A



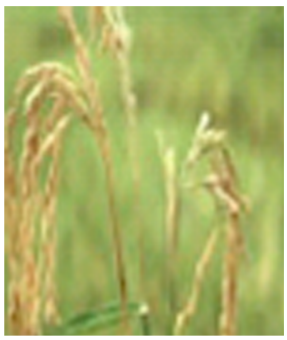
B



C



D



E

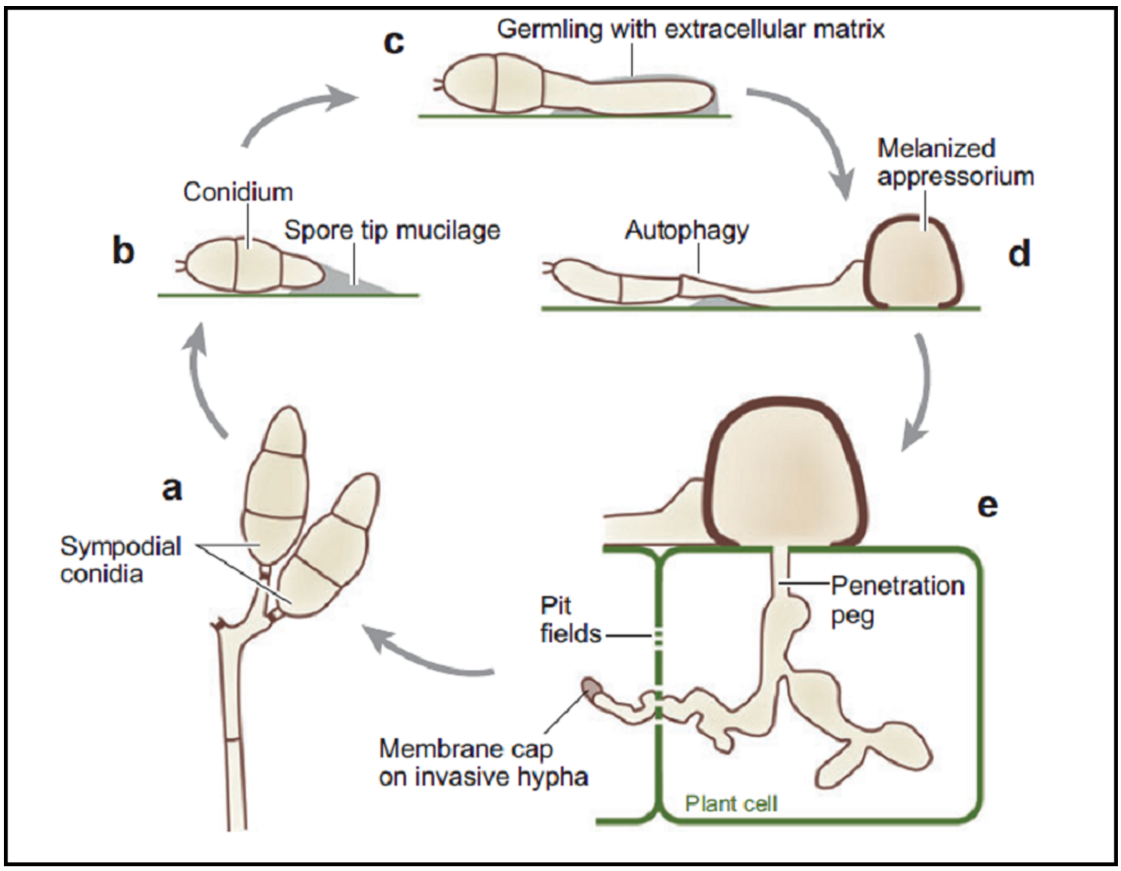


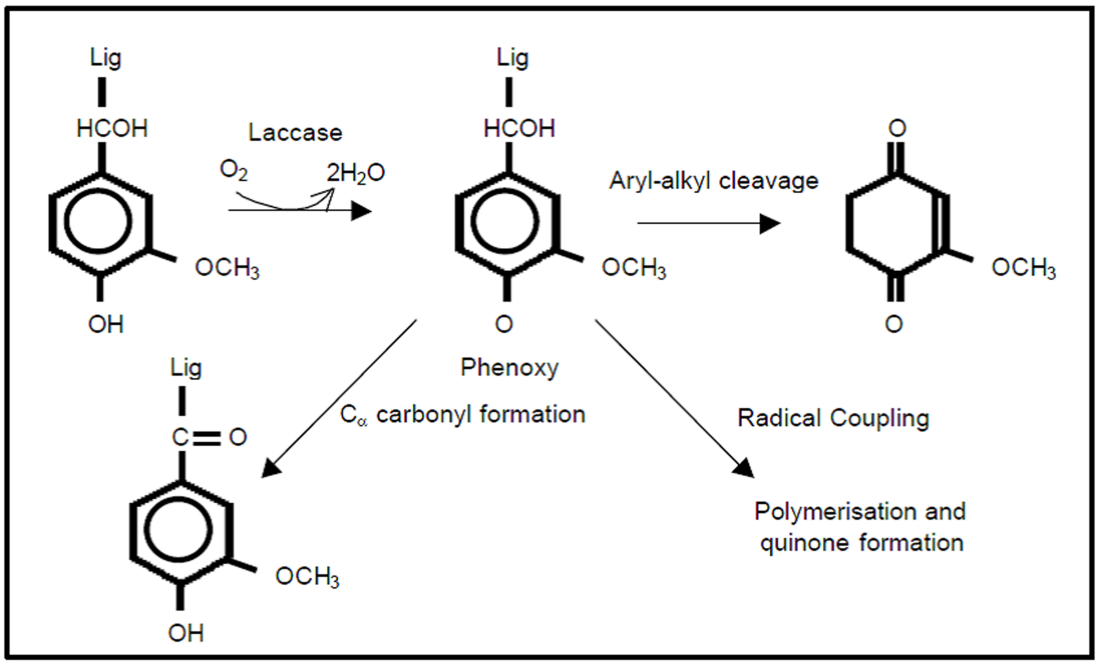
F

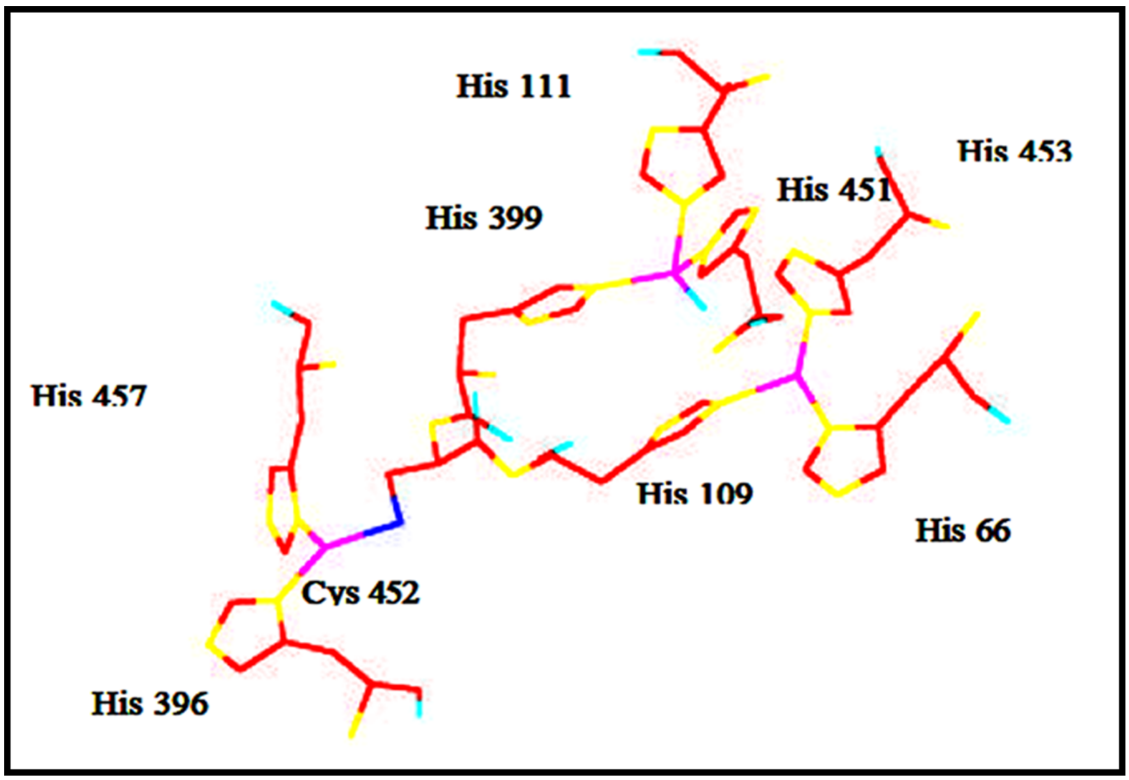


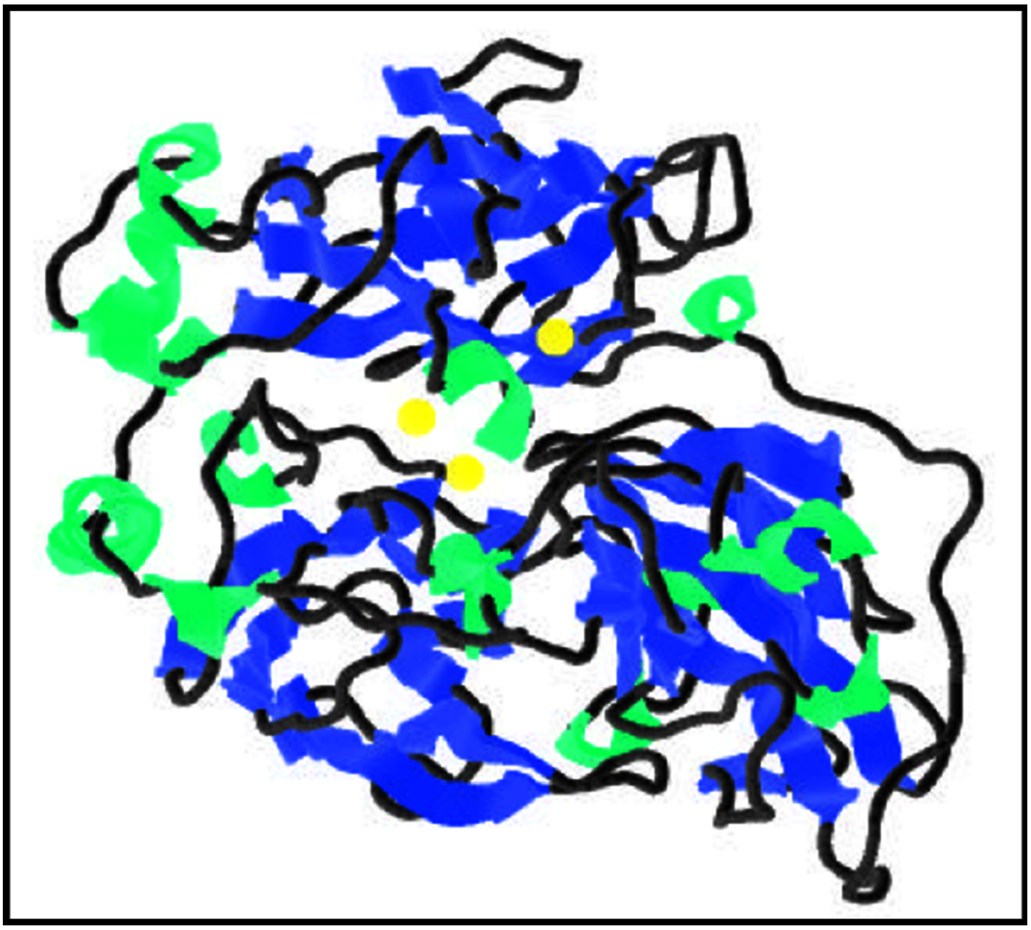
G



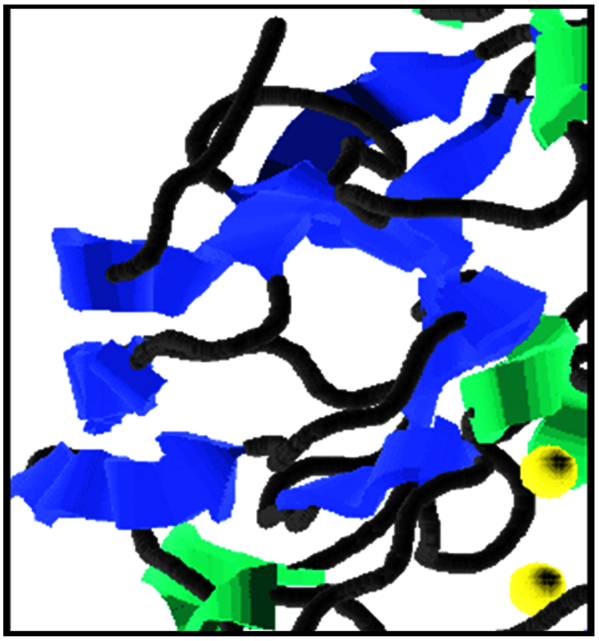

c
Germling with extracellular matrix
Melanized
appressorium
Conidium
Spore tip mucilage
b
Autophagy
d
a
Sympodial
conidia
e
Penetration
peg
Pit
fields
Membrane cap
on invasive hypha
Plant cell


Lig
HCOH
OCH3
OH
Laccase
O2
2H2O
Lig
HCOH
OCH3
O
Aryl-alkyl cleavage
O
OCH3
O
Phenoxy
Lig
C O
OCH3
OH
Cα carbonyl formation
Radical Coupling
Polymerisation and
quinone formation

