagtagccc 540
MPaB(HQ731031.1)      tgattggtgaaatggtagagaatagtcctacctcgcagagggcattcatctcagtagccc 540
                      ************************************************************

MPaB(Sequenced)       gcacacgattcctacatagcggctatcaagcttcgggcaagatcctcgatgctgatttgc 600
MPaB(HQ731031.1)      gcacacgattcctacatagcggctatcaagcttcgggcaagatcctcgatgctgatttgc 600
                      ************************************************************

MPaB(Sequenced)       tttacacccttgcactctttgcggtccaacctgtgcgatttattgagaatttcgaatggc 660
MPaB(HQ731031.1)      tttacacccttgcactctttgcggtccagcctgtgcgatttattgagaatttcgaatggc 660
                      **************************** ********************************

MPaB(Sequenced)       ggaccttgagtgatttggaactctgtgctattgggacctttggaagagtctaggtgatg 720
MPaB(HQ731031.1)      ggaccctgagtgatttggaactctgtgccattgggacctttggaagagtctaggtgatg 720
                      ***** ********************** ********************************

MPaB(Sequenced)       ctttgggtattagctctgagattcttccatcgggcaagaccggcttcaaagatggcatcc 780
MPaB(HQ731031.1)      ctttgggtattagctctgagattcttccatcgggcaagacaggcttcaaagatggtatcc 780
                      ****************************************** ************** ****

MPaB(Sequenced)       aatggcttgaagaggtggatgtttggagtcaggattatgaggccaagtatatggtcccag 840
MPaB(HQ731031.1)      aatggcttgaagaggtggatgtttggagtcaggattatgaggccaagtacatggtcccag 840
                      ************************************************** **********

MPaB(Sequenced)       atcccaaaaaccgcgagtcagcagaccaagcgacggcagttctgctttacaatctgccga 900
MPaB(HQ731031.1)      atcccaagaatcgcgagtcggcagatcaagcgacggcagttctactttacaatctgccaa 900
                      ******* ** ******** ***** ***************** ************** *

MPaB(Sequenced)       agattttgcatccaataggactgcagtttacatcttatatgatggatgatcggttgagga 960
MPaB(HQ731031.1)      aatttttgcatccaataggactgcaatttacatcttatatgatggatgatcggctgagga 960
                      *  ********************** ***************************** ******

MPaB(Sequenced)       aggcgatgttgtatgtgatatgagtccattcgagatcctactatttggtcctgatacaa 1020
MPaB(HQ731031.1)      aggcgatgctgtatgtgatatgagtccactcgagatcttgaaacttggctctcacgaga 1020
                      ******** ******************** ******** *   * ****  **  *   *

MPaB(Sequenced)       tcacagatactaacttcaaacagatacgaggccccaagtcctggctggagcgctgttttc 1079
MPaB(HQ731031.1)      tcaaagatgctaatttcgaacagatatgaagccccaactcctggctggagcatggttttc 1079
                      *** **** **** *** ******** ** ******* *************   ******

MPaB(Sequenced)       tcatccctttggctactcgcaagttcgttctgcgttatctatcgccaccacggcctgcg 1139
MPaB(HQ731031.1)      tcaaccctcttggctattcgcaagttgattctgcgttacctatcaccaccacggcctgcg 1139
                      *** **** ******* *********  ********** ***** ***************

MPaB(Sequenced)       gctcttgcagtgtcaaacatagctcaaaagcccgacaaagatgatcgctatcaccgcatg 1199
MPaB(HQ731031.1)      gctcttgcggtgtcaaacatagctcaaaagcccgacaaagatgatcgctatcaccgcatg 1199
                      ******** ************************************************
```

```
MPaB(Sequenced)       tcttgggatgcactcccatttttacatcaggcctactttctggaatagatggggtccaatg 1259
MPaB(HQ731031.1)      tcttgggatgcactcccatttttacatcaggcctactttctggaacagatggggtccaatg 1259
                      ********************************************* ***************

