



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

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**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. 2011). 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*

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(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 (Ismail & 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 TCTTGCCTTGCCTCCAG-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<sup>2+</sup>, 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).

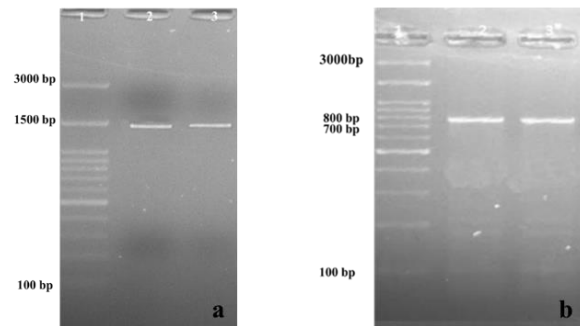
**Nucleotide sequences analysis**

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**

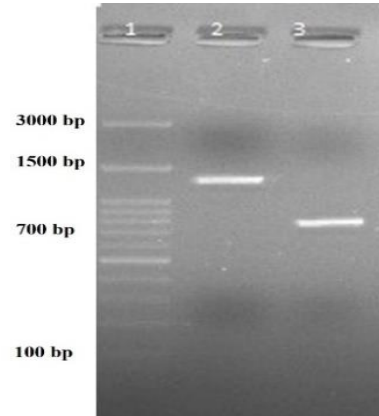
The genomic DNA of *P. brevicompactum* was extracted and *MPaB* and *MPaE* genes were amplified with the resulting fragments of 1477 and 780 bp, respectively, compared to the 100 bp DNA ladder (Fig. 1).



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

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DF126461.1 -----
KQ087361.1 -----MEQLAAGLKNGLAAILTTDVSHVGFGRGYPYILAGLAAYPVLCSEFLRFRKMRWLH
AM270165.1 -----
XM001392397.2 -----
HG792019.1 -----
HQ731031.1 -----
KM595305.1 -----
CDHK01000003.1 -----
JQGA0100086601 MKPDDVEKAHSSPTLGSPSQFHLD-----KSASNSSEVSDFAKPDFVLQSRCHRWIA

DF126461.1 -----
KQ087361.1 RKYNFPPTRESLARMTDDEAWEIQR-VLLQQEFPFF-----FIK
AM270165.1 -----
XM001392397.2 -----
HG792019.1 -----
HQ731031.1 -----
KM595305.1 -----
CDHK01000003.1 -----
JQGA0100086601 SLKNVESCIGI-EPIPIEBEREPIITSSTILHMLLMWFMSMTLATNNIVVGSMTLVLGLSFRD

DF126461.1 -----
KQ087361.1 ALQFALFRTYGIPTISGLLTATSQFSNPETSLKRYTDTSVLIQEFMGNAPSSERACIALA
AM270165.1 -----
XM001392397.2 -----
HG792019.1 -----
HQ731031.1 -----
KM595305.1 -----
CDHK01000003.1 -----
JQGA0100086601 AALCAVFGCLAGNCIVGFISIW----GPRS----GNRTLIVARYFMGYPPSK-VCIIILN

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

DF126461.1 -----
KQ087361.1 WRTLSDEKCALGTFWKSIGDGLDISYENLPSS-----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 YVYVLPQMKSSRTFLATVSAATT--AMSIVLLMGIGLGTVIASSAHFTAKYGSSPPGGV

DF126461.1 -----
KQ087361.1 KFVSFMM-----DDR LRKAMLYDAPPAAYTKVFSFILRVRRFVMRYLALPRPYFLRY
AM270165.1 -----
XM001392397.2 -----
HG792019.1 -----
HQ731031.1 -----
KM595305.1 -----
CDHK01000003.1 -----
JQGA0100086601 LMTAYDGLGGFGKFCVINVLALVANNTPGAYSMGMNFQMIG---GVFGKVPQPVFTTL

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

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AM270165.1      LFSARRFALRYLALPRPYFLRYVSYTDDYENNGRIYMTNWDAAPIYVVKPSLRNRWGPVAV
XM001392397.2  LFSARRFALRYLALPRPYFLRYVSYTDDYENNGRIYMTNWDAAPIYVVKPSLRNRWGPVAV
HG792019.1      -----
HQ731031.1      LLAIRKLILRYLSPPRPAALAVSNIAQKPKDDRYHRMSWDALPFYIRPTFWRNRWGPMAW
KM595305.1      LLATRKFVLRYSPPRPAALAVSNIAQKPKDDRYHRMSWDALPFYIRPTFWRNRWGPMAW
CDHK01000003.1 LLTVRKLFRLYLSLPRPYFLRSVFTDEPDEHNRFFLTQWEAAPYYVVKPTFWNRWGPATL
JQGA0100086601 ILTARRLFLRYLALPRPYFLRFASFTEEPDQNNRFFLTEWEAAPYYVVKPTFWNRWGPMAW