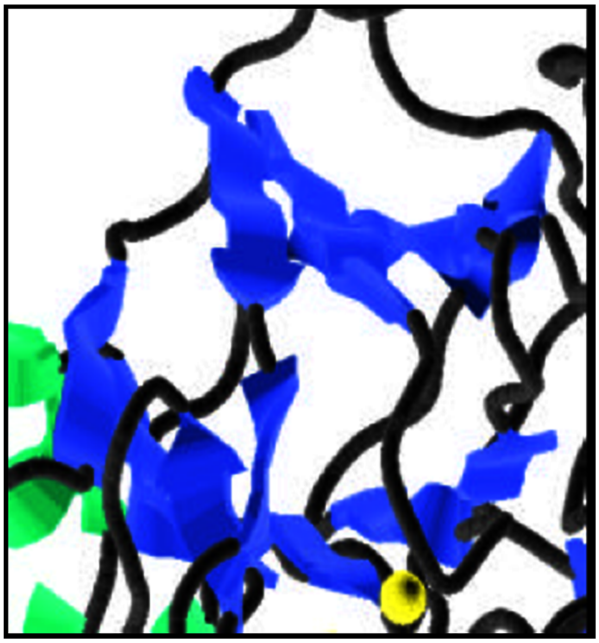

His 111
His 453
His 451
His 399
His 457
His 109
His 66
Cys 452
His 396

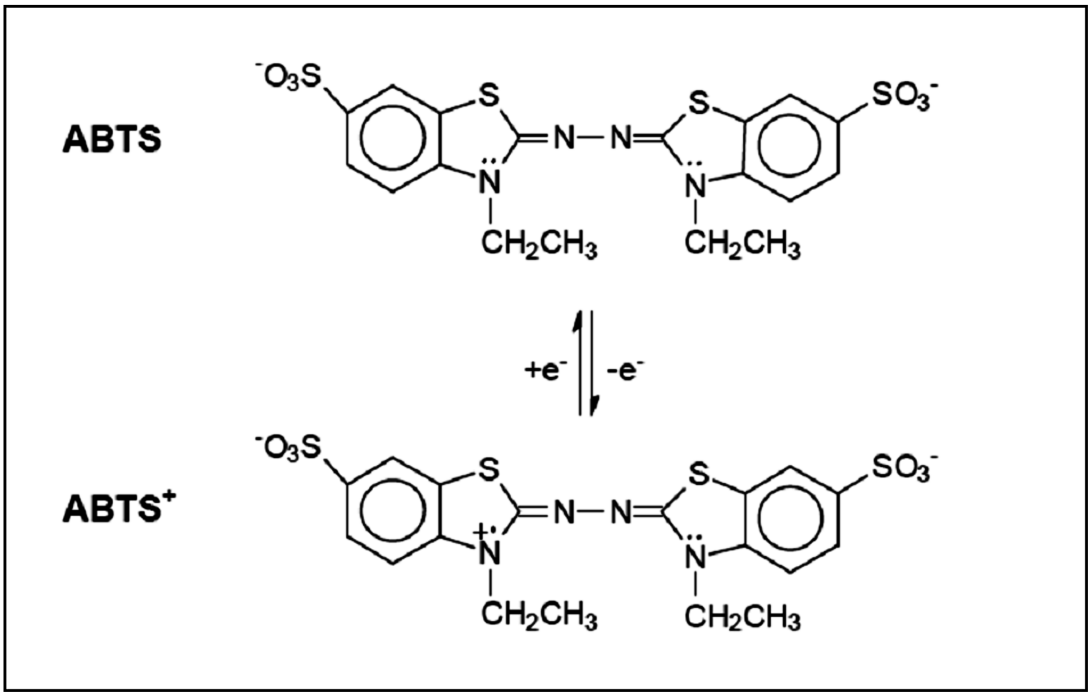


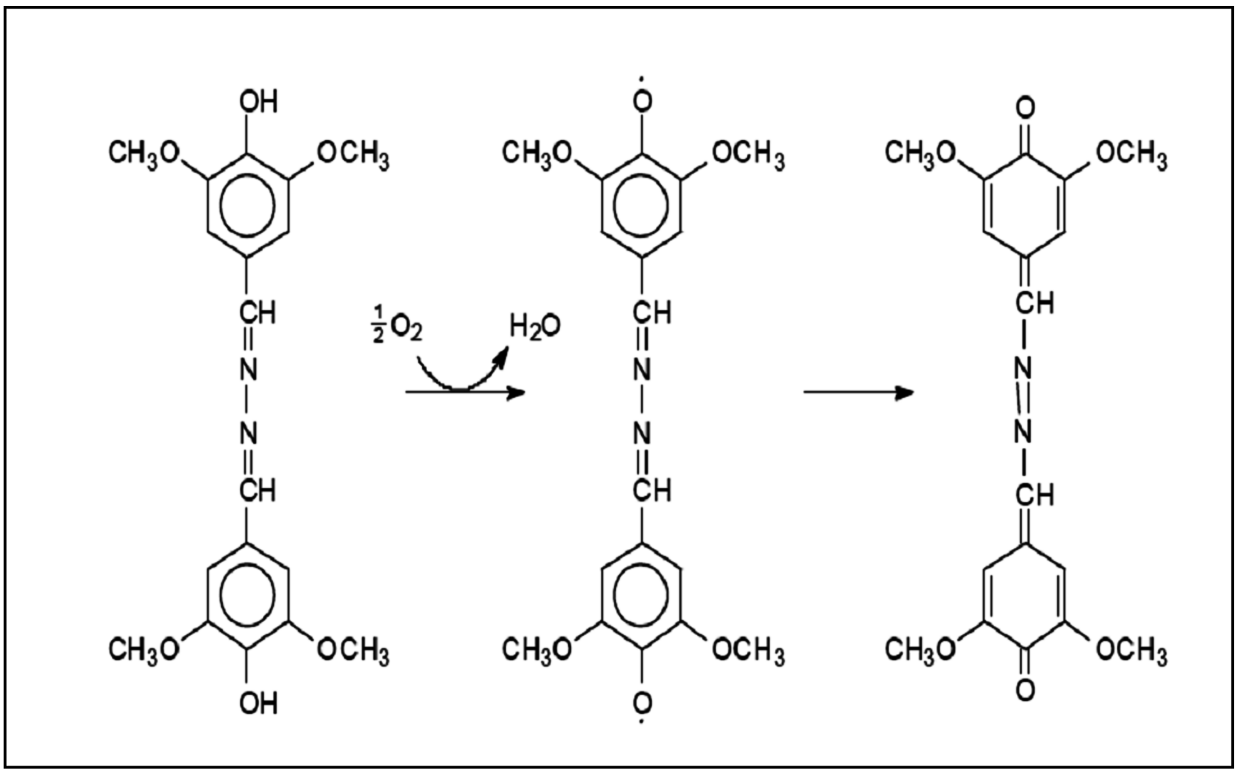
A

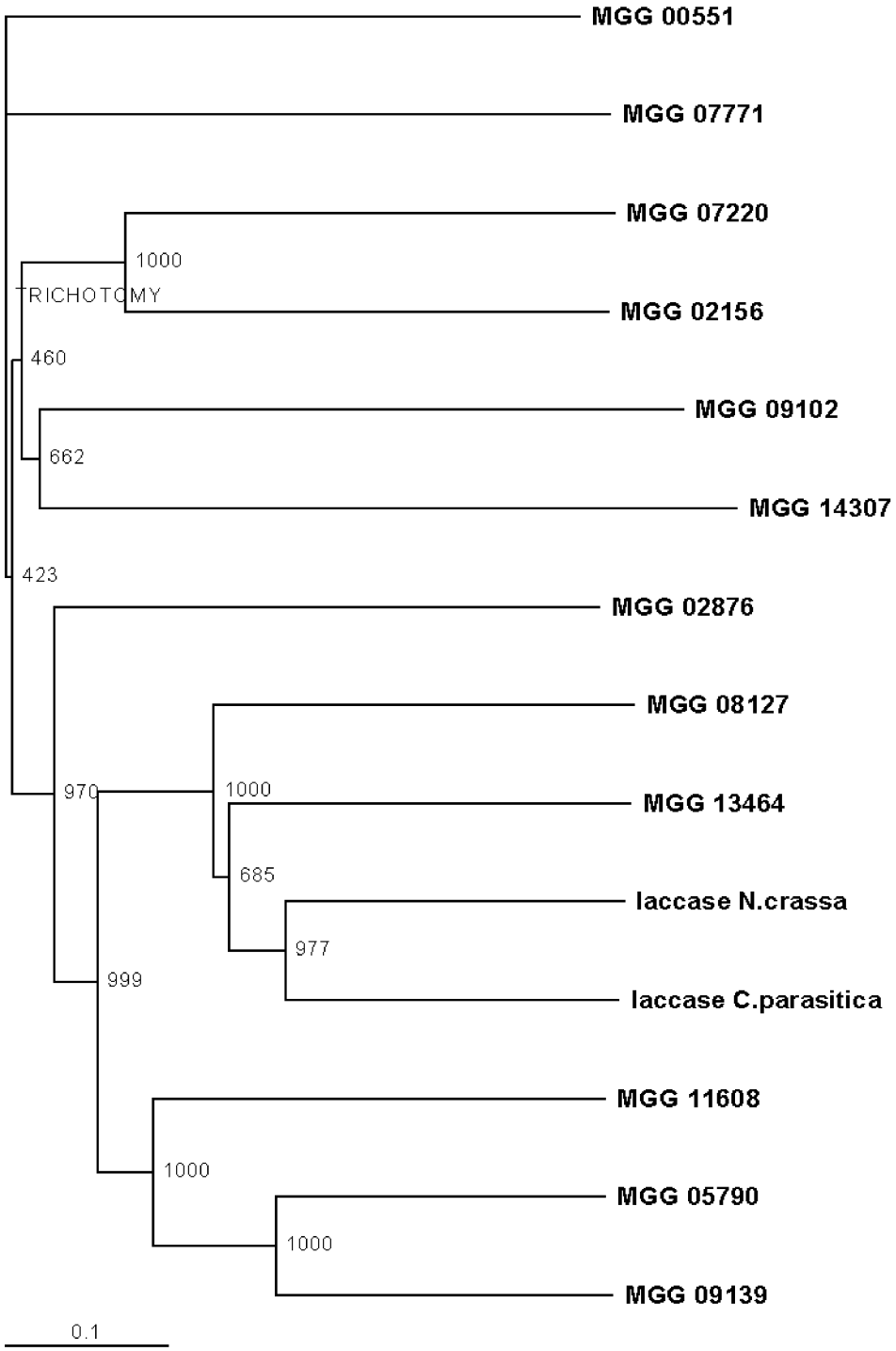


B

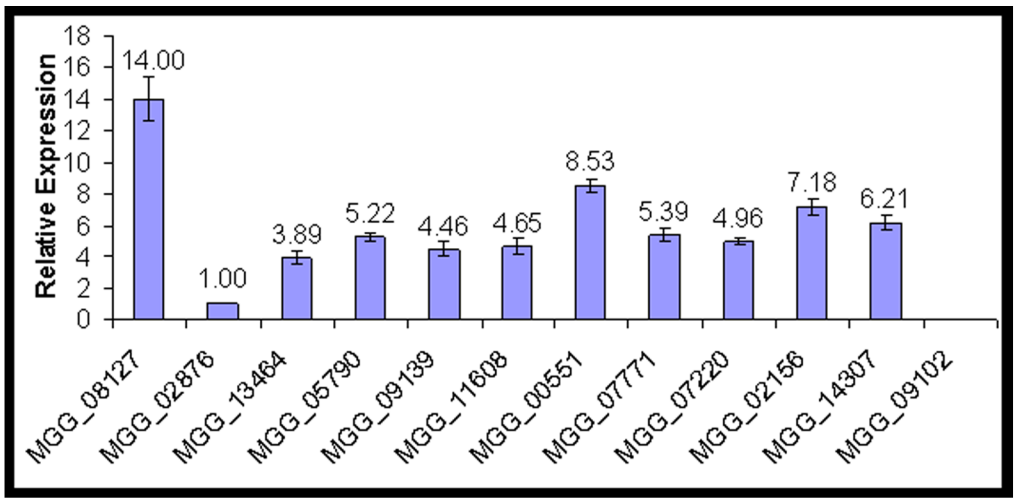



ABTS
$^{-}O_3S$
S
N
N
S
SO_3^-
N
CH_2CH_3
CH_2CH_3
$+e^-$
$-e^-$
$ABTS^+$
$^{-}O_3S$
S
N
N
S
SO_3^-
N
CH_2CH_3
CH_2CH_3

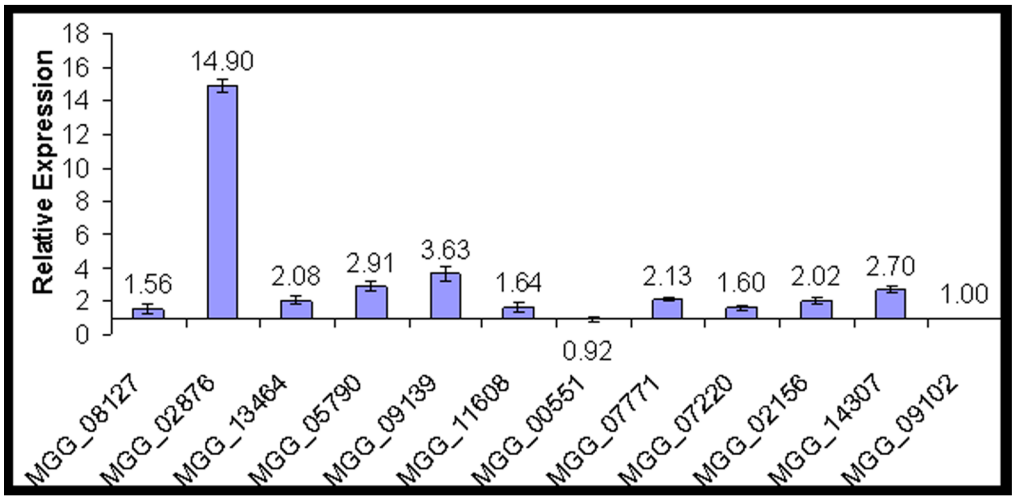

OH
CH3O
OCH3
CH
N
N
CH
CH3O
OCH3
OH
½O2
H2O
O
CH3O
OCH3
CH
N
N
CH
CH3O
OCH3
O
O
CH3O
OCH3
CH
N
N
CH
CH3O
OCH3
O


MGG 00551
MGG 07771
MGG 07220
1000
TRICHOTOMY
MGG 02156
460
MGG 09102
662
MGG 14307
423
MGG 02876
MGG 08127
970
1000
MGG 13464
685
laccase N.crassa
977
999
laccase C.parasitica
MGG 11608
1000
MGG 05790
1000
MGG 09139
0.1

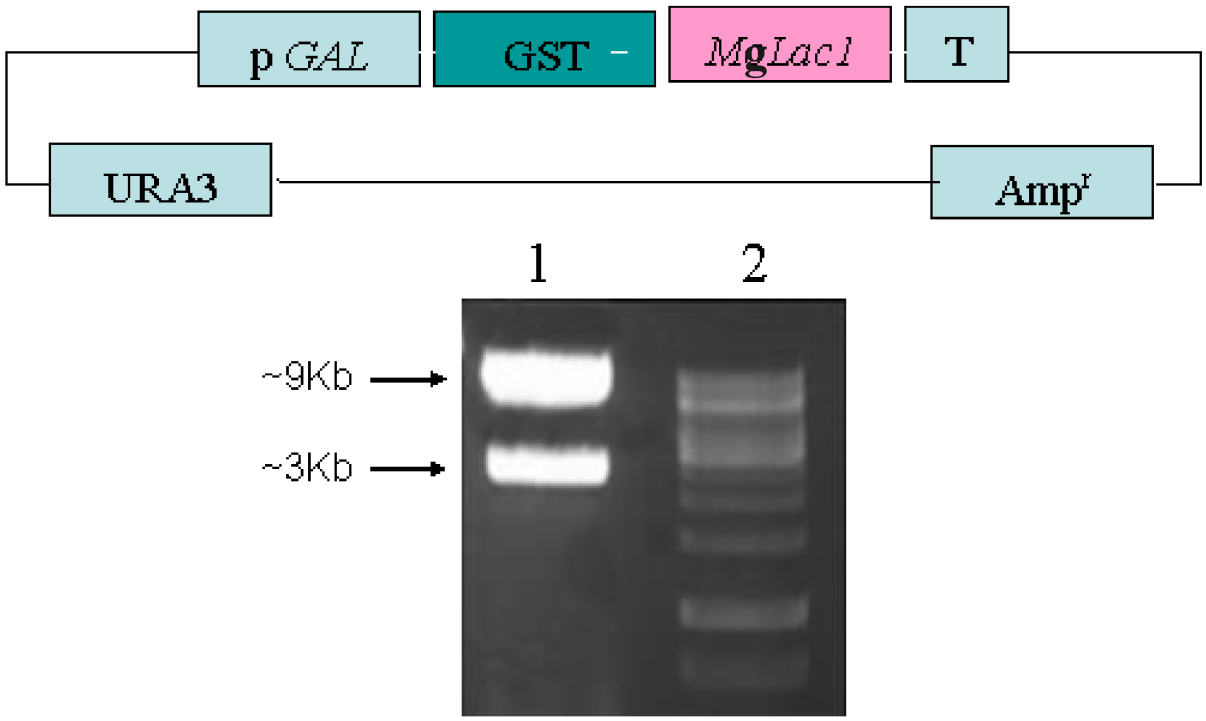
A


Relative Expression
18
16
14
12
10
8
6
4
2
0
14.00
1.00
3.89
5.22
4.46
4.65
8.53
5.39
4.96
7.18
6.21
MGG_08127
MGG_02876
MGG_13464
MGG_05790
MGG_09139
MGG_11608
MGG_00551
MGG_07771
MGG_07220
MGG_02156
MGG_14307
MGG_09102

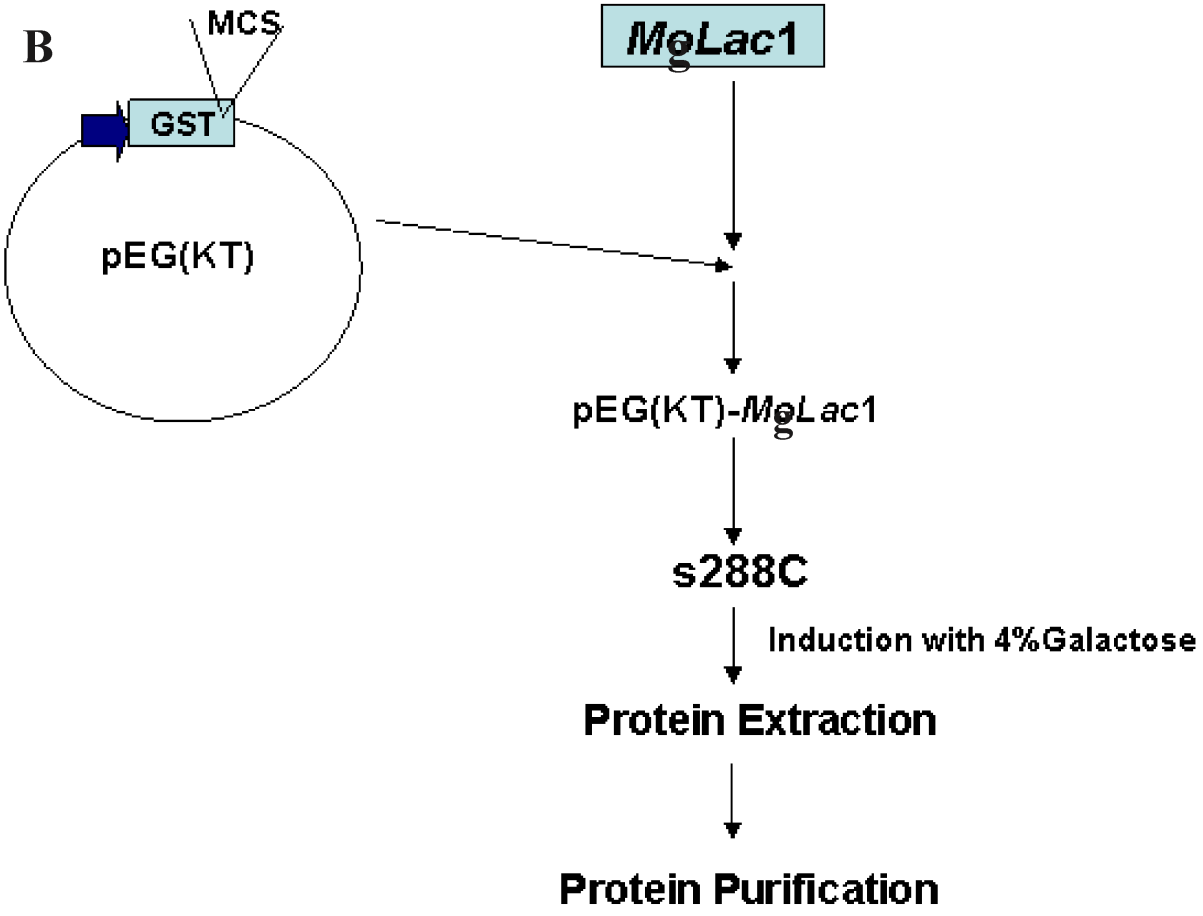
B

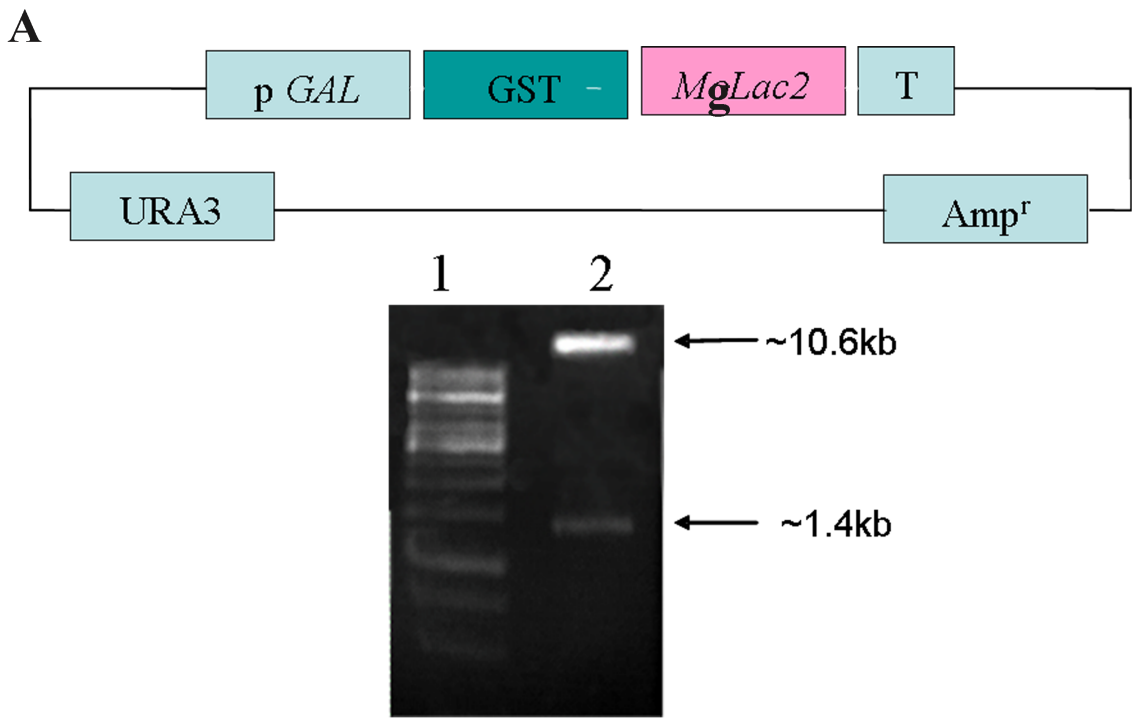

Relative Expression
18
16
14
12
10
8
6
4
2
0
1.56
14.90
2.08
2.91
3.63
1.64
0.92
2.13
1.60
2.02
2.70
1.00
MGG_08127
MGG_02876
MGG_13464
MGG_05790
MGG_09139
MGG_11608
MGG_00551
MGG_07771
MGG_07220
MGG_02156
MGG_14307
MGG_09102