MPaB(Sequenced)       gcttggatctcttggttgatgggccaccctgtcccaggcgatcatggccagaagtactat 1319
MPaB(HQ731031.1)      gcgtggatctcttggttgatgggccaccctgtcccaggcgatcttggccagaagtactat 1319
                      ** ****************************************** ***************

MPaB(Sequenced)       ccacagggatatcatatacaagatattggaccaaaatactttgaaggaaagggccacaag 1378
MPaB(HQ731031.1)      ccacagggatatcatatacaagatattggaccaaaatactttgaaggaaagggccacaag 1378
                      ************************************************** **********

MPaB(Sequenced)       gagatccaggaaatgatgaaggaattaagtacttgcaggacggggaaatgtcccttccat 1438
MPaB(HQ731031.1)      gagatccaggaaatgatgaaggaattaaagatctcaaggacggggaaatgtcccttccat 1438
                      *****************************  *  *  ***********************

MPaB(Sequenced)       tagtctggagcaagtaccatgctacaacaaatgattaa 1477
MPaB(HQ731031.1)      tagtctggagcaagtaccatgctacaacaaatgattaa 1477
                      ************************************
```

Fig. 6. Nucleotide alignment of *MPaB* and *MPaB* accession number: HQ731031.1 from *P. brevicompactum* IBT23078. Asterisks represent identical nucleotides.

```
MPaE(Sequenced)       atgatcaaatcgcaaacggtcatccaagagccacgcaacatctcagatgttcttgactca 60
MPaE(HQ731031.1)      atgatcaaatcgcaaacggtcatccaagagccacgcaacatctcagatgttcttgactca 60
                      ************************************************************

MPaE(Sequenced)       gacgagtcctctctgggggtccggagcaaagatattgaagcgatcatctggtcgcttgcc 120
MPaE(HQ731031.1)      gacgagtcctctctgggggtccggagcaaagatattgaagcgattatctggtcacatgcc 120
                      ***** ************************************ ******** * ****

MPaE(Sequenced)       cattttgaccacattggtgacccatcaactttcccccccatctaccgagctagtcgtcgga 180
MPaE(HQ731031.1)      cattttgaccacattggtgacccatcaactttcccccccgtctaccgagctagtcgtcgga 180
                      ************************************ ********************

MPaE(Sequenced)       cctggcatccgagacacccactggcccggcttcccaactaacccagacgcaatcaacctc 240
MPaE(HQ731031.1)      cctggcatccgagacacccactggcccggcttcccaactaacccagacgcaatcaacctc 240
                      ************************************************************

MPaE(Sequenced)       aacaccgacatccaaggtcgcaatgtgcgagaaatttctttcgaaaagacacagaaggga 300
MPaE(HQ731031.1)      aacaccgacatccaaggtcgcaatgtgcgagaaatttccttcgaaaagacacagaaggga 300
                      ************************************ ********************

MPaE(Sequenced)       gccaccaagattggctctttcgacgccgtggactatttcggtgatgggtcgttgtatctt 360
MPaE(HQ731031.1)      gccaccaagatcggttctttcgacgccgtggactattttggcgatgggtccttttatctt 360
                      *********** ** ********************** ** ******** ** ******

MPaE(Sequenced)       ctagatgctgcgggtcattccgtgggccatatcggtgctcttgctcgtgtgactacctct 420
MPaE(HQ731031.1)      ctagatgctgcgggtcattccgtgggccatatcggtgctcttgctcgcgtgactacctct 420
                      ********************************************** ************

MPaE(Sequenced)       ccagactcgtttgtcttcatgggtggtgactcatgtcaccatgccggagtgcttcgaccc 480
MPaE(HQ731031.1)      ccagtctcgtttgtcttcatgggtggtgactcatgtcaccatgccggagtgcttcgaccc 480
                      **** ********************************************************

MPaE(Sequenced)       acaaaatatcttccttgtccactcgactctggtgacacttcacttccatgcaaatccgac 540
MPaE(HQ731031.1)      acaaaatatcttccttgtccacttgactctggtgacacttcacttccatgcaaatccgac 540
                      ********************** ************************************