DF126461.1      LTWVLGRPLPGDEGDKYYPKGYDNLNVGPKYFEGKGRQGMEDIMKELETTRRGQCPFH
KQ087361.1      LTWALGRPLPGDEGDKYYPKGYDNLNVGPKYFEGKGRQGMEDIMKELENTTRRGQCPFH
AM270165.1      LTWALGRPLPGDEGDKYYPKGYDNLNVGPKYFEGKGRQGMEDIMKELENTTRRGQCPFH
XM001392397.2  LTWALGRPLPGDEGDKYYPKGYDNLNVGPKYFEGKGRQGMEDIMKELENTTRRGQCPFH
HG792019.1      -----
HQ731031.1      ISWLMGHPVPGDLGQKKGPQGDGPNDEGIKDLKDGEM-SLPLVMSKYHATTND-----
KM595305.1      ISWLMGHPVPGDHGQKYYPPQGYHIQDIGPKYFEGKGHKEIQEMMKELKISRTGKCPFH
CDHK01000003.1 LTRILGRFPVPGDERDKYYPQGYSIQDVGPKYFEGKGRKAIEEAMEEFKEYRTGKCPFH
JQGA0100086601 LTWALGRFPVPGDDGDKYYPNGYSVPDVGPKYFEGKGGKQLDETI LELKGYRTGKCPFH
    
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**Fig. 3.** Alignment of amino acid sequences of the most recognized MPaB proteins registered in GenBank which their implications are listed in Table 1.

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BBXM01000144.1 -----
HG792019.1      MRVEAGGDMRDKLMWIRLYILGNVQTFGDMKRYIGMWSGMLFPISTKHTRRLISILKVY
KM595305.1      -----
HQ731031.1      -----
BK008023.1      -----

BBXM01000144.1 -----MTMLDKIDIPRLAAAVALYAFYRAFQSFVRLSHVPGPFIAKF
HG792019.1      FIFILNPPAQVYVQDINLYTMDYLIIRITAVAVVLYLTRYVCCLYLHLQDVPGLFAKF
KM595305.1      -----MESLSLTWITAIHAVVLYLVQRYVRSYWRLEKDI PGVFLAKL
HQ731031.1      -----
BK008023.1      -----MKLSLTWITAVAVVLYLVQRYVRSYWRLEKDI PGVFLAKL

BBXM01000144.1  TNLQRVWVVKSGRAHEYHRQMHERYGKLVRFGPNMVSISDPGAMSIVYPNRPGFQK----
HG792019.1      TNLQRVWVVKSGRAHEYHRRMHAVYGPVAVRFGPNMVSISDPRTIPAIYPSRPGFPKSDFY
KM595305.1      TDLQRVWVVKSGRAHEFHRRDMHAMYGPIVRFGPNMVSISDPRVIPTIYPSRPGFPKSDFY
HQ731031.1      -----
BK008023.1      TDLQRVWVVKSGRAHEFHRRDMHAMYGPIVRFGPNMVSISDPRVIPTIYPSRPGFPKSDFY

BBXM01000144.1  ---RPYSPKSGVLPVAVFNTQDETLHRQLRKP IASLYSMTSIVGSEPLVDQTL EILFRQLD
HG792019.1      RTQKPYTPNKGAMPAVFNQDEDLHKRLRSPIAPLYSMTNVVKLESFVDQTLAVLLEQLD
KM595305.1      RTQKPYTRNKGAMPAVFNQDEDLHKQLRSPIASLYSMTNVVRLEPLVDETLTVLSKQLD
HQ731031.1      -----
BK008023.1      RTQKPYTRNKGAMPAVFNQDEDLHKQLRSPIASLYSMTNVVRLEPLVDETLTVLSKQLD

BBXM01000144.1  LRFG-ATGRSLDLAEWLQFFAFDVMGMLSFSKRHGFLQGRDVRGILGGIWA FMKTVPV
HG792019.1      GRFLGSNDVPFDLGSWLQYFAFDSMGTLTFSRRYGFLEQGRDMNGILGEIWKFMKRV SVM
KM595305.1      ERFVGTNDKPFDLGDWLQYFAFDSMGTLTFSRRYGFLEQGRDMHGILQE IWNFMTRVAVM
HQ731031.1      -----
BK008023.1      ERFVGTNDKPFDLGDWLQYFAFDSMGTLTFSRRYGFLEQGRDMHGILQE IWNFMTRVAVM

BBXM01000144.1  GQIPWFDPVWNKNPIIALFKQTTGLAVLVVDRFVAERQMSSSQHGAEKGREKRDMLS KF
HG792019.1      GQIPWFDFCNTNPFIALFRSPTGFGVLKVVDFILQRLAPRE---KDEVSDEKDMLSQF
KM595305.1      GQIPWFDEIWNKNSFITLFRKPTGFGVLKVVDFISQRVSSRE---NDEKADEKDMLSQF
HQ731031.1      -----
BK008023.1      GQIPWFDEIWNKNSFITLFRKPTGFGVLKVVDFISQRVSSRE---NDEKADEKDMLSQF

