

**Identification of the role of a MAP kinase Tmk2 in *Hypocrea jecorina***

**(*Trichoderma reesei*)**

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**Supplementary Information 1-4**

## Supplementary Information 1. Sequence alignment between Tmk2 from *H.*

### *jecorina* and Slt2 from *S. cerevisiae*

```
Tmk2      MADLQGRKVFVKVFNQDFVVDERYTVTKELGQGAYGIVCAAVNNQTNEG--VAIKKVTNVE
Slt2      MADKIERHTFKVFNQDFSVDKRFQLIKEIGHGAYGIVCSARFAEAAEDTTVAIKKVTNVE
          ***      *:.***** **:* : **:*:*****:*      :.  *****

Tmk2      SKKILAKRALREIKLLQHFRGHRNITCLYDMDIP-RPD-NFNETYLYEELMECDLAAIIR
Slt2      SKTLLCKRSLRELKLLRHFRGHKNITCLYDMDIVFYPDGSINGLYLYEELMECDMHQIIK
          **.:*.**:*:***:***:*****:***** ** .:* *****: **

Tmk2      SGQPLTDAHFQSFYQILCGLKYIHSANVLHRDLKPGNLLVNADCELKICDFGLARGFSV
Slt2      SGQPLTDAHYQSFTYQILCGLKYIHSADVLHRDLKPGNLLVNADCQLKICDFGLARGYSE
          *****:*** *****:*****:*****:*****:

Tmk2      DPEENAGYMTEYVATRWRAPAIMLSFQSYTKAIDVWSVGCILAELLGGRPFKGRDYVD
Slt2      NPVENSQFLTEYVATRWRAPAIMLSYQGYTKAIDVWSAGCILAFLGGKPIFKGKDYVN
          :* **: :*:*****:*.*****.*****:***:*:***:***:

Tmk2      QLNQILHILGTPNEETLRRIGSPRAQEYVRNLPFMPKPPFALFPDANPDALDLDKMLA
Slt2      QLNQILQVLGTPPDETLRRIGSKNVQDYIHQLGFIPKVPFVNLYPNANSQALDLEQMLA
          *****:***** :***** ..*:*::* *:* ** *:*:***:*****:***

Tmk2      FDPSQRISVEQALEHPYLHIWHDASDEPDCPTTFNDFEVVEDVGEKRMILDEVLRFRQ
Slt2      FDPQKRITVDEALEHPYLSIWHDDAPEVPCSEKFEFESVNDMEDLKQMVIQEVQDFRL
          ***.:**:*:***** *****.:*** *. .*:*.** *:*: ::*:::** **

Tmk2      LVRT-----APASGNQGAGQQIQVPLPSAGGQWTAED-----
Slt2      FVRQPLLEEQRQLQLQQQQQQQQQQQQQQPSDVDNGNAAASEENYPKQMATSNVAPQ
          :**      . :* ** * * .*: :*.:

Tmk2      -----PRPQEYMGQP-----NG----LEQELQAGMD
Slt2      QESFGIHSQNLPRHDADFPPRPQESMMEMRPATGNTADIPPQNDNGTLLDLEKELEFGLD
          ***** * :      **      **:*: *:*

Tmk2      IRR-
Slt2      RKYF
          :
```

**Supplementary Information 2. Sequence alignment between Tmk2 and Tmk3 from *H. jecorina*.**

```

Tmk2      MADLQGRKVKFVFNQDFVVDERYTVTKELGQGAYGIVCAAVNNQTNEGVAIKKVTNVFSK
Tmk3      MAEFVVR--AQIFGTTFEITSRYSDLQFVGMGAFGLVCSARDQLTNQNVAVKKIMKPFST
          **:::      **:.*  * : .**:  : :* **:*:**:* : : **:.**:**: : **.

Tmk2      KILAKRALREIKLLQHFRGHRNITCLYDMDIPRPNDFNETYLYEELMECDLAAIIRSGQP
Tmk3      PVLAKRTYRELKLLKHLR-HENVISLSDIFISP---LEDIYFVTELLGTDLHRLTTS-RP
          :****: **:***:*:* *.*: .* *: *.      ::: *: **: **  : : * :*

Tmk2      LTDAHFQSFYIYQILCGLKYIHSANVLHRDLKPGNLLVNADCELKICDFGLARGFSVDPEE
Tmk3      LEKQFIQYFLYQIMRGLKYVHSAGVVHRDLKPSNILVNENCDLKICDFGLAR--IQDPQ-
          * . .:* *:**: ****:*:*.*:*****.*:** *:*:**:***** **

Tmk2      NAGYMTEYVATRWRAPPEIMLSFQSYTKAIDVWSVGCILAEELGGRPFKGRDYVDQLNQ
Tmk3      ----MTGYVSTRYYRAPEIMLTWQKYDVEVDIWSAGCIFAEMLEGKPLFPGKDHVNQFSI
          ** **.*:**:*****:.*.*      :*:*.*:**:***.* *:*:* *:*:**:*.

Tmk2      ILHILGTPNEETLRRIGSPRAQEYVRNLPFMPKKPFPPALFPDANPDALDLLDKMLAFDPS
Tmk3      ITELLGTPDDVINTIASENTLRFVKS LPKRERQPLRNKFKNADDSAVDLLERMLVFDPK
          * .:**** :::.. *.* .: .:*.**      :*: * :*: .:*:**:***.***.

Tmk2      QRISVEQALEHPYLHIWHDASDEPDCPTTFNDFEVVED-VGEMRKMI LDEVLRFRQLVR
Tmk3      KRITATEALAEHYLAPYHDPTDEPVAEKFDWSFNDADLPVDTWKIMMYSEILDYHNIEG
          :**:. :** * ** :*:*:**. . *::.*: .: * . : * :*:* ::::

Tmk2      TAPASGNQGAGQQIQVPLPSAGGQWTAEDPRPQEYMGQPNGLEQELQAGMDIRR
Tmk3      GVPNMDEQFPFQ-----
          .* .:* . *