MPaE(Sequenced)       tctgttttcacgttatcgcctgcactgccaactgattacactgctgctttgaggacagtc 600
MPaE(HQ731031.1)      tctgttttcacgttatcgcctgcactgccaactgattacactgctgctttgaggacagtc 600
                      ************************************************************

MPaE(Sequenced)       gagaatattaaggagctcgatgcctgtgaggatgtattcgtcgtccttgctcatgatgct 660
MPaE(HQ731031.1)      gagaatattaaggagctcgatgcctgtgaggatgtattcgtcgtccttgctcatgatgct 660
                      ************************************************************

MPaE(Sequenced)       accttgaaagggaaggttgacttttaccccttcgaaaatcaatgattggaaggcgaaagag 720
MPaE(HQ731031.1)      accttgaaagggaaggtcgacttttaccccttcgaaaatcaatgattggaaggcgaaagag 720
                      ***************** ******************************************

MPaE(Sequenced)       tacggcaagaagacaaaaatggctttttataaggatattgagaattccatagaagaac 778
MPaE(HQ731031.1)      tacggcaagaagacaaagtggctttttacaaggatattgagaattccatagaaggac 778
                      *****************   *  *********************************

MPaE(Sequenced)       ataaagtaa 786
MPaE(HQ731031.1)      ataaagtaa 786
                      *********
```

Fig. 7. Nucleotide alignment of *MPaE* and *MPaE* accession number: HQ731031.1 from *P. brevicompactum* IBT23078. Asterisks represent identical nucleotides.

```
MPaB(Sequenced)       MSLPLPPALSELARAPSYSRTQWLPILVGFLIGYPLLIKALRYKRLGEMKKKFYFPTRES 60
MPaB(HQ731031.1)      MSLPLPPALSELARALPYSRTQWLPIFVGFLIGYPILIRALRYKRHGEMKKKFYFPTRES 60
                      ***************  ********* ******** ** ****** **************

MPaB(Sequenced)       MAEMTDEEAFLIQKEMAQLEFPFMFLTSGQFALFRTYGIPTISHLLTKTGQFSKPETSFK 120
MPaB(HQ731031.1)      MAEMTDEEAFLIQKEMAQLEFPFMFLTSGQFALFRTYGIPTISHLLTKTGQFSKPETSFK 120
                      ************************************************************

MPaB(Sequenced)       RYTDTAALIGEMVENSPTSQRAFISVARTRFLHSGYQASGKILDADLLYTLALFAVQPVR 180
MPaB(HQ731031.1)      RYTDTAALIGEMVENSPTSQRAFISVARTRFLHSGYQASGKILDADLLYTLALFAVQPVR 180
                      ************************************************************

MPaB(Sequenced)       FIENFEWRTLSDLELCAIGTFWKSLGDALGISSEILPSGKTGFKDGIQWLEEVDVWSQDY 240
MPaB(HQ731031.1)      FIENFEWRTLSDLELCAIGTFWKSLGDALGISSEILPSGKTGFKDGIQWLEEVDVWSQDY 240
                      ************************************************************

MPaB(Sequenced)       EAKYMVPDPKNRESADQATAVLTSNRYEAPSPGWSAVFSSLLATRKFVLRYLSPPRPAAL 300
MPaB(HQ731031.1)      EAKYMVPDPKNRESADQATAMLISNRYEAPTPGWSMVFSTLLAIRKLILRYLSPPRPAAL 300
                      ******************* * ******* **** *** *** **  ************

MPaB(Sequenced)       AVSNIAQKPDKDDRYHRMSWDALPFYIRPTFWNRWGPMAWISWLMGHPVPGDHGQKYYPQ 360
MPaB(HQ731031.1)      AVSNIAQKPDKDDRYHRMSWDALPFYIRPTFWNRWGPMAWISWLMGHPVPGDLGQKKGPQ 360
                      ************************************************* ***  **

MPaB(Sequenced)       GDPGNDEGIKYLQDGEMSLPLVWSKYHATTND*  392
MPaB(HQ731031.1)      GDPGNDEGIKDLKDGEMSLPLVWSKYHATTND*  392
                      ********** * ********************
```

Fig. 8. Amino acid alignment of *MPaB* and *MPaB* accession number: HQ731031.1from *P. brevicompactum* IBT23078. Different amino acids are in bold and underlined. Asterisks represent identical amino acids between two sequences.