BBXM01000144.1  LEIQAKDPK-IPAWAPKAWTFSNMLAGSDTTATALTAVMYNLLNCR TSMDTLARELSNAQ
HG792019.1      LNIQASNPD-VMPWAPRAWTFSNIMAGSDSTANVMRTIMYNLLVHRD TSLRQLDELLESE
KM595305.1      LNIQASNPHSIMPWAPRAWTFSNVMAGSDSTANVMRTMMYNLLVDRD TSLRSLRAELLEAE
HQ731031.1      -----
BK008023.1      LDIQASNPHSIMPWAPRAWTFSNVMAGSDSTANVMRTMMYNLLVDRD TSLRSLRAELLEAE

BBXM01000144.1  RKGRLSRYPSPWHEVRELPYLDACIMEALRLHPPFCLPFERVVP EGGVTVCETYLAAGTV
HG792019.1      SSNGLSRTCPSEWVKVRLPYLDACVLEALRLHPPFCLPFERVVP GGLTVCETYLPAGTI
KM595305.1      SSNGLSRSLSWDGVRSLPYLDACVLEALRLHPPFCLPFERVVP EGGITVCETYLPAGTV
HQ731031.1      -----
BK008023.1      NSNGLSRSLSWDGVRSLPYLDACVLEALRLHPPFCLPFERVVP EGGITVCETYLPAGTV

BBXM01000144.1  VGMSPIVNRDRD TYGDDADEWRPERWLN LGEGDRRLENGILTFGSGRR TCGLRNLAIF
HG792019.1      VGISPYMANRDKETFGNDADEWRPERWLGSLHEDRKRLENSLLTF GAGRRTCGKNIAIL
KM595305.1      VGISPYLANRDKQTFGDDADKWRPSRWL DLSREDRVKLENSILTFGAGRR TCGKNIAIL
HQ731031.1      -----
BK008023.1      VGISPYLANRDKQTFGDDADKWRPSRWL DLSREDRVKLENSILTFGAGRR TCGKNIAIL

BBXM01000144.1  EMKKLLPALMRYEITAVEPLQLKLENSWLFKQWDLHVHVR LN-----EALQPP
HG792019.1      EIKKLI PVLLLNYDIQIVNPENYKTENAWFFKQTGLQAVIRKRAKMERGSSNKDKPTLPP
    
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KM595305.1	EIKKLFPMLLLNYEIEIVNPENYQTTNAWFFRQWGLHAVIRKLPAPERDDTIEQKASIPP
HQ731031.1	-----
BK008023.1	EIKKLFPMLLLNYEIEIVNPENYQTTNAWFFRQWGLQAVIRKLPAPERDDTIEQKASIPP
BBXM01000144.1	PLDVPSSTSTALVRVIDPGTTLDLKPLGFWQFALNGLDKLTVPMYCFLISSGERHILFDL
HG792019.1	VLNIPSSSTVDVVRVIDPGTLLDLRDLFWQPELPLGRKVTAPTYCFLISVGRHVLFDL
KM595305.1	ALNIPSSSTVDVRIIDSGTLLDLRDLFWTPDLPLGLKVTAPTYCFLISNGSRHVLFDL
HQ731031.1	-----
BK008023.1	ALNIPSSSTVEVRIIDSGTLLDLRDLFWTPDLPLGLKVTAPTYCFLISNGTRHVLFDL
BBXM01000144.1	GVRADWENLAPAAAALIRNTTIVNSRNIADILDTPPIPESSIRTTNIEAI IWSHDHFDH
HG792019.1	GVRQDWERLPPSVVAMIKSQTTIQNPRNIDILDSDA-SSLGIRSTDIEAI IWSHAHFDH
KM595305.1	AVRQDWERLPPSIVAMIKSQTVIQEPRNIDVLDSDSDE-SSLGIRSKDIEAI IWSHAHFDH