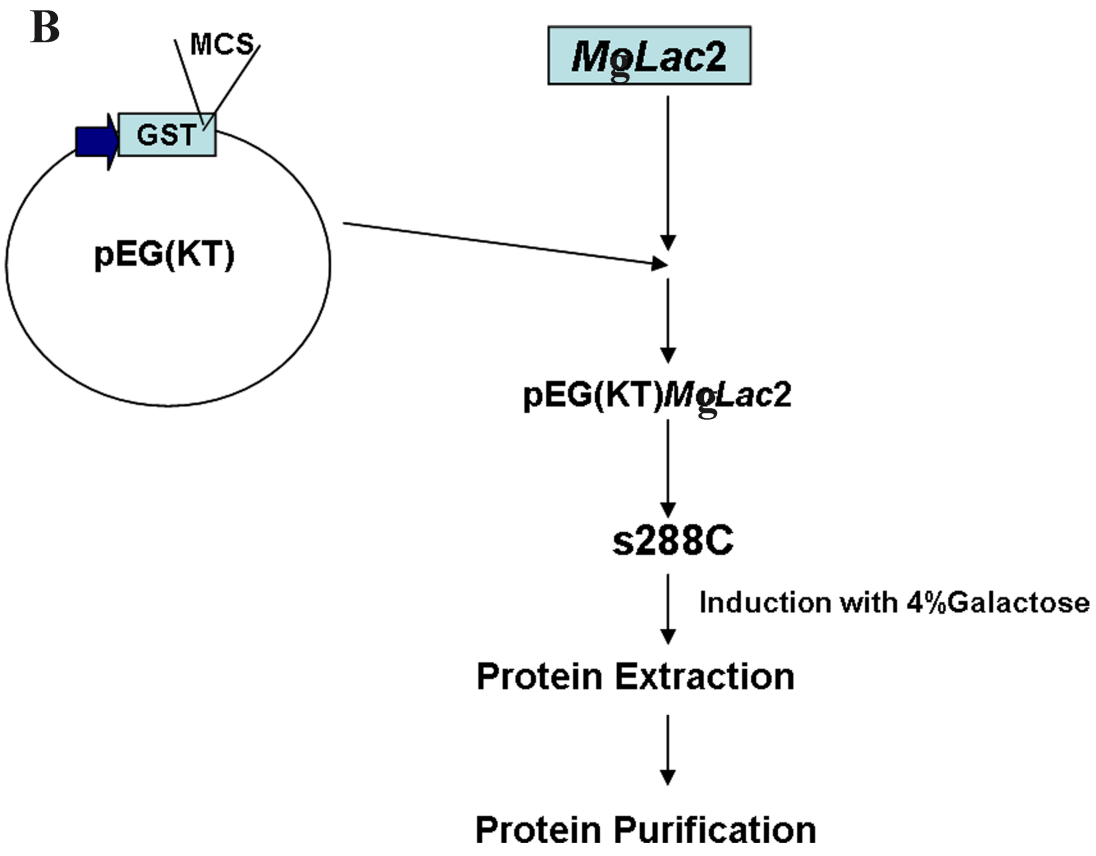
A

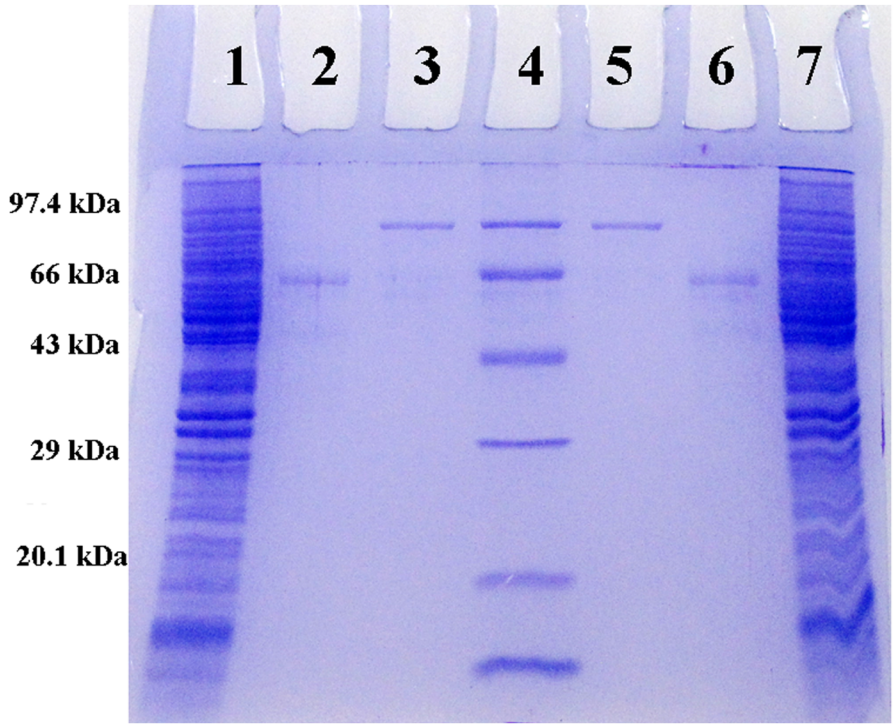

p GAL
GST
MgLac1
T
URA3
Amp^r
1
2
~9Kb
~3Kb

B

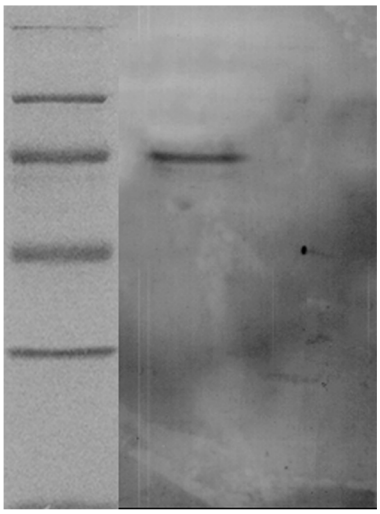

MCS
GST
pEG(KT)
MgLac1
pEG(KT)-MgLac1
s288C
Induction with 4%Galactose
Protein Extraction
Protein Purification


A
p GAL
GST
MgLac2
T
URA3
Amp^r
1
2
~10.6kb
~1.4kb

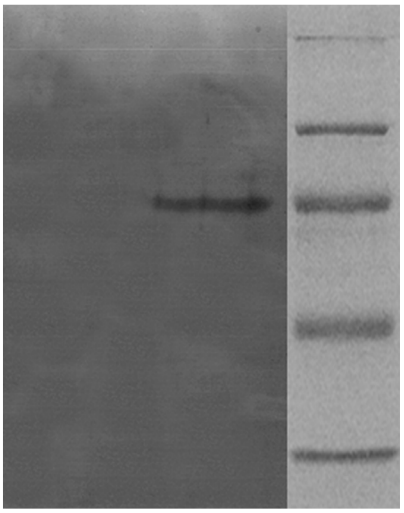

B
MCS
GST
pEG(KT)
MgLac2
pEG(KT)MgLac2
s288C
Induction with 4%Galactose
Protein Extraction
Protein Purification

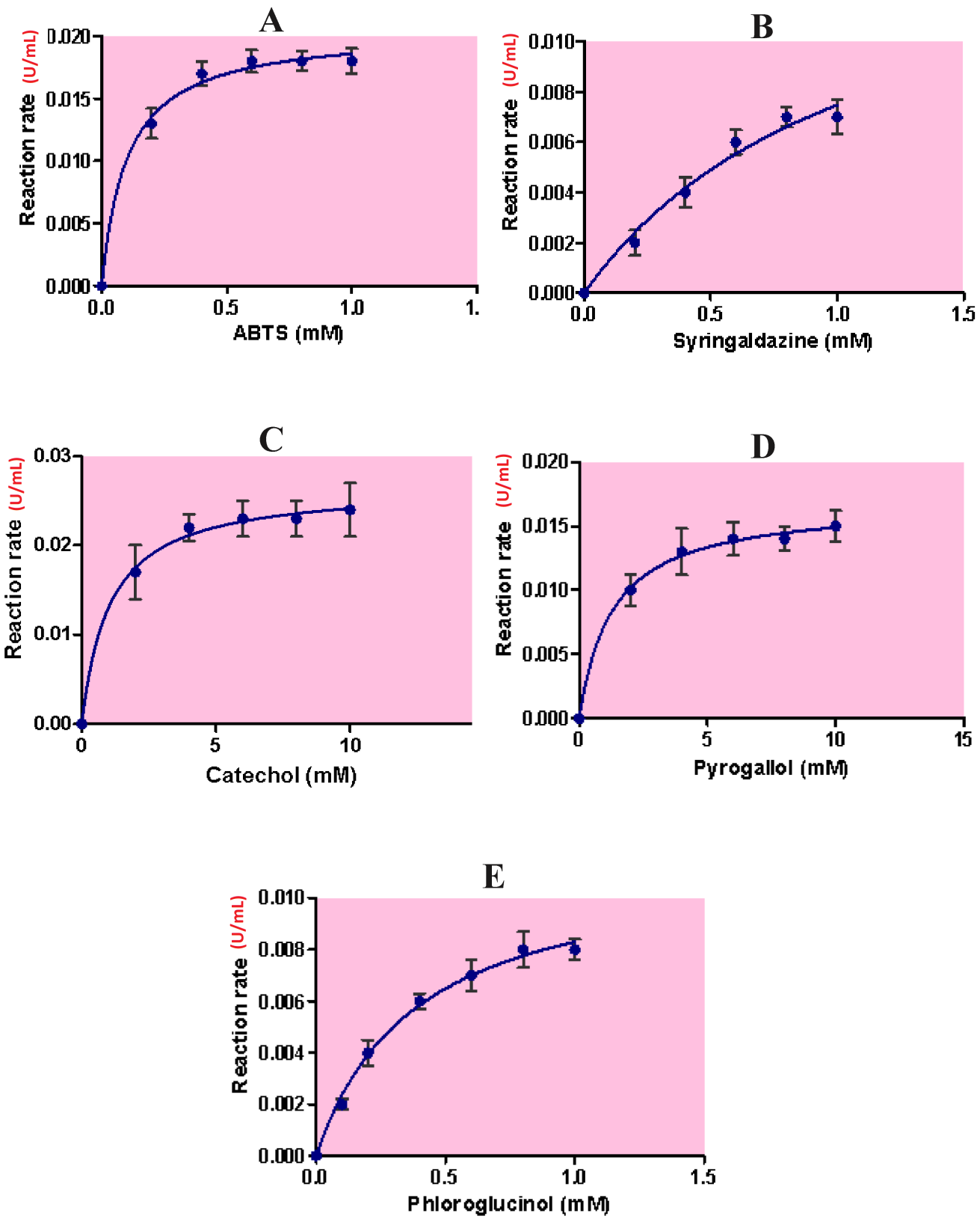

1
2
3
4
5
6
7
97.4 kDa
66 kDa
43 kDa
29 kDa
20.1 kDa

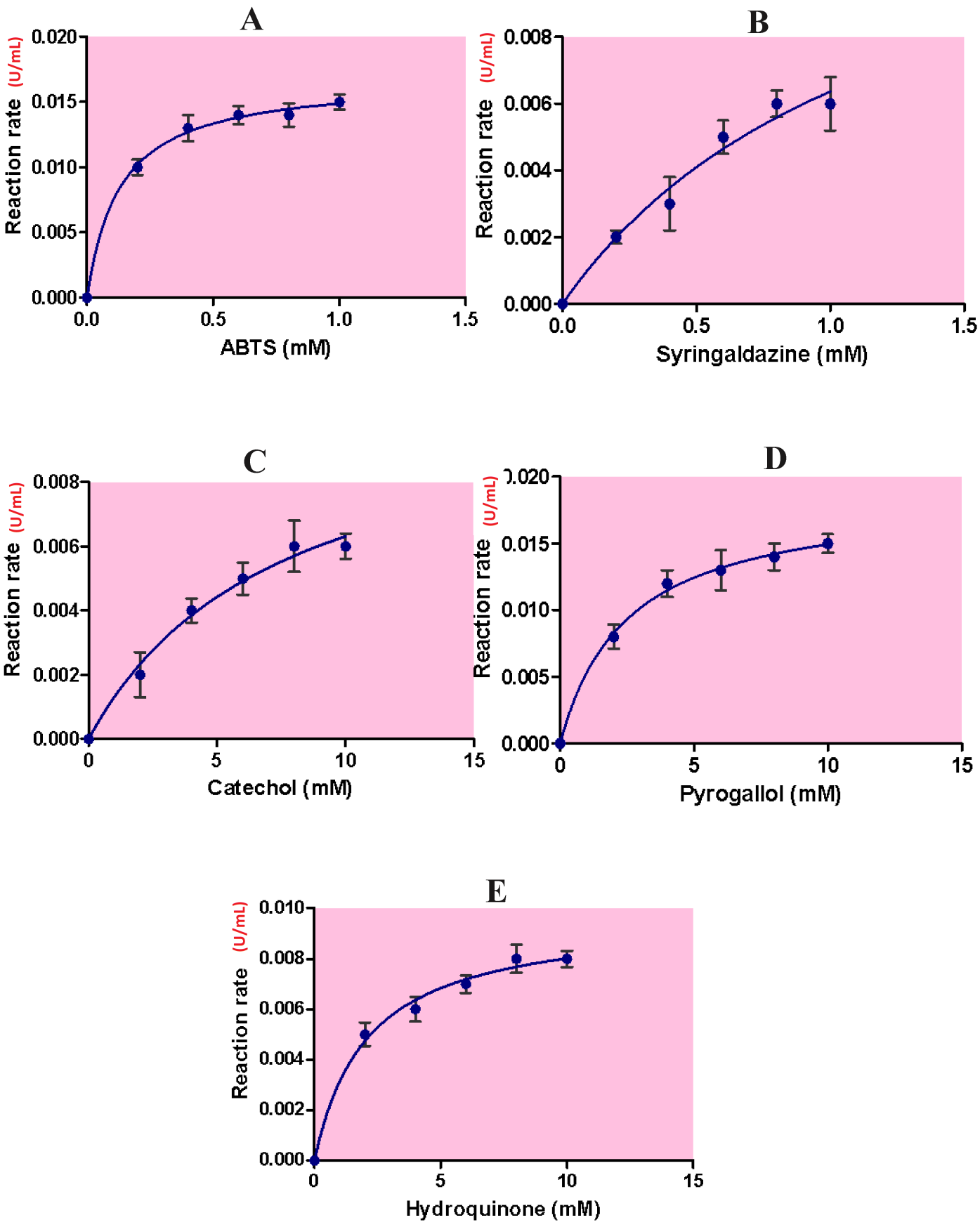
A.