```
MPaE(Sequenced)       MIKSQTVIQEPRNISDVLDSDESSLGVRSKDIEAIIWSLAHFDHIGDPSTFPPSTELVVG 60
MPaE(HQ731031.1)      MIKSQTVIQEPRNISDVLDSDESSLGVRSKDIEAIIWSHAHFDHIGDPSTFPPSTELVVG 60
                      ************************************ ********************

MPaE(Sequenced)       PGIRDTHWPGFPTNPDAINLNTDIQGRNVREISFEKTQKGATKIGSFDAVDYFGDGSLYL 120
MPaE(HQ731031.1)      PGIRDTHWPGFPTNPDAINLNTDIQGRNVREISFEKTQKGATKIGSFDAVDYFGDGSFYL 120
                      ************************************************************:**

MPaE(Sequenced)       LDAAGHSVGHIGALARVTTSPDSFVFMGGDSCHHAGVLRPTKYLPCPLDSGDTSLPCKSD 180
MPaE(HQ731031.1)      LDAAGHSVGHIGALARVTTSPVSFVFMGGDSCHHAGVLRPTKYLPCPLDSGDTSLPCKSD 180
                      ******************** ************************************

MPaE(Sequenced)       SVFTLSPALPTDYTAALRTVENIKELDACEDVFVVLAHDATLKGKVDFYPSKINDWKAKE 240
MPaE(HQ731031.1)      SVFTLSPALPTDYTAALRTVENIKELDACEDVFVVLAHDATLKGKVDFYPSKINDWKAKE 240
                      ************************************************************