HQ731031.1	-----MIKSQTVIQEPRNIDVLDSDSDE-SSLGVRSKDIEAI IWSHAHFDH
BK008023.1	AVRQDWERLPPSIVAMIKSQTVIQEPRNIDVLDSDSDE-SSLGVRSKDIEAI IWSHAHFDH :*. * . : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : *
BBXM01000144.1	IGDPSTFFPPSTNLVVGPGVRD-AWPGYPSNPTSRLVLDSDIEGRLLREISFGQT---PLKV
HG792019.1	IGDPSTFFPLSTELVVGPGIRDHSHWPGFPTNPDAINLNSDIQGRKREISFERTEKEAIKI
KM595305.1	IGDPSTFFPSTELVVGPGIRDTHWPGFPTNPDAINLNTDIQGRNVREISFEKTQKGATKI
HQ731031.1	IGDPSTFFPSTELVVGPGIRDTHWPGFPTNPDAINLNTDIQGRNVREISFEKTQKGATKI
BK008023.1	IGDPSTFFPSTELVVGPGIRDTHWPGFPTNPDAINLNTDIQGRNVREISFEKTQKGATKI ***** * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : *
BBXM01000144.1	GPFDADFDFDGSFYLLNAPGHSIGHMCGLARVTTSPDTFVFMGADACHHPGLRPTKYR
HG792019.1	GSFDALDYFDGGSFYLLNAGHSIGHIGALARVTTSPDSFVFMGGDSCHHAGVLRPISKYL
KM595305.1	GSFDAMDYFDGGSFYLLDAAGHSVGHIGALARVTTSPDSFVFMGGDSCHHAGVLRPISKYL
HQ731031.1	GSFDADVDFDGSFYLLDAAGHSVGHIGALARVTTSPVSVFVFMGGDSCHHAGVLRPISKYL
BK008023.1	GSFDADVDFDGSFYLLDAAGHSVGHIGALARVTTSPVSVFVFMGGDSCHHAGVLRPISKYL * * * . * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : *
BBXM01000144.1	PLPPGQRSPPLSPCAACPLTWDESFLFKVSPVLASDHARALETVEKIKELDASDDVFVIL
HG792019.1	PCPSHSRHIPL-SS-----ESESVFTLSPVLPDSDYDAALKTVDNKELDAYDNVFLIL
KM595305.1	PCPLDSGDTSL-PC-----KSDSVFTLSPALPTDYTAALRTVENIKELDACEDVFVVL
HQ731031.1	PCPLDSGDTSL-PC-----KSDSVFTLSPALPTDYTAALRTVENIKELDACEDVFVVL
BK008023.1	PCPLDSGDTSL-PC-----KSDSVFTLSPALPTDYTAALRTVENIKELDACEDVFVVL * * . . . . . : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : *
BBXM01000144.1	SHDYTLRGRIRFFPDTINDWQEMGYGSSTRWLFCKDLAAL*-----
HG792019.1	AHDSLKGNMDFYPLTINDWKAKGYGKQTKWLFYKDIENAIIEGQK*
KM595305.1	AHDATLKGKVDYFYPKINDWKAKKEYGKTKWLFYKDIENAIIEGQK*
HQ731031.1	AHDATLKGKVDYFYPKINDWKAKKEYGKTKWLFYKDIENAIIEGQK-
BK008023.1	AHDATLKGKVDYFYPKINDWKAKKEYGKTKWLFYKDIENAIIEGQK* : * * * * : * * * * * : * * * * * : * * * * * : * * * * * : * * * * * : * * * * * : * * * * *