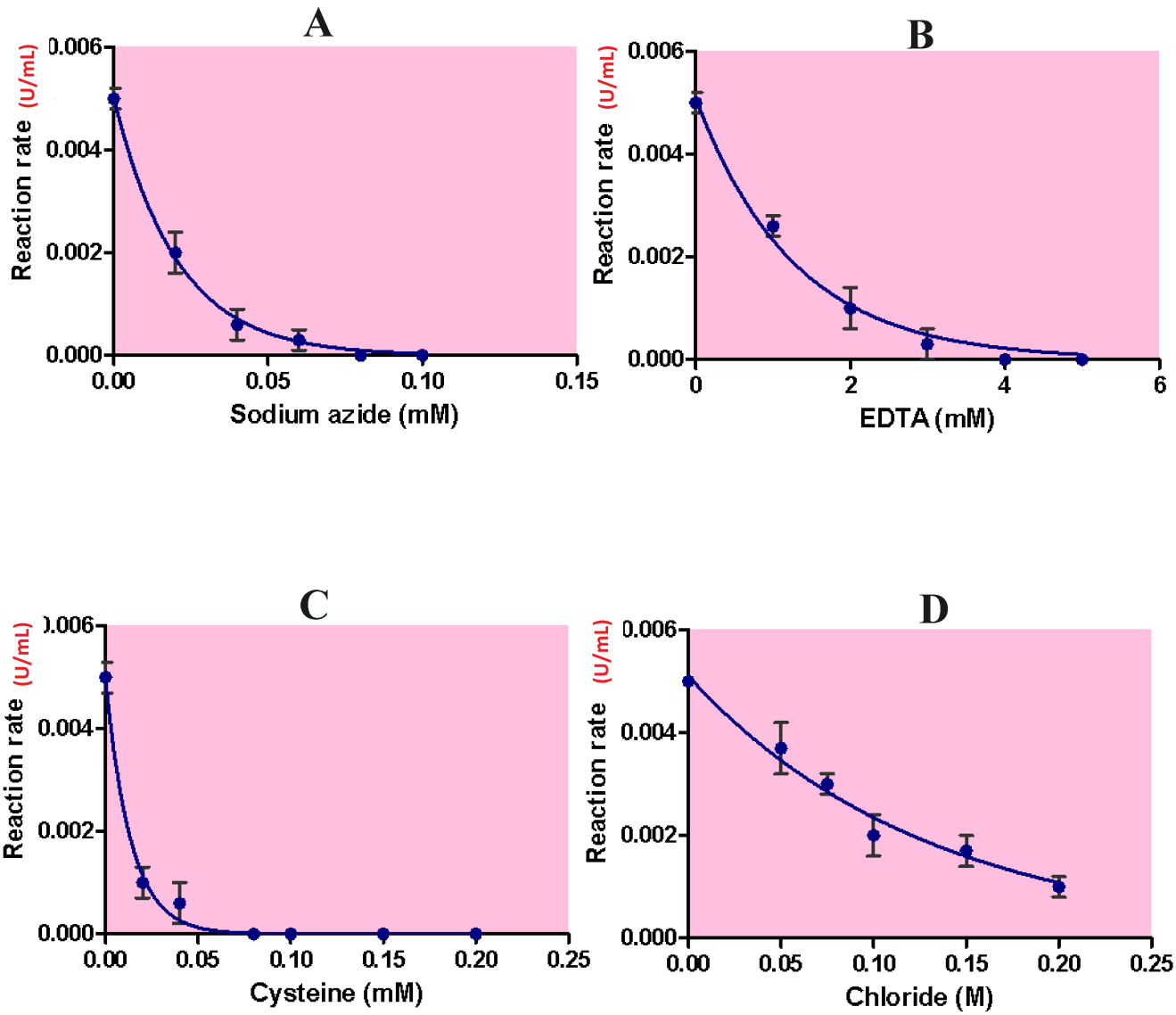


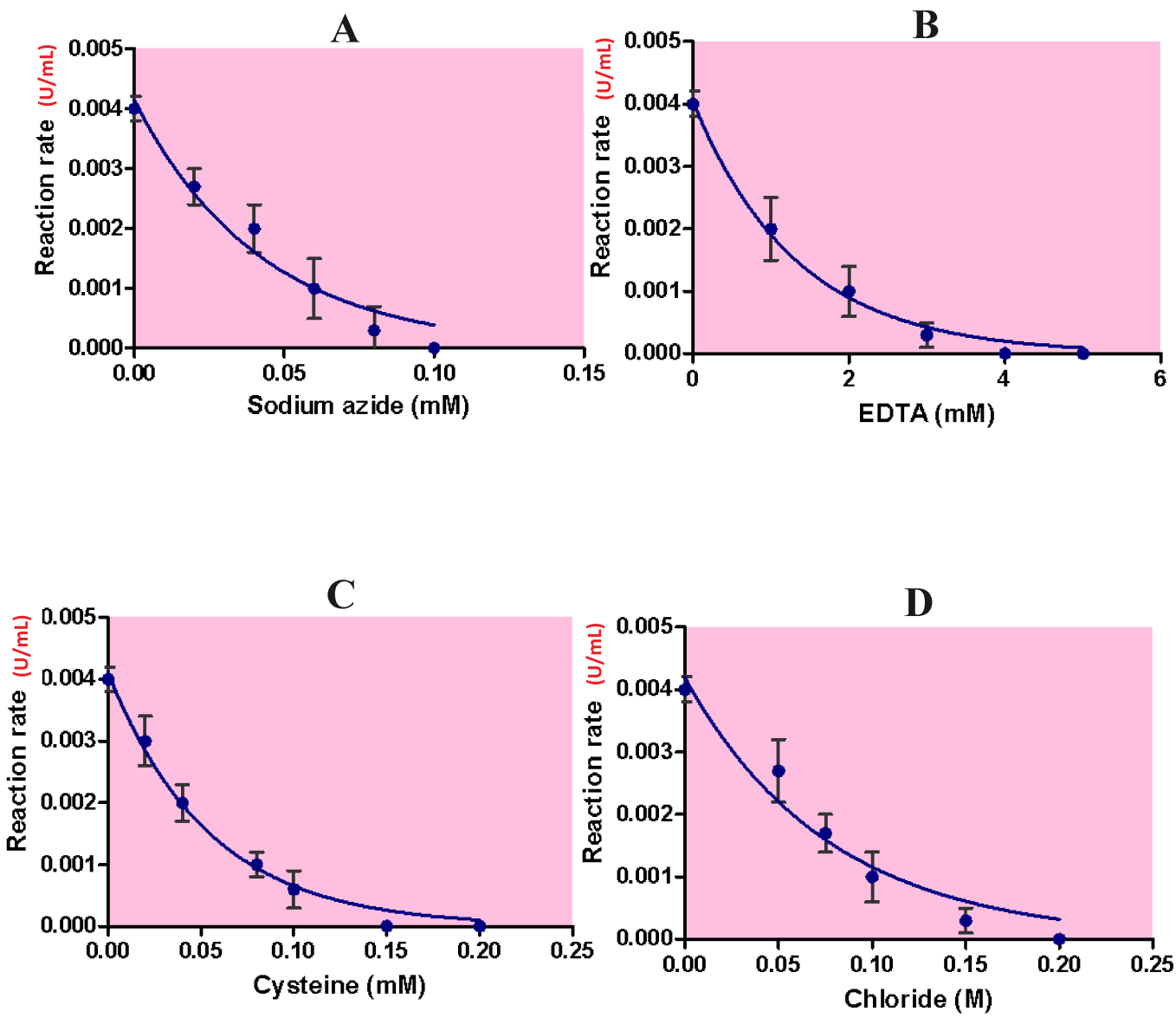
B.



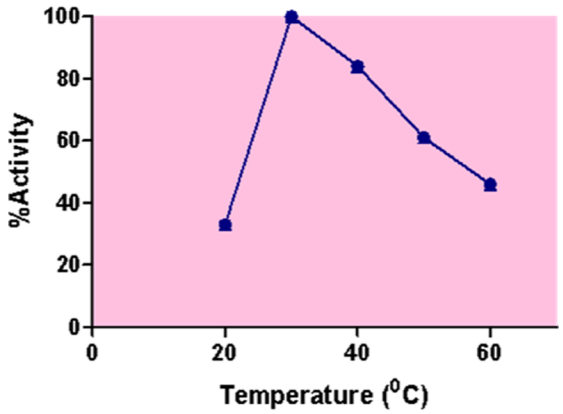

A
Reaction rate (U/mL)
0.020
0.015
0.010
0.005
0.000
0.0
0.5
1.0
1.
ABTS (mM)
B
Reaction rate (U/mL)
0.010
0.008
0.006
0.004
0.002
0.000
0.0
0.5
1.0
1.5
Syringaldazine (mM)
C
Reaction rate (U/mL)
0.03
0.02
0.01
0.00
0
5
10
Catechol (mM)
D
Reaction rate (U/mL)
0.020
0.015
0.010
0.005
0.000
0
5
10
15
Pyrogallol (mM)
E
Reaction rate (U/mL)
0.010
0.008
0.006
0.004
0.002
0.000
0.0
0.5
1.0
1.5
Phloroglucinol (mM)


A
Reaction rate (U/mL)
0.020
0.015
0.010
0.005
0.000
0.0
0.5
1.0
1.5
ABTS (mM)
B
Reaction rate (U/mL)
0.008
0.006
0.004
0.002
0.000
0.0
0.5
1.0
1.5
Syringaldazine (mM)
C
Reaction rate (U/mL)
0.008
0.006
0.004
0.002
0.000
0
5
10
15
Catechol (mM)
D
Reaction rate (U/mL)
0.020
0.015
0.010
0.005
0.000
0
5
10
15
Pyrogallol (mM)
E
Reaction rate (U/mL)
0.010
0.008
0.006
0.004
0.002
0.000
0
5
10
15
Hydroquinone (mM)

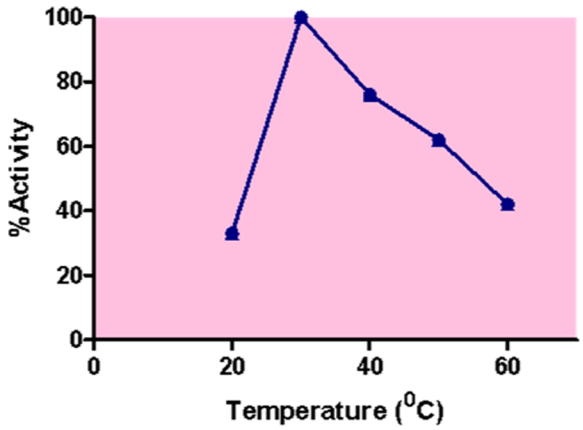

A
Reaction rate (U/mL)
0.006
0.004
0.002
0.000
0.00
0.05
0.10
0.15
Sodium azide (mM)
B
Reaction rate (U/mL)
0.006
0.004
0.002
0.000
0
2
4
6
EDTA (mM)
C
Reaction rate (U/mL)
0.006
0.004
0.002
0.000
0.00
0.05
0.10
0.15
0.20
0.25
Cysteine (mM)
D
Reaction rate (U/mL)
0.006
0.004
0.002
0.000
0.00
0.05
0.10
0.15
0.20
0.25
Chloride (M)

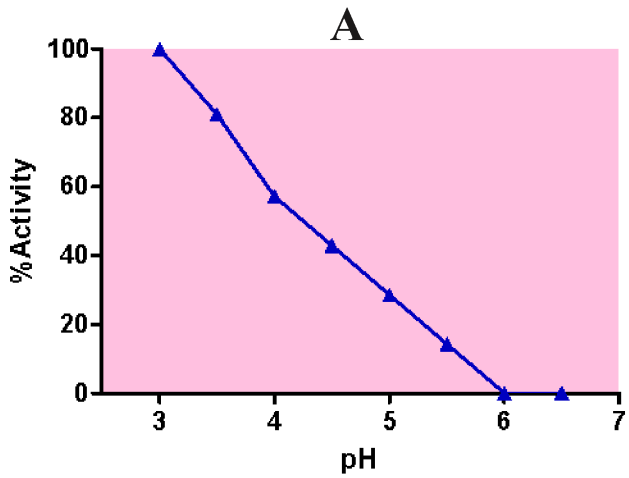

A
Reaction rate (U/mL)
0.005
0.004
0.003
0.002
0.001
0.000
0.00
0.05
0.10
0.15
Sodium azide (mM)
B
Reaction rate (U/mL)
0.005
0.004
0.003
0.002
0.001
0.000
0
2
4
6
EDTA (mM)
C
Reaction rate (U/mL)
0.005
0.004
0.003
0.002
0.001
0.000
0.00
0.05
0.10
0.15
0.20
0.25
Cysteine (mM)
D
Reaction rate (U/mL)
0.005
0.004
0.003
0.002
0.001
0.000
0.00
0.05
0.10
0.15
0.20
0.25
Chloride (M)

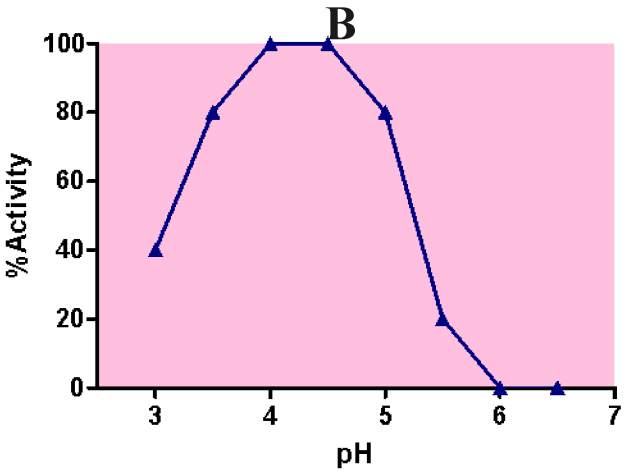
A.

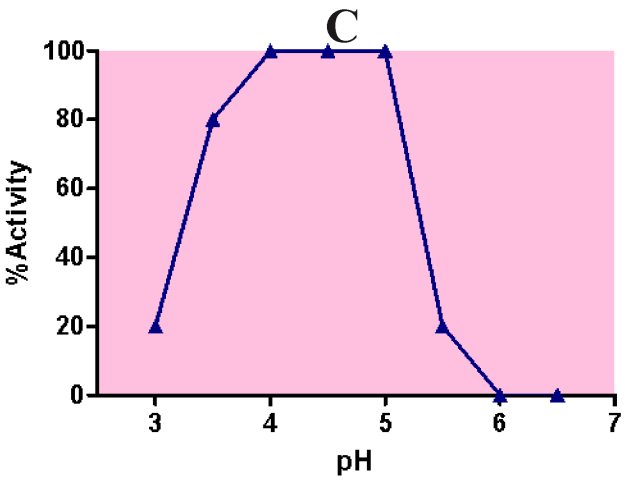

100
80
60
40
20
0
%Activity
0
20
40
60
Temperature (0C)

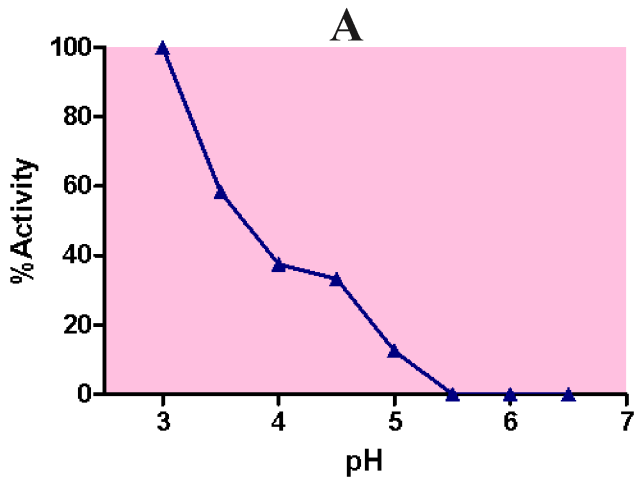
B.

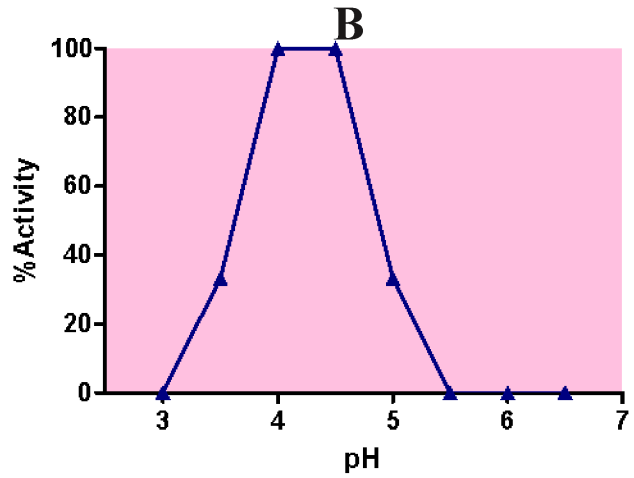

100
80
60
40
20
0
%Activity
0
20
40
60
Temperature (0C)

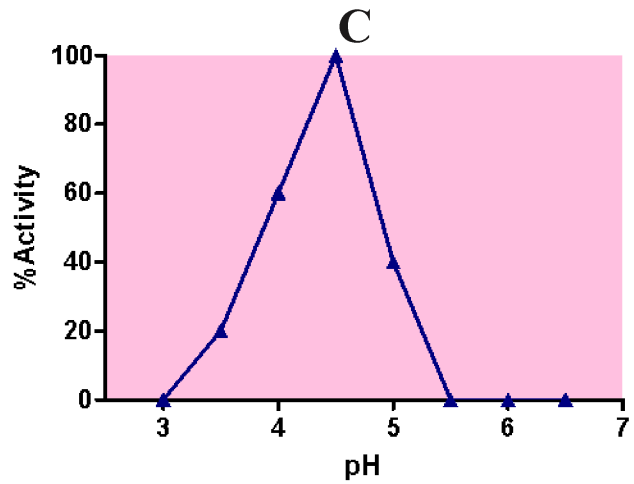

A
100
80
60
40
20
0
%Activity
3
4
5
6
7
pH

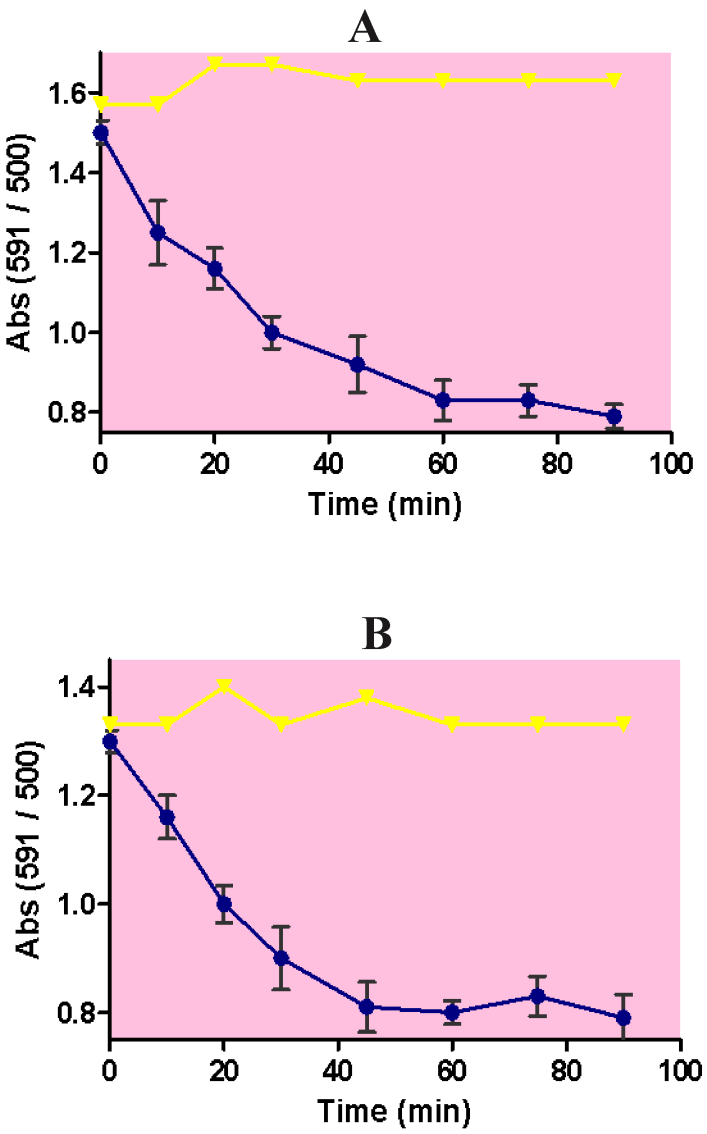

B
100
80
60
40
20
0
%Activity
3
4
5
6
7
pH


C
100
80
60
40
20
0
%Activity
3
4
5
6
7
pH

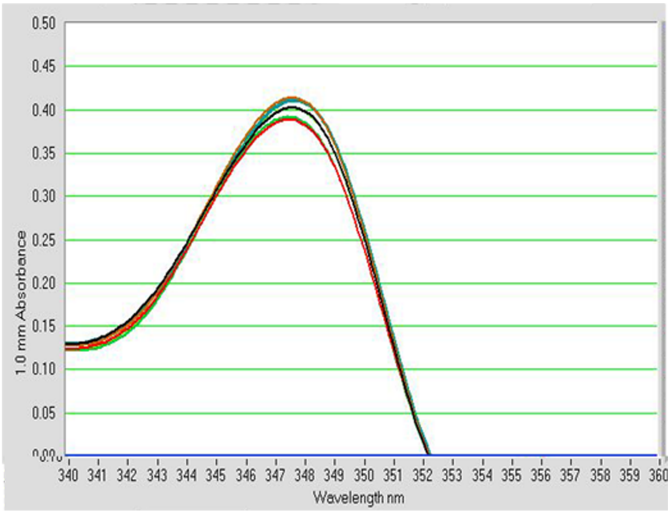

A
100
80
60
40
20
0
% Activity
3
4
5
6
7
pH


B
100
80
60
40
20
0
% Activity
3
4
5
6
7
pH

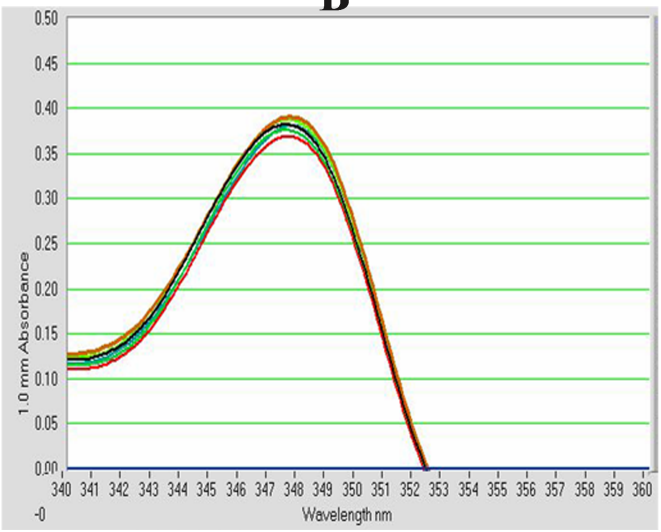

C
100
80
60
40
20
0
% Activity
3
4
5
6
7
pH


A
1.6
1.4
1.2
1.0
0.8
Abs (591 / 500)
0
20
40
60
80
100
Time (min)
B
1.4
1.2
1.0
0.8
Abs (591 / 500)
0
20
40
60
80
100
Time (min)