```

**Supplementary Information 3. Colony diameters of *H. jecorina* parent and**

***Δtmk2* strains grown on plates.** All values are expressed in mean±s.d. (n=4 for PDA plates, n=19 for *H. jecorina* parent strain grown on glucose plates, n=17 for *H. jecorina Δtmk2* grown on glucose plates, n=3 for all other plates).

Plates	Parent (mm)	<i>Δtmk2</i> (mm)	Plates	Parent (mm)	<i>Δtmk2</i> (mm)
PDA	78.0±0.8	28.5±2.1	PDA+1 mM H <sub>2</sub> O <sub>2</sub>	63.0±5.0	28.3±7.5
PDA+0.2 M NaCl	59.7±5.8	30.3±0.6	PDA+2 mM H <sub>2</sub> O <sub>2</sub>	46.3±10.2	22.3±1.2
PDA+0.4 M NaCl	43.3±2.9	27.0±1.0	PDA+3 mM H <sub>2</sub> O <sub>2</sub>	0.0±0.0	0.0±0.0
PDA+0.6 M NaCl	34.3±2.1	24.3±0.6	PDA+4 mM H <sub>2</sub> O <sub>2</sub>	0.0±0.0	0.0±0.0
PDA+0.8 M NaCl	23.3±0.6	18.3±1.2	Lactose	33.0±1.0	23.7±0.6
PDA+1.0 M NaCl	14.0±0.0	11.3±0.6	Glucose	48.4±2.5	21.5±0.9
Glycerol	23.0±1.7	16.7±0.6	Avicel	59.3±1.5	37.3±1.1

**Supplementary Information 4. Primers used in real-time PCR reactions and amplicon sizes.**

Primer	Sequence (5' to 3')	Amplicon size (bp)
actin-F	TTAAGAAAGCCGCCACCCCC	134
actin-R	GTTGGTTCGACAGGGAGAGGATG	
cbh1-F	CTGCGACTGGAACCCATAACC	132
cbh1-R	AAGTGACGCCATTCTGGACAT	
cbh2-F	CGTTGCTGGATTCGTTTGTC	178
cbh2-R	GATGGGTTTGCGTTTGTGAG	
egl1-F	CCCAGAGTCCATCCCAAG	100
egl1-R	TCCAGCGGTAGTTCCAGTCAA	
egl2-F	CAACGACGACGGGATGACTA	246
egl2-R	GATGCCAACTGCGACCAAA	
bgl1-F	TGGGCGGGTCTTCCTTC	104
bgl1-R	GGGCTCTTCGCAATGGTGTA	
xyr1-F	ACAGTGGAGCGGTAACAGACA	209
xyr1-R	CACGAATCCTTCCGACGAG	
cre1-F	CTCCTTCTTCTCCACCTT	140
cre1-R	CTGCAGGGACAGGTTTCT	
ace1-F	AGACCCTGATCTTCATGGCAC	110
ace1-R	GTCGGTGAGATATTCGACTGT	
ace2-F	TCAACATCCTCCACCACCAG	117
ace2-R	CTTGAGACGTGATGCATGTC	
Trire2_112271-F	TCTTCATCGCAGTCAAGGCCATC	204
Trire2_112271-R	TAGGCGTCAGGAGCATGTACTGG	
Trire2_58188-F	GCGAACCTTCCCAAGAGAAAGC	251
Trire2_58188-R	CGACATAGAAGGTGAAGGCGA	
Trire2_55341-F	CCATGGACCGTTGAGCCAATACTT	203
Trire2_55341-R	AGATGAACTCGGGGACGGAATCG	
Trire2_51492-F	ATGGCTACAAGTCCTTCCGTACC	118
Trire2_51492-R	GAAGCAGTTCCACGAAACCAGAC	
Trire2_124228-F	TAGTGACGCTACCACTTCCGAGC	266
Trire2_124228-R	CACCGAGTTGATGGCCGTGAT	
Trire2_67600-F	CAACGACGTGATGACCCAGCA	195
Trire2_67600-R	TCGTTGGAGATGCTACCGAGG	
Trire2_122172-F	ATGGTTGGCCTTCCGAGCGA	172
Trire2_122172-R	GGAGAGCTTCGGTGGCACTATTG	

Trire2_71563-F	TGCGTTGTCTTGGGCTCTCTG	194
Trire2_71563-R	AGG TTCAGAGACAGAGTCACGCC	
Trire2_122993-F	AATGTCGTCGGCTGCCTACTC	206
Trire2_122993-R	TCATCGCTCCTCTCATCCCGT	
fks-F	CAACCAGAAGCCTACCGACTCTC	225
fks-R	CCCTCGAGCAAGAAGAGCACT	