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.

**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.

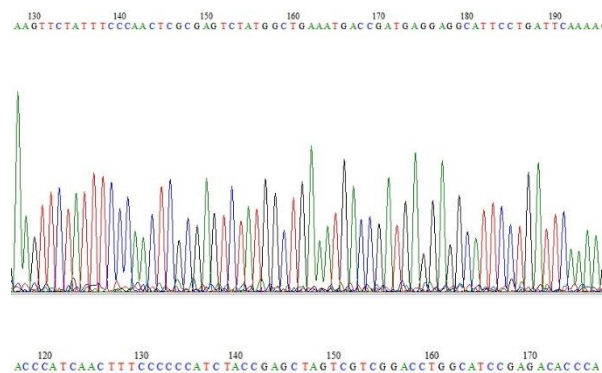


Fig. 5. Partial nucleotide sequence of the *MPaB* (up) and *MPaE* (down) gene sequencing diagram. Peaks represent position of nucleotides.

MPaB (Sequenced)	atgtccttgcctttgcctccagcactttctgagcttgcgagggcgcttccctacagcaga	60
MPaB (HQ731031.1)	atgtccttgcctttgcctccagcactttctgagcttgcgagggcacttccctacagcaga	60
	***** * * *****	
MPaB (Sequenced)	actcaatggcttccaatcttgggtgggatttctgattgggtaccocctcctcatcaaggca	120
MPaB (HQ731031.1)	actcaatggcttccaatcttctgtgggatttctgattgggtaccocctcctcatcaaggca	120
	***** ***** * * * * *	
MPaB (Sequenced)	ttgcggtataagcgacttgagagatgaagaagaagtctatttcccaactcgcgagtct	180
MPaB (HQ731031.1)	ttgcggtataagcgacatggagagatgaagaagaattctatttcccaactcgcgagtcc	180
	***** ***** *****	
MPaB (Sequenced)	atggctgaaatgaccgatgaggaggcattcctgattcaaaggaatggcacagctcgag	240
MPaB (HQ731031.1)	atggctgaaatgaccgatgaggaggcattcctgattcaaaggaatggcacagctcgag	240
	***** *****	
MPaB (Sequenced)	ttcccatcatgttcttgacatctgggcagtttgcaactattccgggtatgcagttcaatt	300
MPaB (HQ731031.1)	ttcccatcatgttcttgacatctgggcagtttgcaactattccgggtatgcagttcaatt	300
	***** *****	
MPaB (Sequenced)	tcattaattatagttctatacagaaagccacacgggcaccttggcttgagcttgatt	360
MPaB (HQ731031.1)	tcattaattatagttctataatgaagccacacggacaccttggcttgaaacttgatt	360
	***** * ***** *****	
MPaB (Sequenced)	tatcttttctaatacatttgcagacatattggcattccaacaatctctcatcttctaacga	420
MPaB (HQ731031.1)	tatcttttctaatacatttgcagacatattggcattccaacaatctctcatcttctaacga	420
	***** ***** *****	
MPaB (Sequenced)	aaaccgggcaattctccaagccagaaacatccttcaaagcttatacagacacagctgctc	480
MPaB (HQ731031.1)	aaaccgggcaattctccaagccagaaacatccttcaaagcttatacagacacagctgctc	480
	***** *****	
MPaB (Sequenced)	tgattgggtgaaatggtagagaatagtcctacctcgcagagggcattcatctcagtagccc	540
MPaB (HQ731031.1)	tgattgggtgaaatggtagagaatagtcctacctcgcagagggcattcatctcagtagccc	540
	***** *****	
MPaB (Sequenced)	gcacacgatcctacatagcggctatcaagcttcgggcaagatcctcagtgctgatttgc	600
MPaB (HQ731031.1)	gcacacgatcctacatagcggctatcaagcttcgggcaagatcctcagtgctgatttgc	600
	***** *****	
MPaB (Sequenced)	tttacacccttgactccttgcggtccaacctgtgagttattgagaatttcgaaatggc	660
MPaB (HQ731031.1)	tttacacccttgactccttgcggtccaacctgtgagttattgagaatttcgaaatggc	660
	***** *****	
MPaB (Sequenced)	ggaccttgagtgatttggaaactctgtgctattgggaccttttggaaagagtctaggtgatg	720
MPaB (HQ731031.1)	ggaccttgagtgatttggaaactctgtgctattgggaccttttggaaagagtctaggtgatg	720
	***** *****	
MPaB (Sequenced)	ccttgggtattagctctgagattcttccatcgggcaagaccggcttcaaagatggcatcc	780
MPaB (HQ731031.1)	ccttgggtattagctctgagattcttccatcgggcaagaccggcttcaaagatggatcc	780
	***** ***** *	
MPaB (Sequenced)	aatggcttgaagaggtggatgttggagtcaggattatgaggccaagtatatggtcccag	840
MPaB (HQ731031.1)	aatggcttgaagaggtggatgttggagtcaggattatgaggccaagtatatggtcccag	840
	***** *****	
MPaB (Sequenced)	atcccaaaaaccgagtcagcagaccaagcgacggcagttctgctttacaactctgccga	900
MPaB (HQ731031.1)	atcccaagaatcgcgagtcggcagatcaagcgacggcagttctactttacaactctgccaa	900
	***** * ***** ***** *	
MPaB (Sequenced)	agattttgatccaataggactgcagtttacatcttatatgatggatgatcggttgagga	960
MPaB (HQ731031.1)	agattttgatccaataggactgcaatttacatcttatatgatggatgatcggttgagga	960
	* ***** *****	
MPaB (Sequenced)	aggcgatgttgatgtgatatgagtcactcagatcctactatttggctcctgatacaa	1020
MPaB (HQ731031.1)	aggcgatgttgatgtgatatgagtcactcagatcctactatttggctcctcagcaga	1020
	***** ***** * * * * *	
MPaB (Sequenced)	tcacagataacttcaaacagatagcaggcccaagtcctggctggagcgtgttttc	1079
MPaB (HQ731031.1)	tcaaagatgctaatttcaaacagatagcaggcccaactcctggctggagcgtgttttc	1079
	** * * * * ***** * ***** *****	
MPaB (Sequenced)	tcatcccttttggctactcgcgaagttcgttctcgcgttatctatcgccaccacggcctgcg	1139
MPaB (HQ731031.1)	tcaacccttttggctatttcgcgaagttgattctcgcgttacctatcaccaccacggcctgcg	1139
	** * * * * ***** ***** *****	
MPaB (Sequenced)	gctcttgcagtgcaaacatagctcaaaagcccgacaaagatgatcgctatcacccgatg	1199
MPaB (HQ731031.1)	gctcttgcagtgcaaacatagctcaaaagcccgacaaagatgatcgctatcacccgatg	1199
	***** *****	