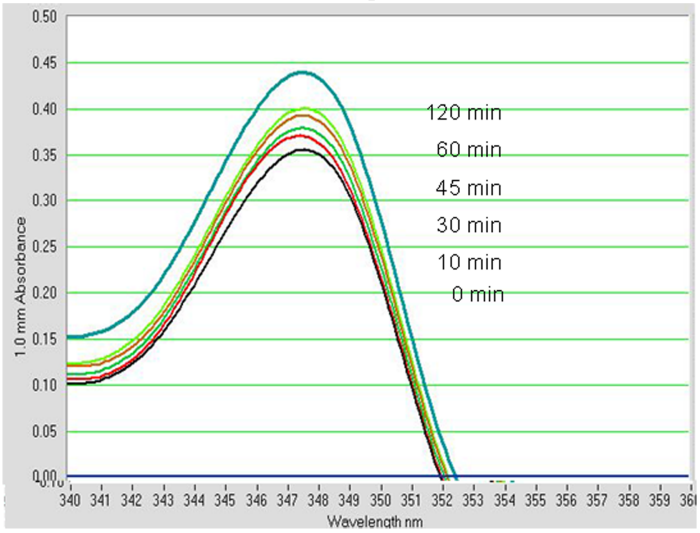
A


1.0 mm Absorbance
Wavelength nm

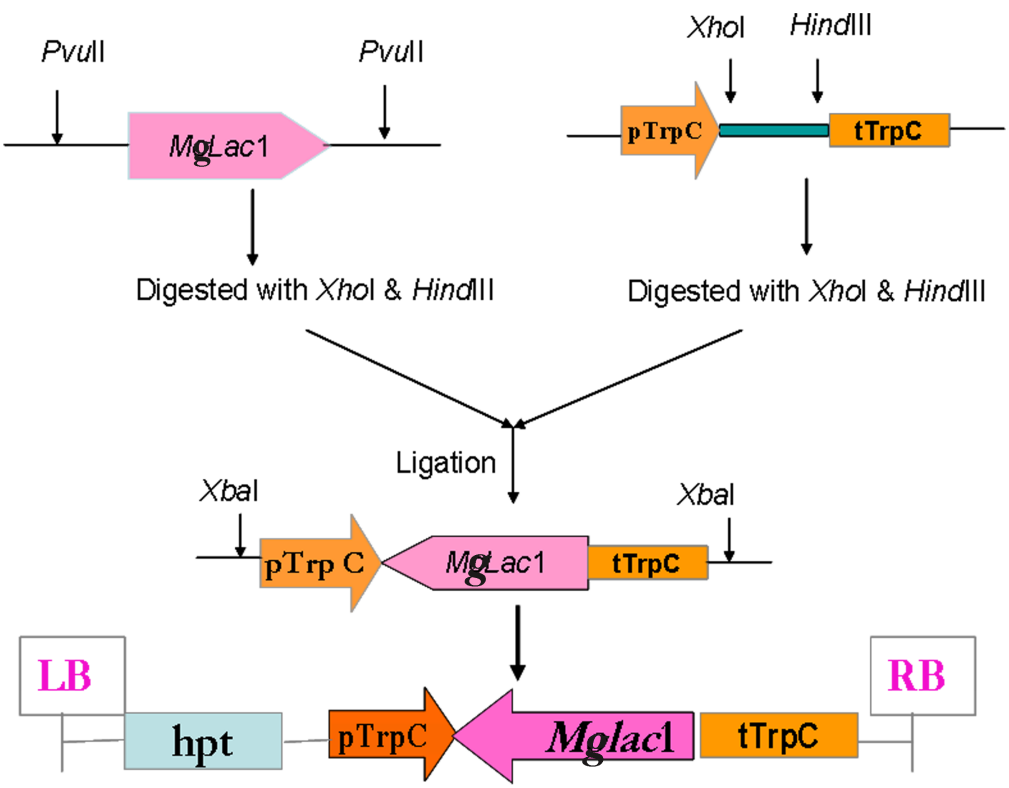
B


1.0 mm Absorbance
Wavelength nm

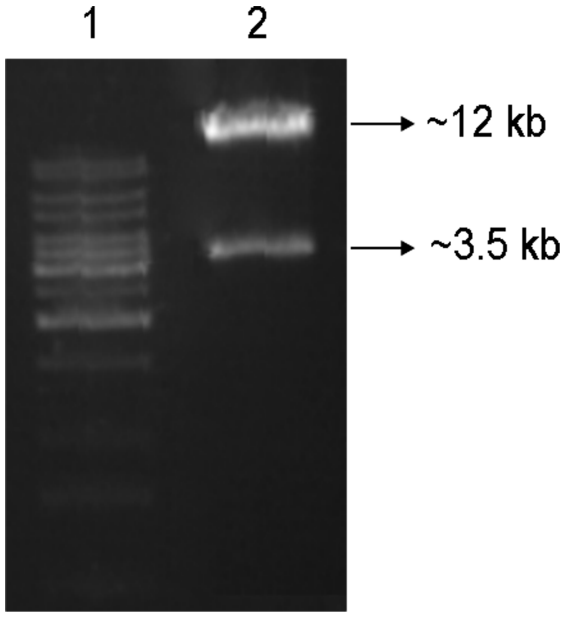
C


120 min
60 min
45 min
30 min
10 min
0 min
1.0 mm Absorbance
Wavelength nm

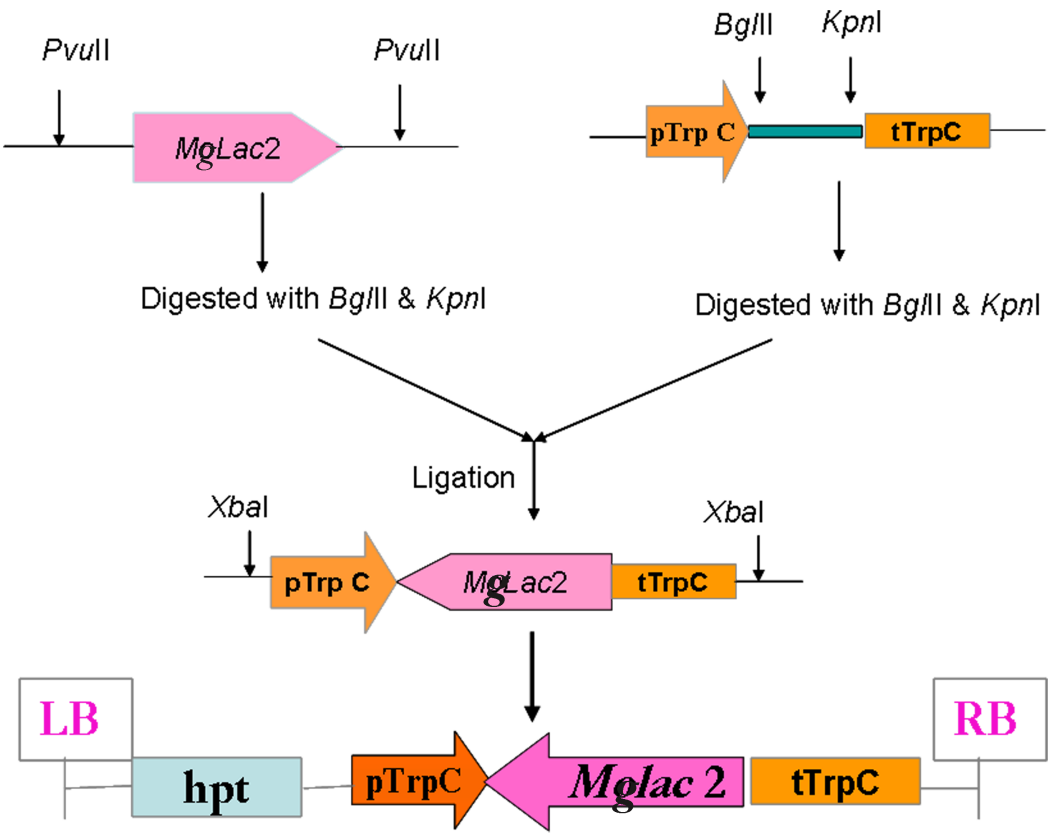
A


PvuII
PvuII
MgLac1
XhoI
HindIII
pTrpC
tTrpC
Digested with XhoI & HindIII
Digested with XhoI & HindIII
Ligation
XbaI
XbaI
pTrp C
MgLac1
tTrpC
LB
RB
hpt
pTrpC
Mglac1
tTrpC

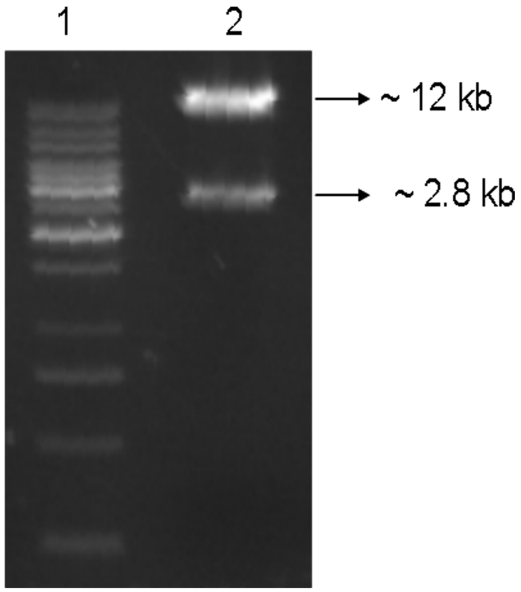
B

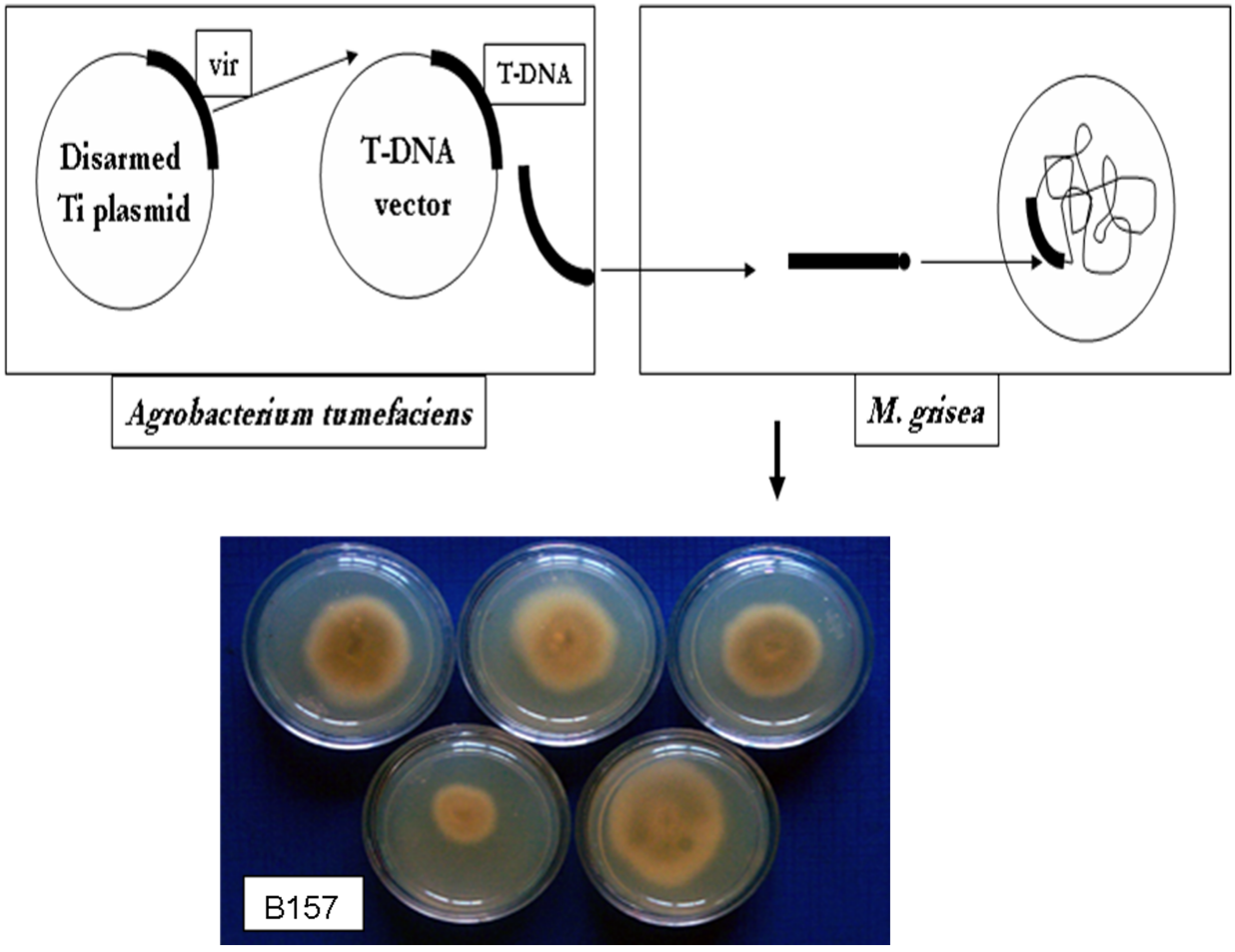

1
2
~12 kb
~3.5 kb

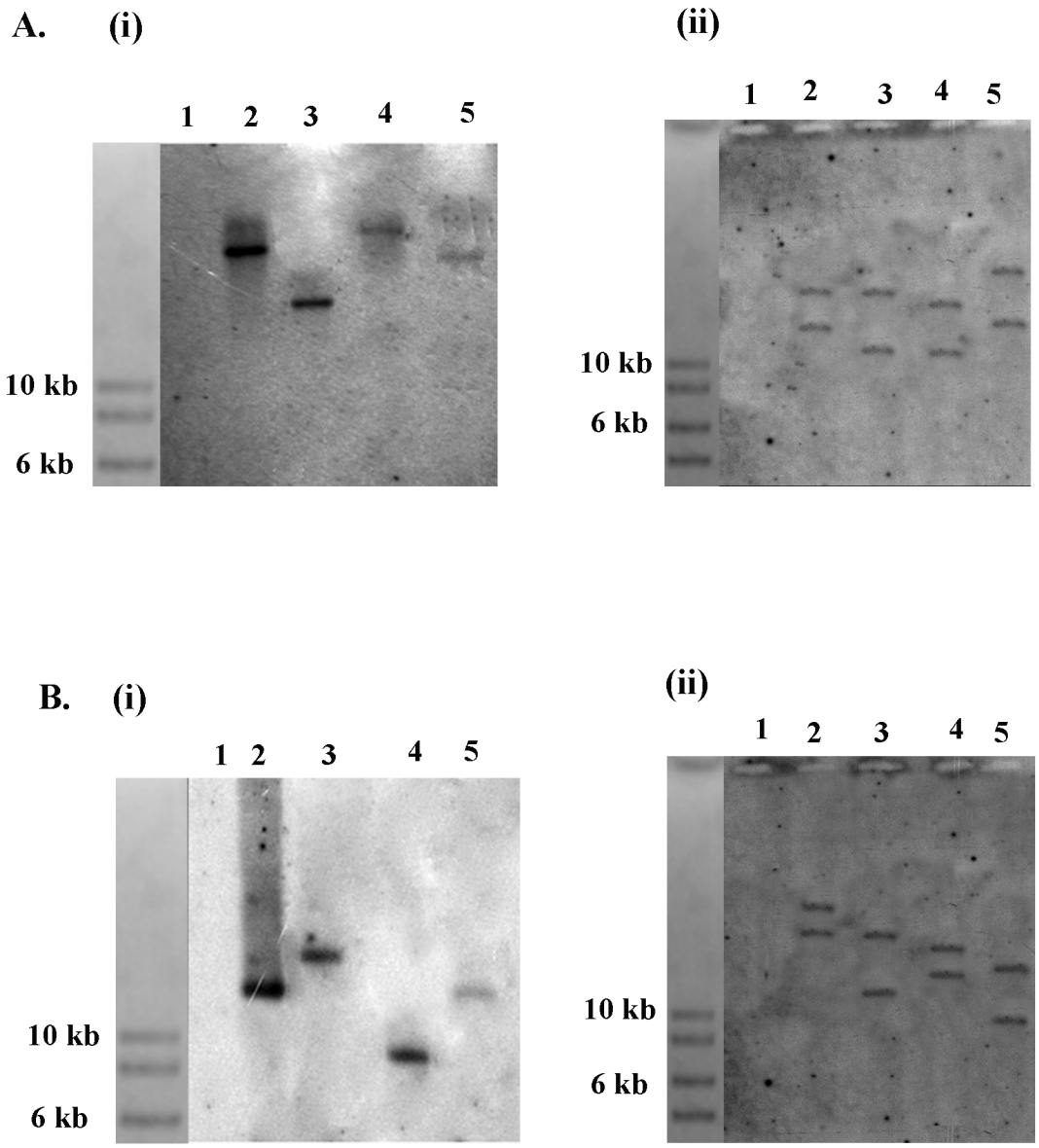
A


PvuII
PvuII
MgLac2
BglII
KpnI
pTrp C
tTrpC
Digested with BglII & KpnI
Digested with BglII & KpnI
Ligation
XbaI
XbaI
pTrp C
MgLac2
tTrpC
LB
hpt
pTrpC
Mglac 2
tTrpC
RB