MPaE(Sequenced)       YGKKTKWLFYKDIENSIEGQIK*  261
MPaE(HQ731031.1)      YGKKTKWLFYKDIENSIEGQIK*  261
                      ***********************
```

Fig. 9. Amino acid alignment of *MPaE* and *MPaE* accession number: HQ731031.1from *P. brevicompactum* IBT23078. Different amino acids are in bold and underlined. Asterisks represent identical amino acids between two sequences

The complete sequence of MPA genes from *P. brevicompactum* IBT23078 has already been published (Regueira et al.2011). The present study provides two homologues for *MPaB* and *MPaE* genes from MPA gene cluster. Together with the IBT23078 previously available sequences, we extended the genetic knowledge of *P. brevicompactum*, by performing a comparative analysis of another strain gene sequences.

The *MPaB* amplification produced amplicons of 1477 nt and the *MpaE* PCR amplification generated amplicons of 780 nt with the same length compared to the *MPaB* and *MpaE* genes recorded in GenBank. The subcloned *MPaB* and *MPaE* genes were aligned with *MPaB* and *MPaE* from the IBT23078 recorded in the GenBank which several differences were observed over the entire length of the both genes. The sequence similarity analyses showed that the amplified products were 95.15 and 98.85 (%) identical in amino acid residues compared to the *MPaB* and *MPaE* from *P. brevicompactum* IBT23078. Although the bioinformatics analysis of the MPaB and MPaE has not designated catalytic tasks of these deduced proteins, future functional analyses will reveal their roles in relation to MPA biosynthesis (Regueira et al. 2011).

This is the first comparative analysis of *MPaB and MPaE* genes from MPA gene cluster at the nucleotide and amino acid levels. These comparisons provide definitive evidence for classifying the members of this species. The identification of alternative *MPaB and MPaE* genes in *P. brevicompactum* strains may accelerate further research on MPA biosynthesis.

ACKNOWLEDGEMENTS

The authors have special thanks to authorities of Urmia Institute of Biotechnology for technical support.

REFERENCES

Asfari M, Berta S, Coffy S, Kergoat M, Charon C, Audet A. 2005. Short–term treatment with mycophenolic acid and tacrolimus is tolerogenic for INS–1 cell clone transplantation and the deleterious effects of the drugs are limited: in vivo and in vitro studies. Journal of Endocrinology 186: 213–220.

Brennan J, Brakeman P. 2019. Kidney transplant in Adolescents: Medical Aspects. In: Adolescents with Chronic Kidney Disease (MN Haddad, E Winnicki & S Nguyen, eds): 201–215. Springer, Cham.

Borroto–Esoda K, Myric F, Feng J, Jeffrey J, Furman PH. 2004. In vitro combination of amdoxovir and the inosine monophosphate dehydrogenase inhibitors mycophenolic acid and ribavirin demonstrates potent activity against wild–type and drug–resistant variants of human immune deficiency virus type 1. Antimicrobial Agents and Chemotherapy 48: 4387–4394.

Chang QY, Guo FC, Li XR, Zhou JH, Cai X, Pan Q, Ma XX. 2018. The IMPDH inhibitors, ribavirin and mycophenolic acid, inhibit peste des petits ruminant's virus infection. Veterinary Research Communications 42: 309–313.

Danafar H, Hamidi M. 2015. Simple and sensitive high–performance liquid chromatography (HPLC) method with UV detection for mycophenolic acid assay in human plasma. Application to a bioequivalence study. Advanced Pharmaceutical Bulletin 5: 563.

Del Cid A, Gil Durán C, Vaca I, Rojas Aedo J F, García Rico R O, Levicán G, Chávez R. 2016. Identification and functional analysis of the mycophenolic acid gene cluster of Penicillium roqueforti. PLoS One 11: e0147047.

Dubus I, Vendrely B, Christophe I, Labouyrie J P, Delmas Y, Bonnet J, Combe C. 2002. Mycophenolic acid antagonizes the activation of cultured human mesangial cells. Kidney International 62: 857–867.

Geissler EK, Schlitt HJ. 2019. Can mycophenolic acid–based immunosuppression benefit liver transplant patients with HCC? Transplantation 103: 873–874.

Guzera M, Szulc Dąbrowska L, Cywińska A, Archer J, Winnicka A. 2016. In Vitro Influence of mycophenolic acid on selected parameters of stimulated peripheral canine lymphocytes. PLoS One 11(5): e0154429.