MPaB (Sequenced)	tcttgggatgcactcccattttacatcaggcctacttttctggaatagatggggtccaatg	1259
MPaB (HQ731031.1)	tcttgggatgcactcccattttacatcaggcctacttttctggaacagatggggtccaatg	1259
	*****	
MPaB (Sequenced)	gcttggatctcttgggtgatggggccaccctgtcccaggcgatcatggccagaagtactat	1319
MPaB (HQ731031.1)	gcttggatctcttgggtgatggggccaccctgtcccaggcgatcttggccagaagtactat	1319
	** *****	
MPaB (Sequenced)	ccacagggatatacatatacaagatattggaccaaatactttgaaggaaagggccacaag	1378
MPaB (HQ731031.1)	ccacagggatatacatatacaagatattggaccaaatactttgaaggaaagggccacaag	1378
	*****	
MPaB (Sequenced)	gagatccaggaaatgatgaaggaattaagtacttgcaggacgggaaatgtcccttccat	1438
MPaB (HQ731031.1)	gagatccaggaaatgatgaaggaattaagtacttgcaggacgggaaatgtcccttccat	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)	atgatcaaatcgcaaacgggtcatccaagagccacgcaacatctcagatgttcttactca	60
MPaE (HQ731031.1)	atgatcaaatcgcaaacgggtcatccaagagccacgcaacatctcagatgttcttactca	60
	*****	
MPaE (Sequenced)	gacgagtcctctctgggggtccggagcaagatattgaagcgatcatctggtcgcttgcc	120
MPaE (HQ731031.1)	gacgagtcctctctgggggtccggagcaagatattgaagcgatcatctggtcgcttgcc	120
	**** * ***** * ****	
MPaE (Sequenced)	cattttgaccacattgggtgaccatcaactttcccccatctaccgagctagctcgctgga	180
MPaE (HQ731031.1)	cattttgaccacattgggtgaccatcaactttcccccgctctaccgagctagctcgctgga	180
	*****	
MPaE (Sequenced)	cctggcatccgagacaccactggccggcttcccaactaaccagacgcaatcaacctc	240
MPaE (HQ731031.1)	cctggcatccgagacaccactggccggcttcccaactaaccagacgcaatcaacctc	240
	*****	
MPaE (Sequenced)	aacaccgacatccaaggtcgcaatgtgagagaaatttcttcgaaaagacacagaagga	300
MPaE (HQ731031.1)	aacaccgacatccaaggtcgcaatgtgagagaaatttcttcgaaaagacacagaagga	300
	*****	
MPaE (Sequenced)	gccaccaagattggctctttcgacgcccgtggactatttgcggtgatgggtcgttgtatctt	360
MPaE (HQ731031.1)	gccaccaagattggctctttcgacgcccgtggactatttgcggtgatgggtcgttgtatctt	360
	***** * * ***** * * ***** * * *****	
MPaE (Sequenced)	ctagatgctgcccgtcattccgctggccatatacgggtgctcttgcgctgactacctct	420
MPaE (HQ731031.1)	ctagatgctgcccgtcattccgctggccatatacgggtgctcttgcgctgactacctct	420
	*****	
MPaE (Sequenced)	ccagactcgtttgtcttcatgggtgggtgactcatgtcaccatgccggagtgcttcgaccc	480
MPaE (HQ731031.1)	ccagactcgtttgtcttcatgggtgggtgactcatgtcaccatgccggagtgcttcgaccc	480
	*** *****	
MPaE (Sequenced)	acaaaatatcttcttcttgcactcgcactctgggtgacacttcaacttccatgcaaatccgac	540
MPaE (HQ731031.1)	acaaaatatcttcttcttgcactcgcactctgggtgacacttcaacttccatgcaaatccgac	540
	*****	
MPaE (Sequenced)	tctgttttcacggttatcgctgactgccaactgattacactgctgctttgaggacagtc	600
MPaE (HQ731031.1)	tctgttttcacggttatcgctgactgccaactgattacactgctgctttgaggacagtc	600
	*****	
MPaE (Sequenced)	gagaatattaaggagctcgatgcctgtgaggatgtattcgtcgtccttgcctatgatgct	660
MPaE (HQ731031.1)	gagaatattaaggagctcgatgcctgtgaggatgtattcgtcgtccttgcctatgatgct	660
	*****	
MPaE (Sequenced)	acctgaaaggaaggttgactttacccttcgaaaatcaatgattggaagggcaagag	720
MPaE (HQ731031.1)	acctgaaaggaaggttgactttacccttcgaaaatcaatgattggaagggcaagag	720