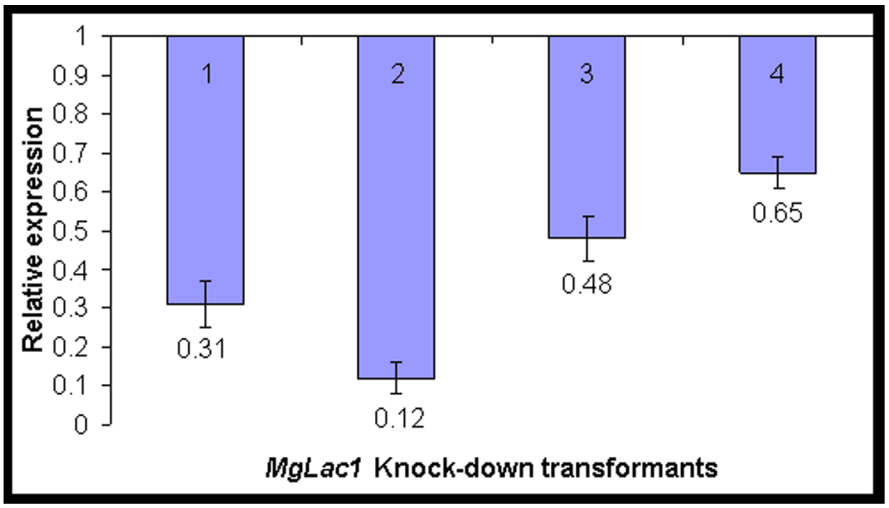
B


1
2
~ 12 kb
~ 2.8 kb

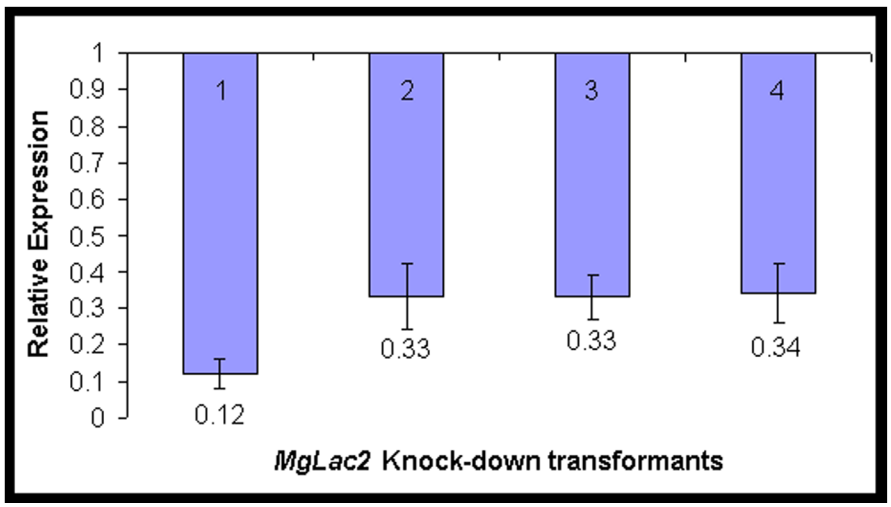

vir
T-DNA
Disarmed
Ti plasmid
T-DNA
vector
Agrobacterium tumefaciens
M. grisea
B157


A.
(i)
1 2 3 4 5
10 kb
6 kb
(ii)
1 2 3 4 5
10 kb
6 kb
B.
(i)
1 2 3 4 5
10 kb
6 kb
(ii)
1 2 3 4 5
10 kb
6 kb

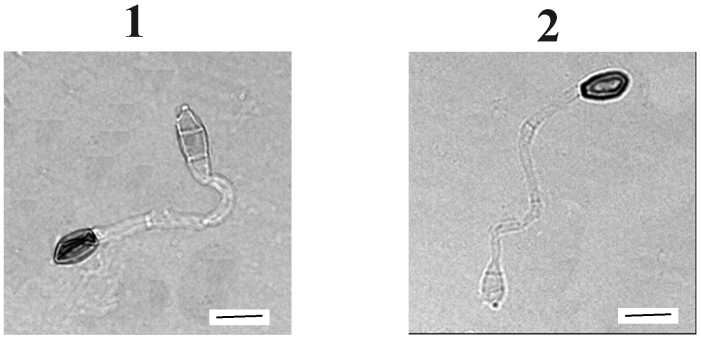
A.


Relative expression
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
0.31
0.12
0.48
0.65
MgLac1 Knock-down transformants

B.


Relative Expression
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
0.12
0.33
0.33
0.34
MgLac2 Knock-down transformants

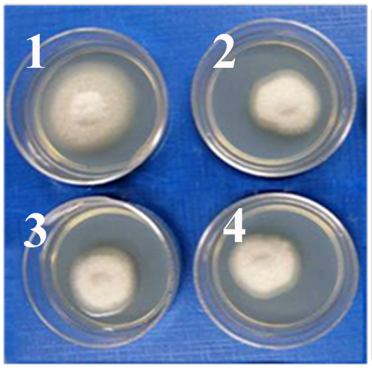
A



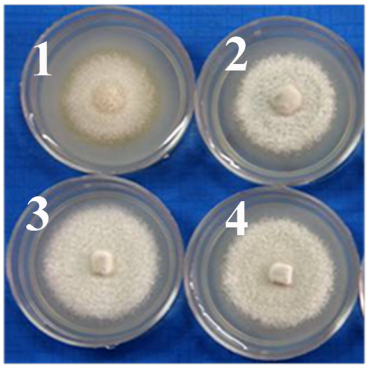
B



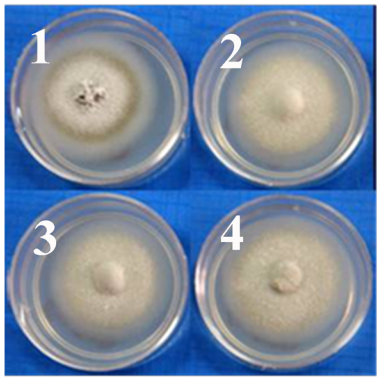
A.



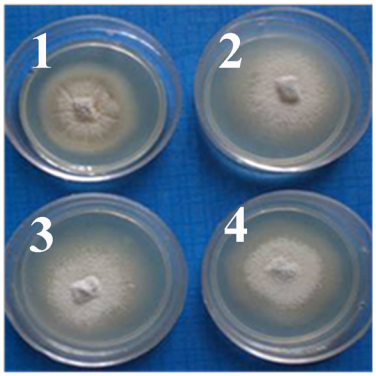
B.



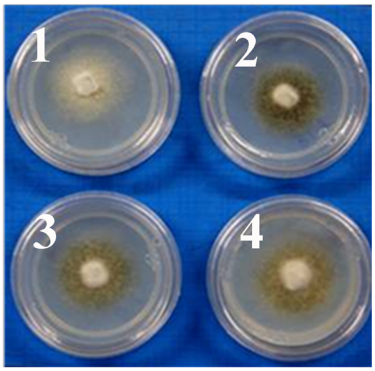
C.

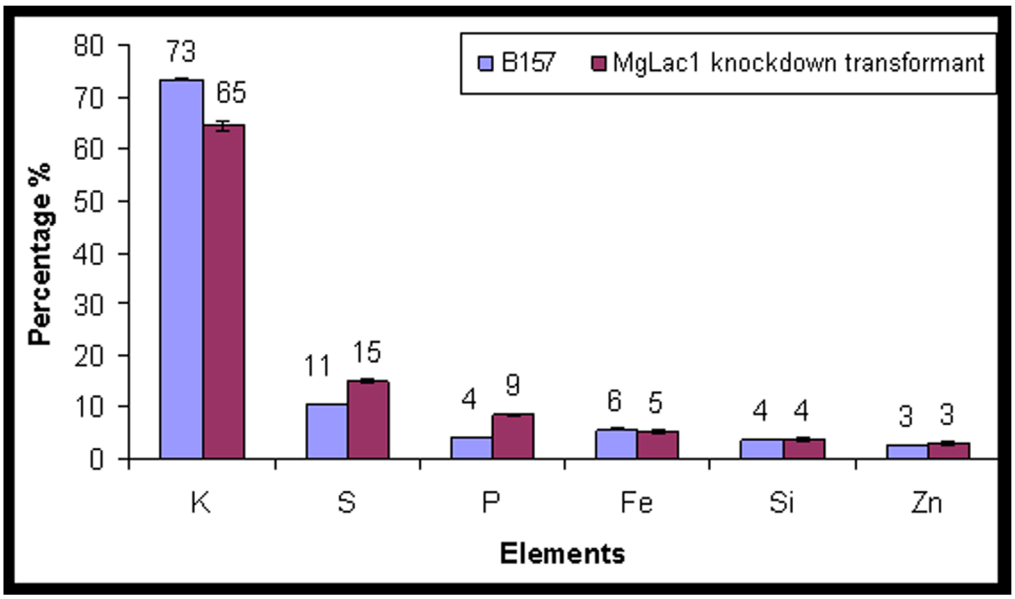


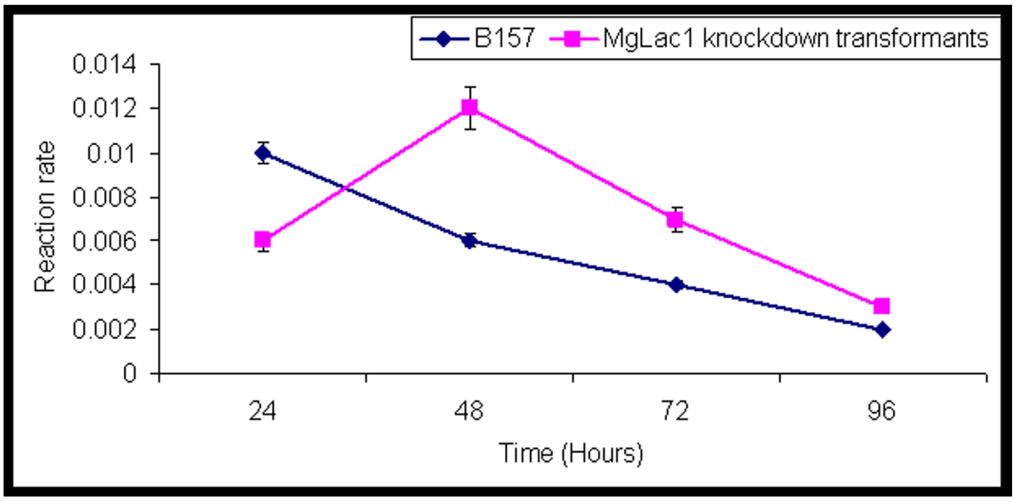
D.



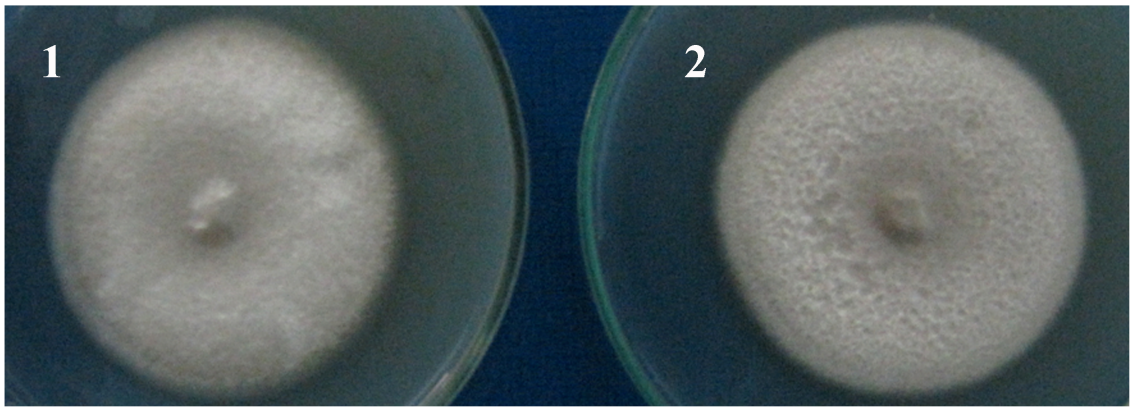
E.



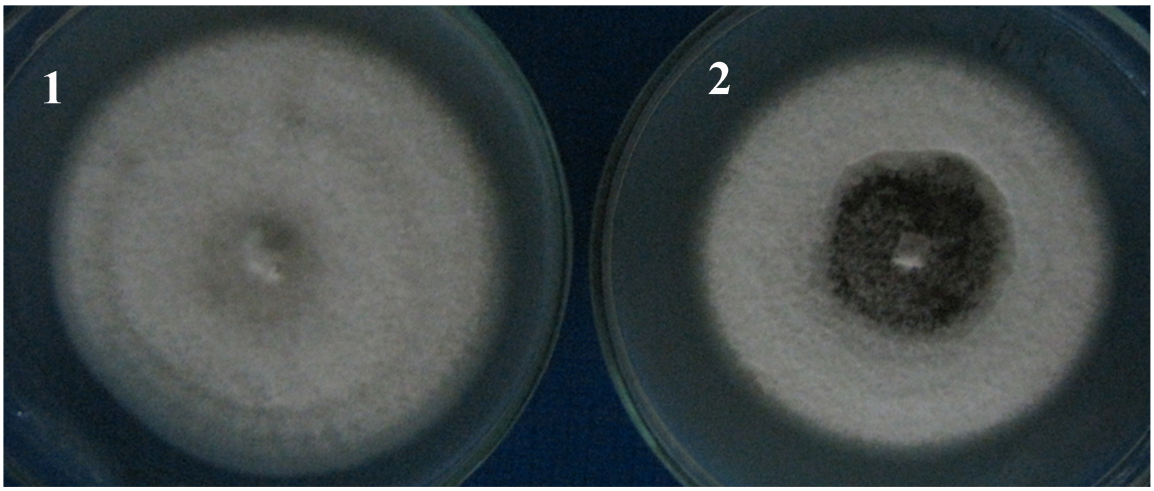

B157
MgLac1 knockdown transformant
Percentage %
80
70
60
50
40
30
20
10
0
73
65
11
15
4
9
6
5
4
4
3
3
K
S
P
Fe
Si
Zn
Elements


B157
MgLac1 knockdown transformants
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
Reaction rate
24
48
72
96
Time (Hours)