Hansen BG, Mnich E, Nielsen KF, Nielsen JB, Nielsen MT, Mortensen UH, Larsen TO, Patil KR. 2012. A natural fusion of a cytochrome P450 and a hydrolase is involved in mycophenolic acid biosynthesis. Applied and Environmental Microbiology 78: 4908–4913.

Hao C, Anwei M, Bing C, Baiyong S, Weixia Z, Chuan S, Erzhen C, Xiaxing D, Weihua Q, Weiping Y, Chenghong P. 2008. Monitoring mycophenolic acid pharmacokinetic parameters in liver transplant recipients: prediction of occurrence of leukopenia. Liver Transplantation 14: 165–1173.

Ismaiel A, Papenbrock J. 2015. Mycotoxins: producing fungi and mechanisms of phytotoxicity. Agriculture 5: 492–537.

Kavanagh F. 1947. Activities of twenty–two antibacterial substances against nine species of bacteria. Journal of Bacteriology 54: 761.

Lapointe HR, Li S, Mortazavi S, Zeng J. 2016. Expression and Purification of a Potato Type II Proteinase Inhibitor in Escherichia coli Strain BL21 (DE3). Microbiology & Immunology 2:34–40.

Lee PY, Costumbrado J, Hsu CY, Kim YH. 2012 Agarose Gel Electrophoresis for the Separation of DNA Fragments. Journal of Visualized Experiments 62: e3923.

Muth WL, Nash CH. 1975. Biosynthesis of mycophenolic acid: purification and characterization of S–adenosyl–L–methionine: demethylmycophenolic acid O–methyltransferase. Antimicrobial Agents and Chemotherapy 8: 321–327.

Nicoletti R, De Stefano M, De Stefano S, Trincone A, Marziano F. 2004. Antagonism against Rhizoctonia solani and fungitoxic metabolite production by some Penicillium isolates. Mycopathologia 158: 465–474.

Patil US, Jaydeokar AV, Bandawane DD. 2012. Immunomodulators: A pharmacological review. International Journal of Pharmacy and Pharmaceutical Sciences 4: 30–36.

Regueira T B, Kildegaard K R, Hansen B G, Mortensen U H, Hertweck C, Nielsen J. 2011. Molecular basis for mycophenolic acid biosynthesis of Penicillium brevicompactum. Applied and Environmental Microbiology 77: 3035–3043.

Tressler RJ, Garvin LJ, Slate DL. 1994. Anti-tumor activity of mycophenolate mofetil against human and mouse tumors in vivo. International Journal of Cancer 57: 568–573.

Yew SS, Caroll R, Tran H, Coates PT .2018. Predicting mycophenolic acid area under the curve with mycophenolic acid through in de novo renal transplantation. Transplantation 102: S404–S405.

Zhang W, Cao S, Qiu L, Qi F, Li Z, Yang Y, Huang S, Bai F, Liu C, Wan X, Li S. 2015. Functional characterization of MpaG′, the O-methyltransferase involved in the biosynthesis of mycophenolic acid. Chembiochem 16: 565–569.

شناسایی مولکولی ژن های *MPaB* و *MPaE* از خوشه ژنی MAP در یک سوش جدید از قارچ *Penicillium brevicompactum*

نرمین وطنی[۲،۱]، فاطمه رحمانی[۲،۱]✉، یوبرت قوستا[۳]

۱- گروه بیولوژی، دانشکده علوم، دانشگاه ارومیه، ارومیه

۲- موسسه بیوتکنولوژی، دانشگاه ارومیه، ارومیه

۳- گروه گیاهپزشکی، دانشکده کشاورزی، دانشگاه ارومیه، ارومیه

چکیده: مایکوفنولیک اسید (MPA) یک متابولیت ثانویه قارچی با خاصیت ضدویروسی، ضدقارچی، ضدباکتریایی، ضدتومور و ضد پسوریاس می‌باشد. این ماده به عنوان داروی سرکوب کننده سیستم ایمنی بدن در بیماران پیوند اعضای قلب، کلیه و کبد کاربرد داشته که در حضور آن تکثیر لنفوسیت‌های B و T محدود می‌شود. ژن های *MpaB* و *MpaE* از مجموعه ژنی ۲۵ کیلو دالتونی از ژنوم *P. brevicompactum* می‌باشند. در این پژوهش، DNA ژنومی از قارچ *P. brevicompactum* استخراج گردید که در محیط potato dextrose (PD) کشت داده شده بود. جهت تکثیر ژن های *MpaB* و *MpaE* آغازگرهای ویژه با استفاده از نرم افزار Gene Runner و بر اساس توالی ژنوم *P. brevicompactum* سویه IBT23078 با شماره دسترسی HQ731031 .1 طراحی شدند. ژن های *MpaB* و *MpaE* تکثیر شده به داخل وکتور کلونینگ PTG19-T کلون شده و درون باکتری *E. coli* ترانسفورم گردیدند. همچنین نتایج بررسی همسانه سازی (check cloning) توسط PCR نیز نشان داد که همسانه ژن های *MpaB* و *MpaE* به درون وکتور PTG19-T و ترانسفورماسیون این ژنها به درون *E. coli* به درستی انجام گرفته است. محصول تکثیر *MpaB*، قطعه ۱۴۷۷ نوکلئوتیدی است و محصول تکثیر ژن *MpaE* قطعه ۷۸۰ نوکلئوتیدی است که درازای آنها مشابه با ژنهای *MpaB* و *MpaE* گزارش شده در بانک ژن می باشد. اما هم ترازی ژن های همسانه سازی شده با ژن های ثبت شده در بانک ژن، نوکلئوتید ها و آمینواسیدهای متفاوتی را در توالی نشان می دهد که معرف سویه جدیدی از قارچ *P. brevicompactum* می باشد.

کلمات کلیدی: اسید میکوفنلیک، توالی، همسانه سازی، پیوند، همردیف سازی

مکاتبه کننده: فاطمه رحمانی Email: f.rahmani@urmia.ac.ir

تاریخ دریافت: ۱۳۹۷/۱۰/۲۸ تاریخ پذیرش: ۱۳۹۸/۰۲/۲۴