	*****	
MPaE (Sequenced)	tacggcaagaagacaaaatggctttttataaggatattgagaattccatagaagaac	778
MPaE (HQ731031.1)	tacggcaagaagacaaaatggctttttataaggatattgagaattccatagaagaac	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)	MSLPLPPALSELAR <b>AP</b> SYSR <b>TQ</b> WLPI <b>LV</b> GF <b>LI</b> GY <b>PL</b> LI <b>KAL</b> RYKR <b>L</b> GEMKKK <b>FY</b> FP <b>TRES</b> 60
MPaB (HQ731031.1)	MSLPLPPALSELAR <u>AP</u> SYSR <u>TQ</u> WLPI <u>LV</u> GF <u>LI</u> GY <u>PL</u> LI <u>RAL</u> RYKR <u>H</u> GEMKKK <b>FY</b> FP <b>TRES</b> 60
*****	
MPaB (Sequenced)	MAEMTDEEAFLIQEKEM <b>AQ</b> LE <b>FP</b> FM <b>LT</b> SG <b>QF</b> ALFR <b>TY</b> GI <b>PT</b> IS <b>HLL</b> TK <b>TG</b> Q <b>FS</b> K <b>PET</b> S <b>FK</b> 120
MPaB (HQ731031.1)	MAEMTDEEAFLIQEKEM <b>AQ</b> LE <b>FP</b> FM <b>LT</b> SG <b>QF</b> ALFR <b>TY</b> GI <b>PT</b> IS <b>HLL</b> TK <b>TG</b> Q <b>FS</b> K <b>PET</b> S <b>FK</b> 120
*****	
MPaB (Sequenced)	RYTDTAALIGEMVENS <b>PTS</b> Q <b>RAF</b> IS <b>VART</b> RF <b>LH</b> SGY <b>QAS</b> G <b>KIL</b> DAD <b>LLY</b> TL <b>AL</b> FA <b>VQ</b> P <b>V</b> R 180
MPaB (HQ731031.1)	RYTDTAALIGEMVENS <b>PTS</b> Q <b>RAF</b> IS <b>VART</b> RF <b>LH</b> SGY <b>QAS</b> G <b>KIL</b> DAD <b>LLY</b> TL <b>AL</b> FA <b>VQ</b> P <b>V</b> R 180
*****	
MPaB (Sequenced)	FIENFEW <b>RT</b> LS <b>DLE</b> LCAIG <b>T</b> FW <b>KSL</b> GDAL <b>GI</b> SS <b>EIL</b> PS <b>GKT</b> G <b>FKD</b> GI <b>QW</b> LE <b>EV</b> D <b>V</b> W <b>SQ</b> D <b>Y</b> 240
MPaB (HQ731031.1)	FIENFEW <b>RT</b> LS <b>DLE</b> LCAIG <b>T</b> FW <b>KSL</b> GDAL <b>GI</b> SS <b>EIL</b> PS <b>GKT</b> G <b>FKD</b> GI <b>QW</b> LE <b>EV</b> D <b>V</b> W <b>SQ</b> D <b>Y</b> 240
*****	
MPaB (Sequenced)	EAKY <b>MV</b> PD <b>PK</b> NRESAD <b>Q</b> ATA <b>V</b> LT <b>SN</b> RYE <b>AP</b> SP <b>GWS</b> A <b>V</b> FS <b>SL</b> LA <b>TR</b> K <b>FV</b> L <b>RY</b> LS <b>PP</b> RA <b>AL</b> 300
MPaB (HQ731031.1)	EAKY <b>MV</b> PD <b>PK</b> NRESAD <b>Q</b> ATA <b>AM</b> L <b>IS</b> NR <b>YE</b> AP <b>TP</b> FG <b>WS</b> M <b>V</b> FS <b>T</b> LLA <b>IR</b> K <b>L</b> L <b>RY</b> LS <b>PP</b> RA <b>AL</b> 300
*****	
MPaB (Sequenced)	AVSNIA <b>Q</b> PK <b>DK</b> DD <b>RY</b> HRMSWDAL <b>PF</b> Y <b>IR</b> PT <b>F</b> W <b>NR</b> WG <b>PM</b> AWIS <b>W</b> LM <b>GH</b> VP <b>GD</b> H <b>GQ</b> K <b>Y</b> Y <b>PQ</b> 360
MPaB (HQ731031.1)	AVSNIA <b>Q</b> PK <b>DK</b> DD <b>RY</b> HRMSWDAL <b>PF</b> Y <b>IR</b> PT <b>F</b> W <b>NR</b> WG <b>PM</b> AWIS <b>W</b> LM <b>GH</b> VP <b>GD</b> L <b>GQ</b> K <b>K</b> G <b>PQ</b> 360
*****	
MPaB (Sequenced)	GD <b>PG</b> NDEGI <b>KY</b> L <b>Q</b> DGEM <b>S</b> L <b>PL</b> V <b>W</b> S <b>KY</b> HAT <b>TND</b> * 392
MPaB (HQ731031.1)	GD <b>PG</b> NDEGI <b>KD</b> L <b>K</b> DGEM <b>S</b> L <b>PL</b> V <b>W</b> S <b>KY</b> HAT <b>TND</b> * 392
*****	