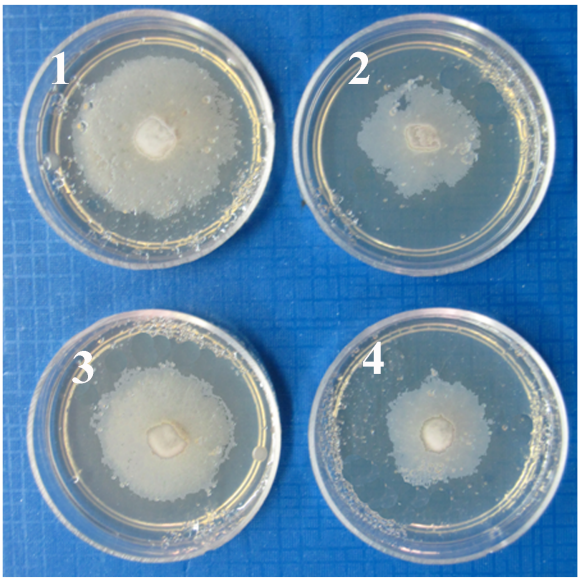
A



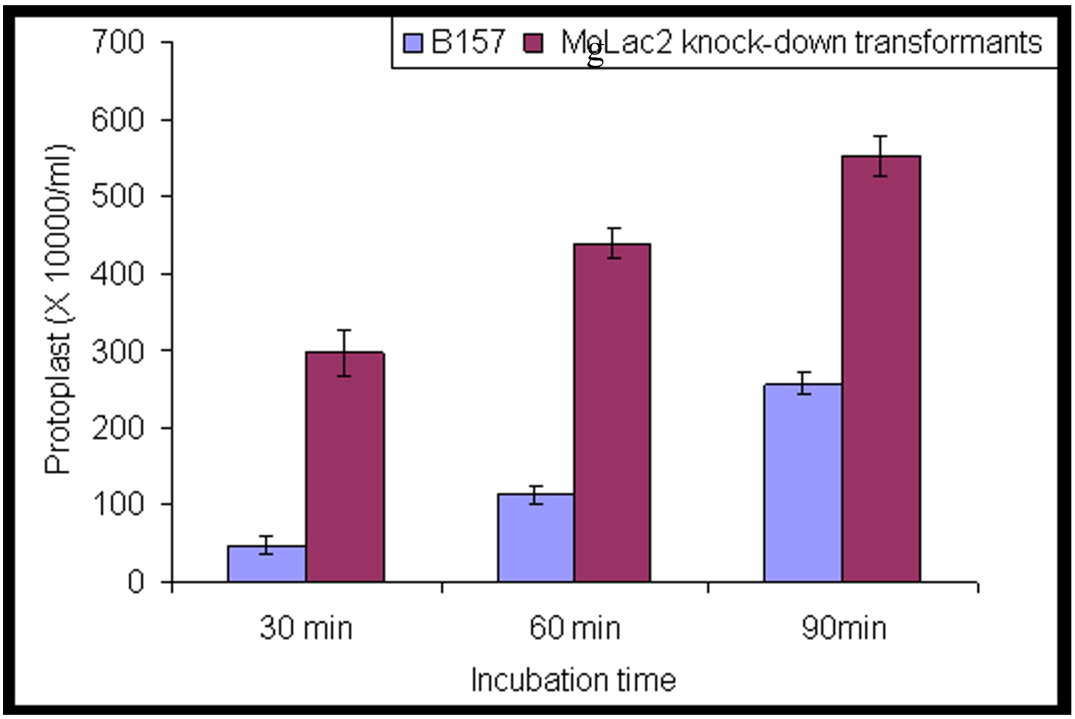
B

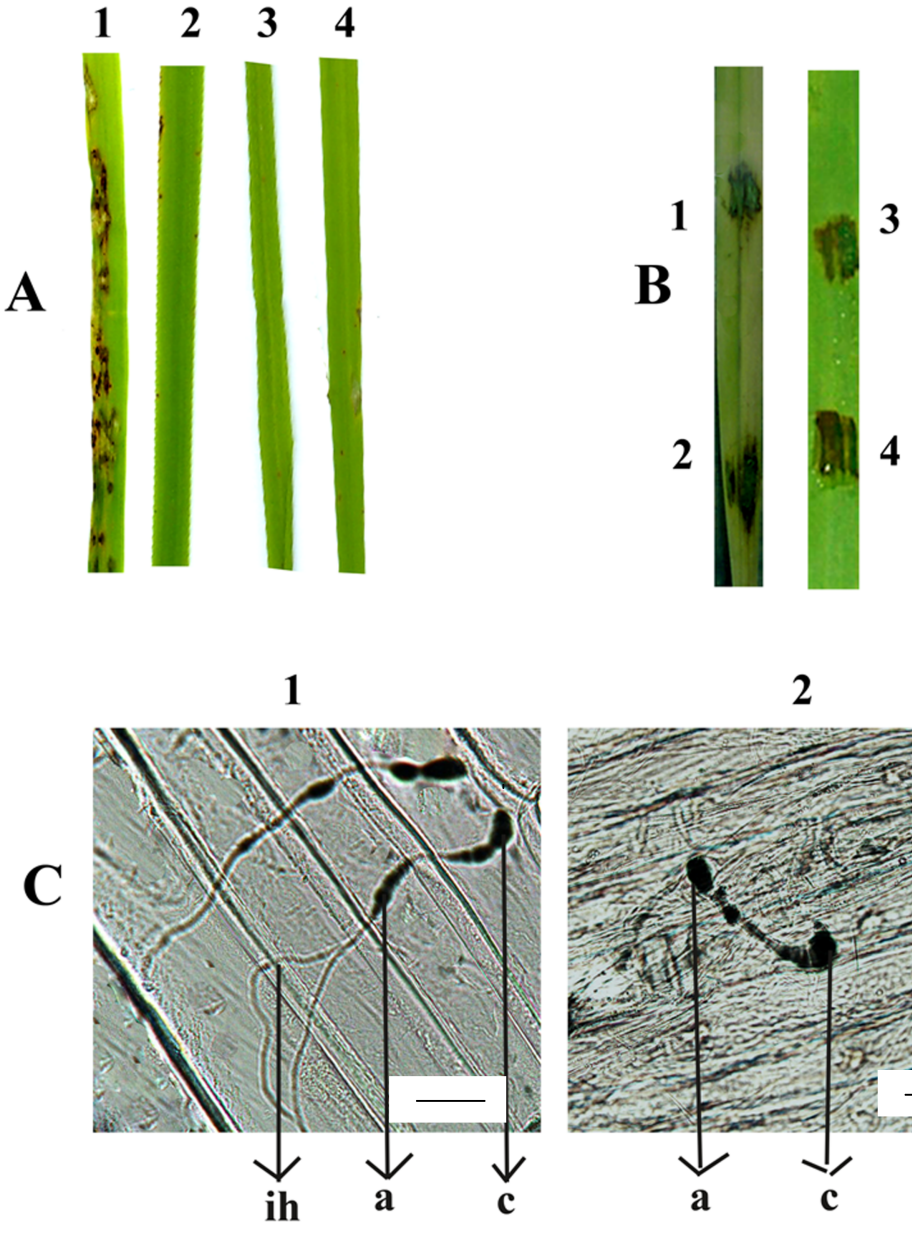


A

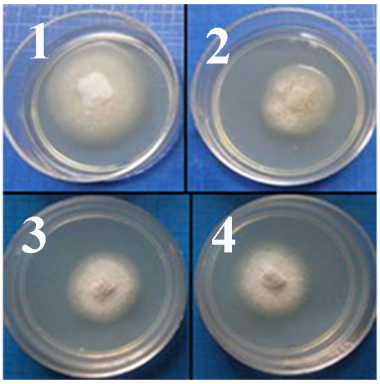

1
2
3
4

B

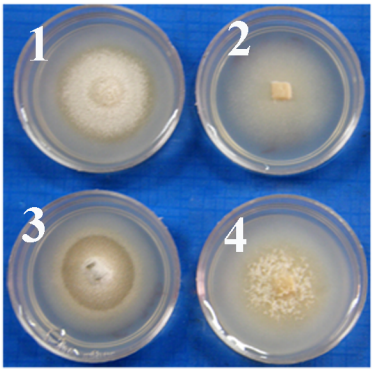

B157
MgLac2 knock-down transformants
Protoplast (X 10000/ml)
700
600
500
400
300
200
100
0
30 min
60 min
90min
Incubation time


A
1
2
3
4
B
1
2
3
4
C
1
2
ih
a
c
a
c

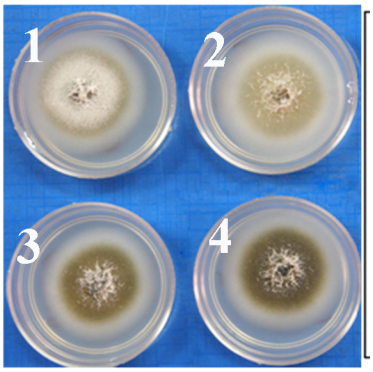
A.



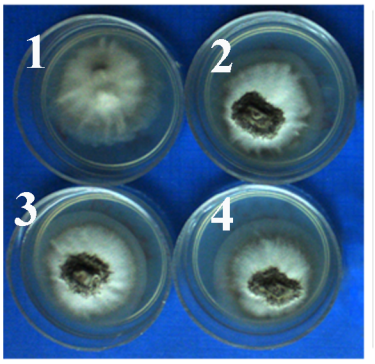
B.



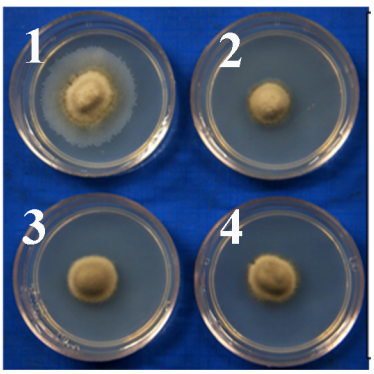
C.

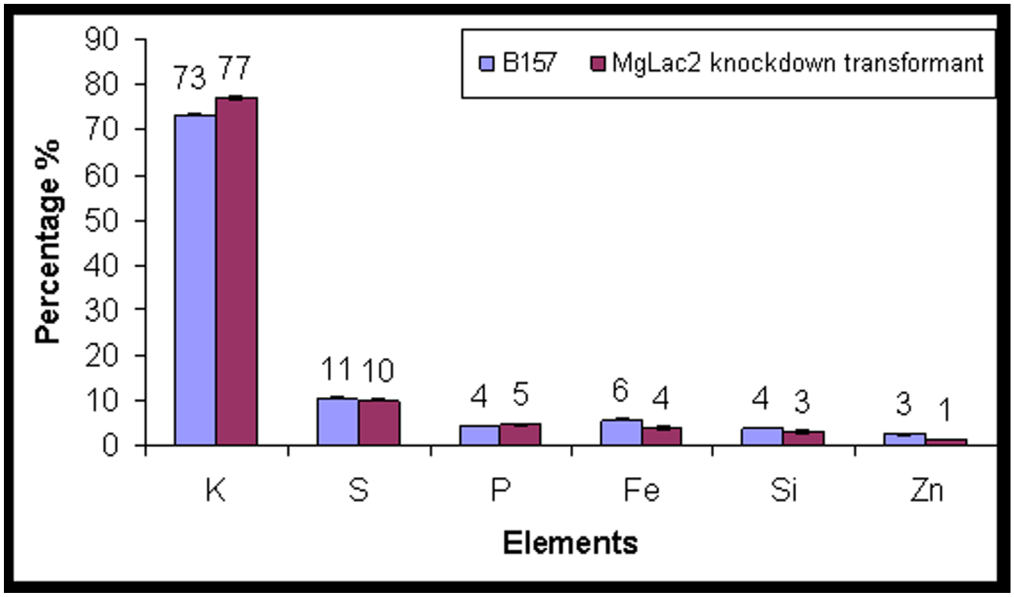


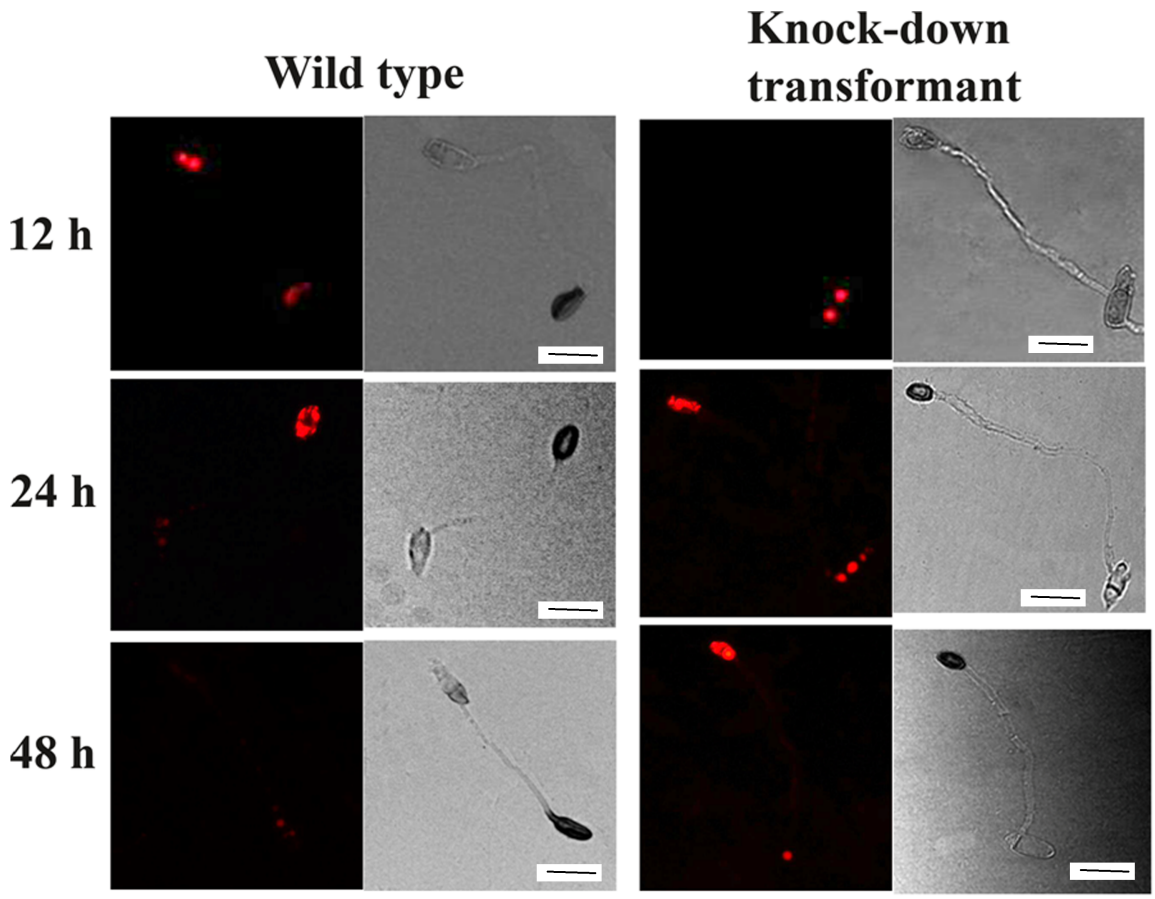
D.

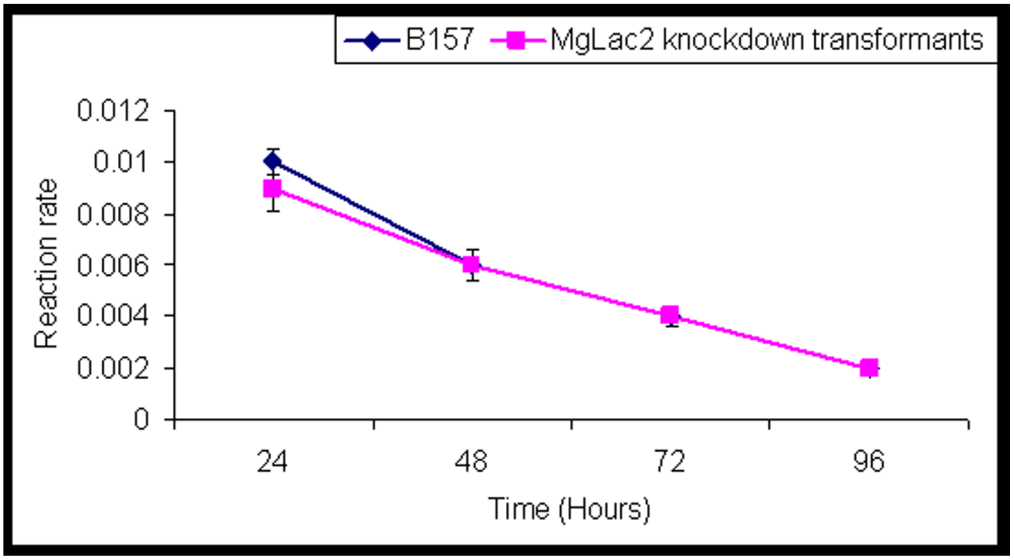


E.

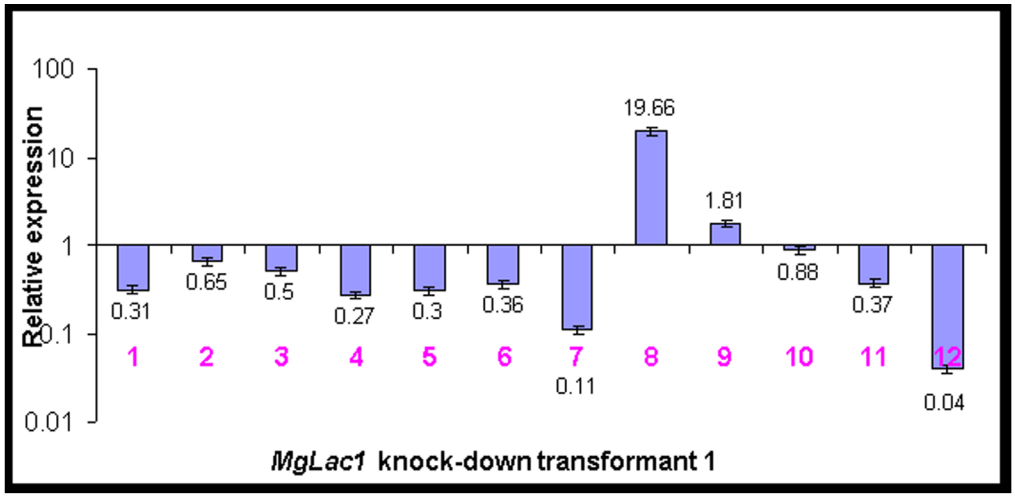



B157
MgLac2 knockdown transformant
Percentage %
90
80
70
60
50
40
30
20
10
0
73 77
11 10
4 5
6 4
4 3
3 1
K
S
P
Fe
Si
Zn
Elements

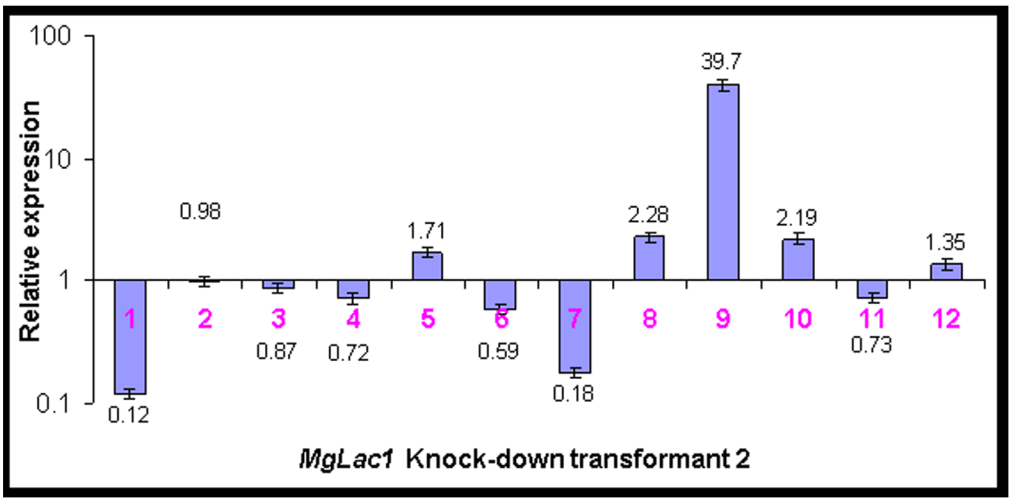

Wild type
Knock-down transformant
12 h
24 h
48 h


B157
MgLac2 knockdown transformants
0.012
0.01
0.008
0.006
0.004
0.002
0
Reaction rate
24
48
72
96
Time (Hours)

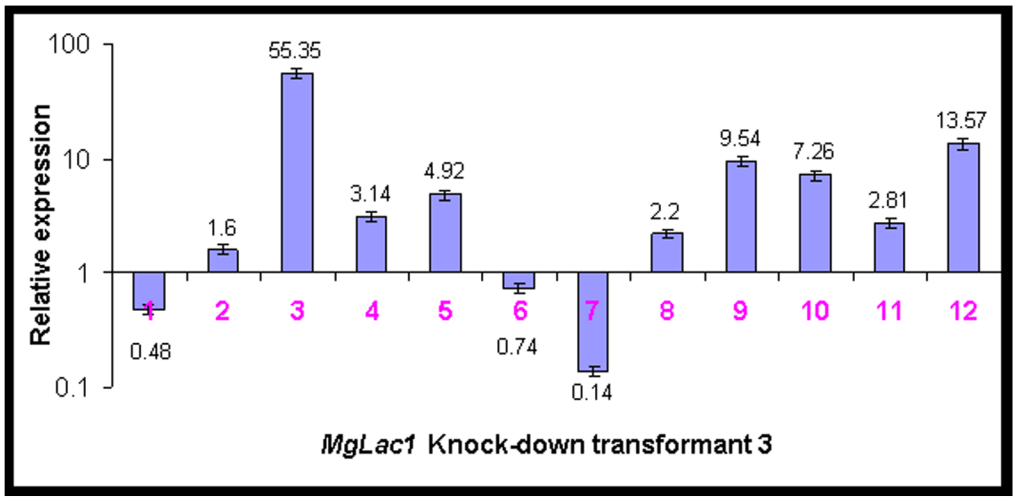
A.


Relative expression
100
10
1
0.1
0.01
0.31
0.65
0.5
0.27
0.3
0.36
0.11
19.66
1.81
0.88
0.37
0.04
1
2
3
4
5
6
7
8
9
10
11
12
MgLac1 knock-down transformant 1

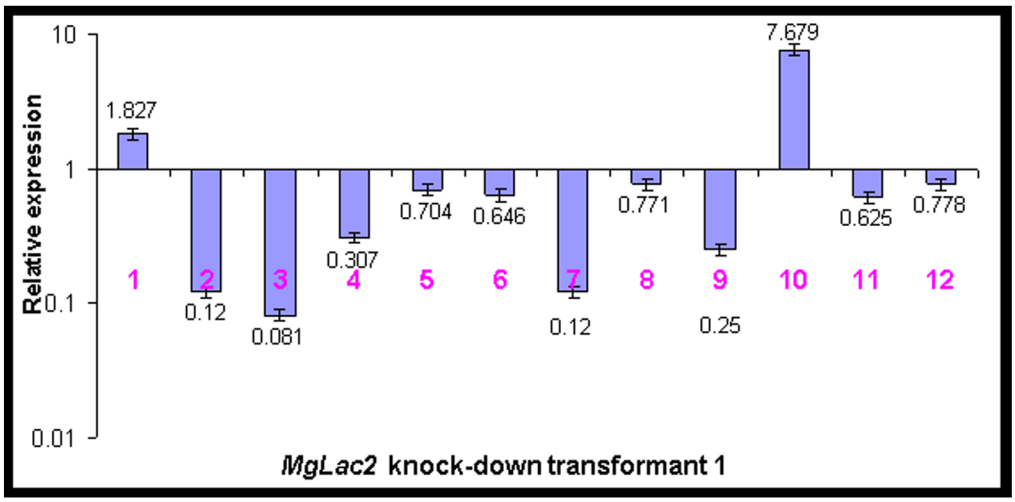
B.