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

MPaE (Sequenced)	MIKSQ <b>T</b> VI <b>Q</b> E <b>PR</b> NI <b>S</b> D <b>V</b> L <b>D</b> S <b>D</b> E <b>SS</b> L <b>G</b> V <b>R</b> S <b>K</b> D <b>IE</b> AI <b>I</b> W <b>S</b> L <b>A</b> H <b>F</b> D <b>H</b> I <b>G</b> D <b>P</b> S <b>T</b> F <b>P</b> P <b>S</b> T <b>E</b> L <b>V</b> V <b>G</b> 60
MPaE (HQ731031.1)	MIKSQ <b>T</b> VI <b>Q</b> E <b>PR</b> NI <b>S</b> D <b>V</b> L <b>D</b> S <b>D</b> E <b>SS</b> L <b>G</b> V <b>R</b> S <b>K</b> D <b>IE</b> AI <b>I</b> W <b>S</b> <u>HA</u> H <b>F</b> D <b>H</b> I <b>G</b> D <b>P</b> S <b>T</b> F <b>P</b> P <b>S</b> T <b>E</b> L <b>V</b> V <b>G</b> 60
*****	
MPaE (Sequenced)	PG <b>IR</b> D <b>TH</b> W <b>P</b> G <b>F</b> P <b>T</b> NP <b>D</b> A <b>IN</b> L <b>NT</b> D <b>I</b> Q <b>GR</b> NV <b>RE</b> I <b>S</b> F <b>E</b> K <b>T</b> Q <b>K</b> G <b>A</b> T <b>K</b> I <b>G</b> S <b>F</b> D <b>A</b> V <b>D</b> Y <b>F</b> G <b>D</b> G <b>S</b> L <b>Y</b> L 120
MPaE (HQ731031.1)	PG <b>IR</b> D <b>TH</b> W <b>P</b> G <b>F</b> P <b>T</b> NP <b>D</b> A <b>IN</b> L <b>NT</b> D <b>I</b> Q <b>GR</b> NV <b>RE</b> I <b>S</b> F <b>E</b> K <b>T</b> Q <b>K</b> G <b>A</b> T <b>K</b> I <b>G</b> S <b>F</b> D <b>A</b> V <b>D</b> Y <b>F</b> G <b>D</b> G <b>S</b> <u>Y</u> L 120
*****	
MPaE (Sequenced)	L <b>D</b> A <b>A</b> G <b>H</b> S <b>V</b> G <b>H</b> I <b>G</b> A <b>L</b> A <b>R</b> V <b>T</b> T <b>S</b> P <b>D</b> S <b>F</b> V <b>F</b> M <b>G</b> G <b>D</b> S <b>C</b> H <b>H</b> A <b>G</b> V <b>L</b> R <b>P</b> T <b>K</b> Y <b>L</b> P <b>C</b> P <b>L</b> D <b>S</b> G <b>D</b> T <b>S</b> L <b>P</b> C <b>K</b> S <b>D</b> 180
MPaE (HQ731031.1)	L <b>D</b> A <b>A</b> G <b>H</b> S <b>V</b> G <b>H</b> I <b>G</b> A <b>L</b> A <b>R</b> V <b>T</b> T <b>S</b> <u>P</u> <b>V</b> S <b>F</b> V <b>F</b> M <b>G</b> G <b>D</b> S <b>C</b> H <b>H</b> A <b>G</b> V <b>L</b> R <b>P</b> T <b>K</b> Y <b>L</b> P <b>C</b> P <b>L</b> D <b>S</b> G <b>D</b> T <b>S</b> L <b>P</b> C <b>K</b> S <b>D</b> 180
*****	
MPaE (Sequenced)	SV <b>F</b> T <b>L</b> S <b>P</b> A <b>L</b> P <b>T</b> D <b>Y</b> A <b>A</b> L <b>R</b> T <b>V</b> E <b>N</b> I <b>K</b> E <b>L</b> D <b>A</b> C <b>E</b> D <b>V</b> F <b>V</b> L <b>A</b> H <b>D</b> A <b>T</b> L <b>K</b> G <b>K</b> V <b>D</b> F <b>Y</b> P <b>S</b> K <b>I</b> N <b>D</b> W <b>K</b> A <b>K</b> E 240
MPaE (HQ731031.1)	SV <b>F</b> T <b>L</b> S <b>P</b> A <b>L</b> P <b>T</b> D <b>Y</b> A <b>A</b> L <b>R</b> T <b>V</b> E <b>N</b> I <b>K</b> E <b>L</b> D <b>A</b> C <b>E</b> D <b>V</b> F <b>V</b> L <b>A</b> H <b>D</b> A <b>T</b> L <b>K</b> G <b>K</b> V <b>D</b> F <b>Y</b> P <b>S</b> K <b>I</b> N <b>D</b> W <b>K</b> A <b>K</b> E 240
*****	
MPaE (Sequenced)	Y <b>G</b> K <b>K</b> T <b>K</b> W <b>L</b> F <b>Y</b> K <b>D</b> I <b>E</b> N <b>S</b> I <b>E</b> G <b>Q</b> I <b>K</b> * 261
MPaE (HQ731031.1)	Y <b>G</b> K <b>K</b> T <b>K</b> W <b>L</b> F <b>Y</b> K <b>D</b> I <b>E</b> N <b>S</b> I <b>E</b> G <b>Q</b> I <b>K</b> * 261
*****	

**Fig. 9.** Amino acid alignment of *MPaE* and *MPaE* accession number: HQ731031.1 from *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.

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## شناسایی مولکولی ژن های *MpaE* و *MpaB* از خوشه ژنی MAP در یک سوش جدید از قارچ *Penicillium brevicompactum*

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**چکیده:** میکوفنولیک اسید (MPA) یک متابولیت ثانویه قارچی با خاصیت ضدویروسی، ضدقارچی، ضدباکتریایی، ضدتومور و ضد پسروریاس می باشد. 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 لنفوسیت های B و T محدود می شود. ژن های *MpaE* و *MpaB* از مجموعه ژنی ۲۵ کیلو دالتونی از ژنوم *P. brevicompactum* می باشند. 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 *MpaE* و *MpaB* آغازگرهای ویژه با استفاده از نرم افزار Gene Runner و بر اساس توالی ژنوم *P. brevicompactum* سویه IBT23078 با شماره دسترسی 1. HQ731031 طراحی شدند. ژن های *MpaE* و *MpaB* 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 *MpaE* و *MpaB* 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 *MpaE* و *MpaB* گزارش شده در بانک ژن می باشد. اما هم ترازای 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 میکوفنولیک، توالی، همسانه سازی، پیوند، همردیف سازی