Relative expression
100
10
1
0.1
0.12
0.98
0.87
0.72
1.71
0.59
0.18
2.28
39.7
2.19
0.73
1.35
1
2
3
4
5
6
7
8
9
10
11
12
MgLac1 Knock-down transformant 2

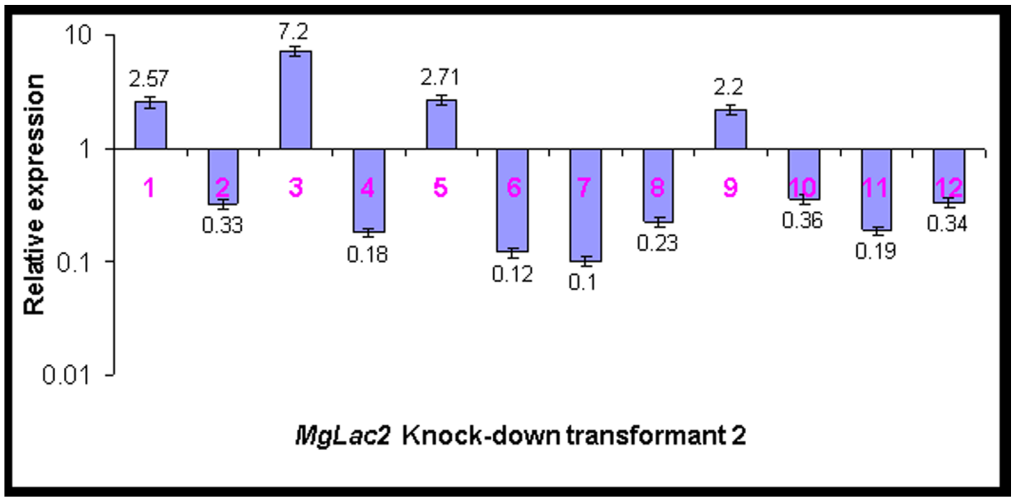
C.


Relative expression
100
10
1
0.1
0.48
1.6
55.35
3.14
4.92
0.74
0.14
2.2
9.54
7.26
2.81
13.57
1
2
3
4
5
6
7
8
9
10
11
12
MgLac1 Knock-down transformant 3

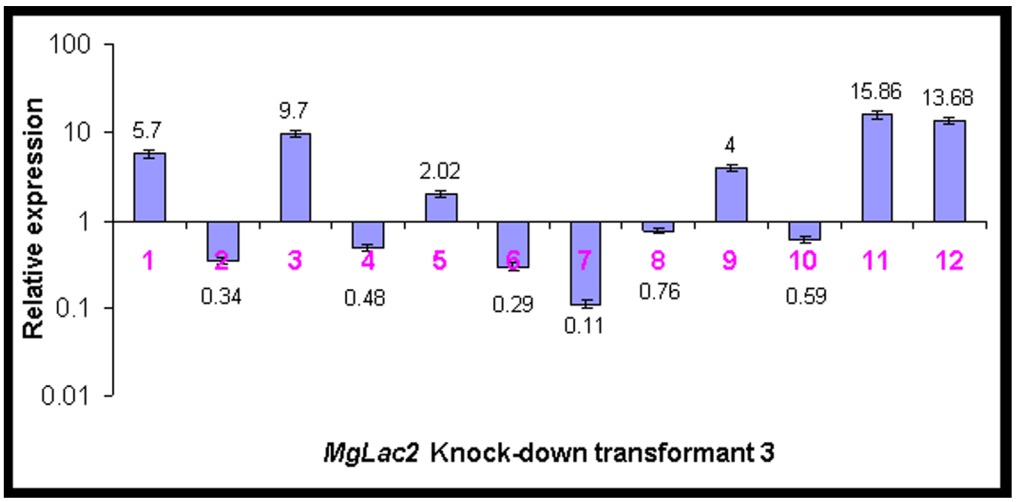
A.


Relative expression
10
1
0.1
0.01
1.827
0.12
0.081
0.307
0.704
0.646
0.12
0.771
0.25
7.679
0.625
0.778
1
2
3
4
5
6
7
8
9
10
11
12
MgLac2 knock-down transformant 1

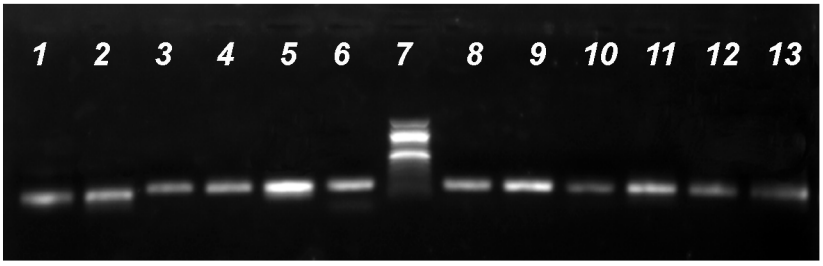
B.


Relative expression
10
1
0.1
0.01
2.57
0.33
7.2
0.18
2.71
0.12
0.1
0.23
2.2
0.36
0.19
0.34
1
2
3
4
5
6
7
8
9
10
11
12
MgLac2 Knock-down transformant 2

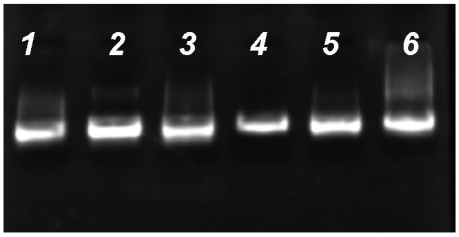
C.


Relative expression
100
10
1
0.1
0.01
5.7
0.34
9.7
0.48
2.02
0.29
0.11
0.76
4
0.59
15.86
13.68
1
2
3
4
5
6
7
8
9
10
11
12
MgLac2 Knock-down transformant 3

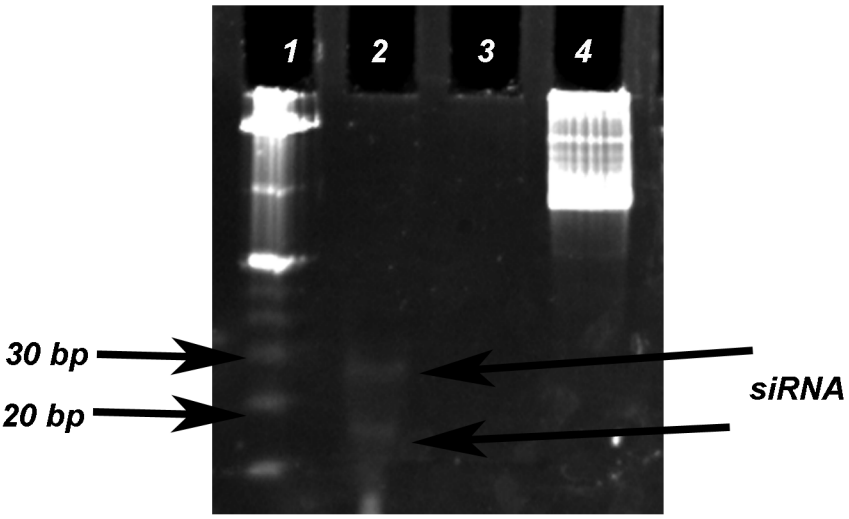
A

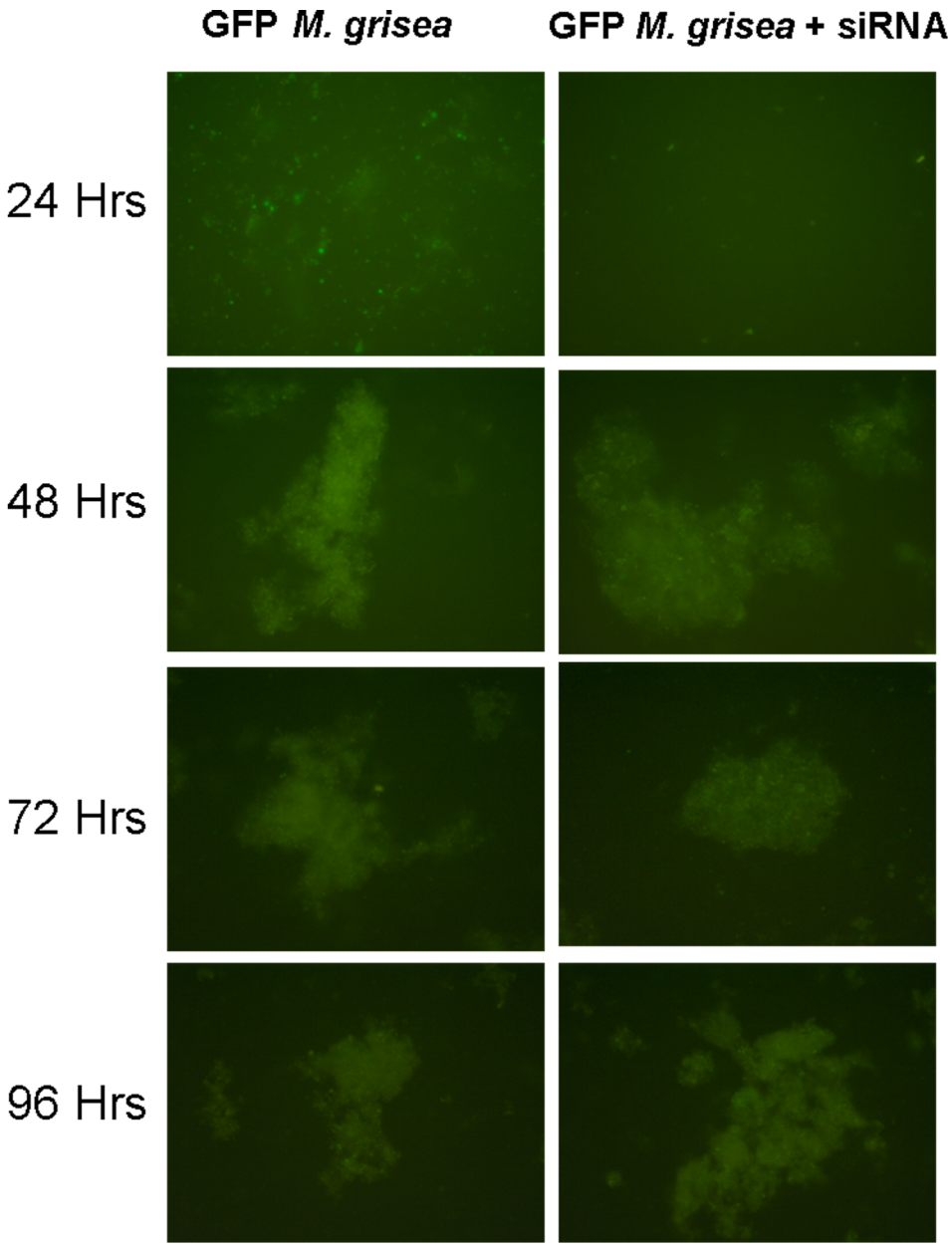


B

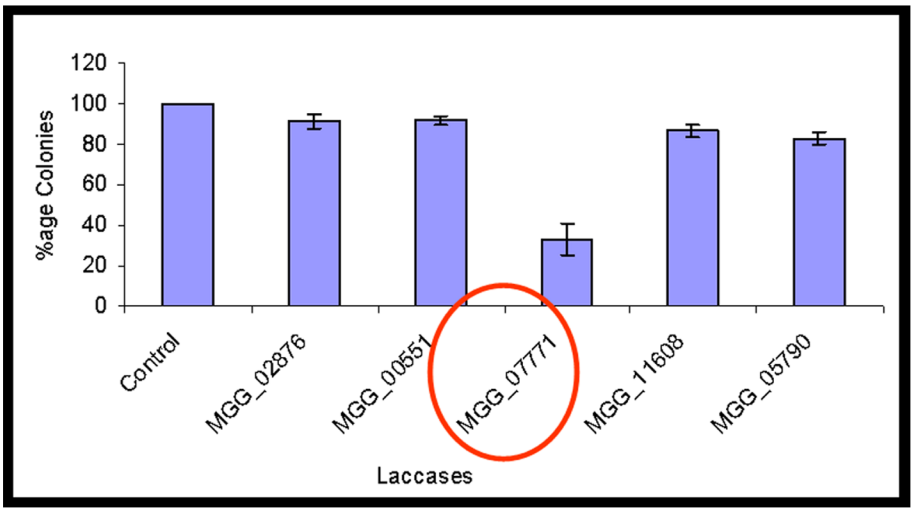


C

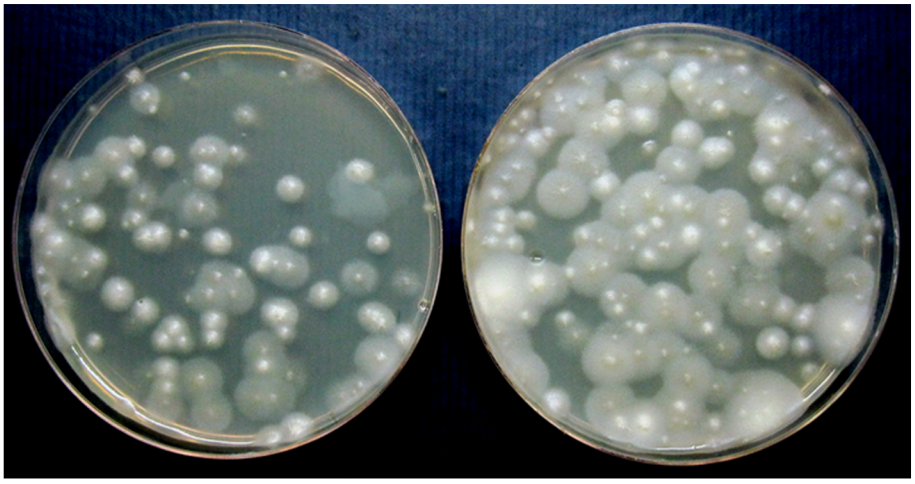



GFP M. grisea
GFP M. grisea + siRNA
24 Hrs
48 Hrs
72 Hrs
96 Hrs

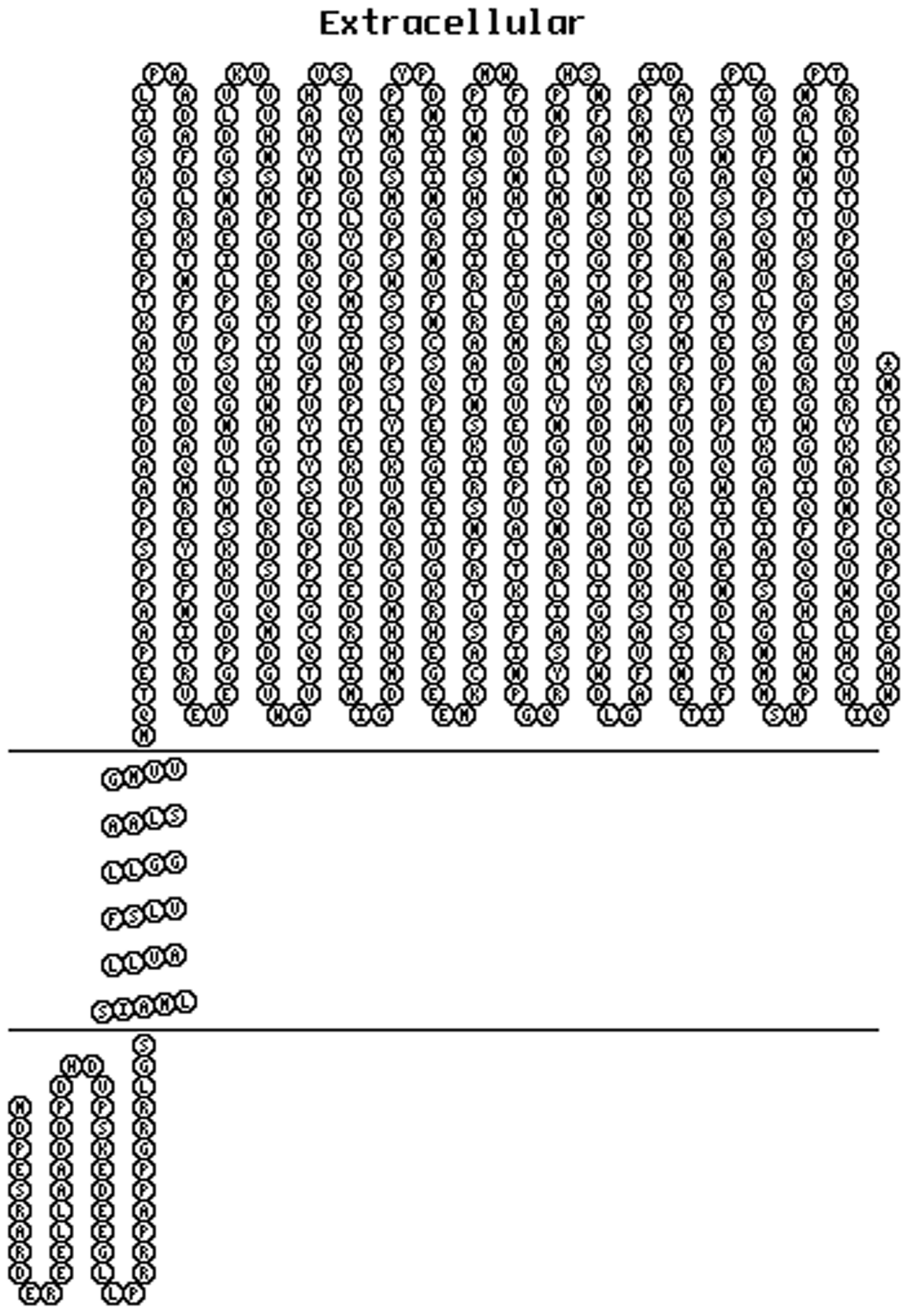
A



B



MGG_07771 siRNA ***Control***


Extracellular
Cytoplasm