```
LOCUS       AY344587  1107 bp DNA linear   PLN 25-JUN-2004
DEFINITION  Rhizopus oryzae high-affinity iron permease gene, complete
cds.
ACCESSION   AY344587
VERSION     AY344587.1  GI:33637943

SEQ ID NO:1

   1 atgtctcaag atctcttcaa cgtaccgatc ttctttatcc tttttcgtga aacgactgag
  61 gcagccatca ttatttctgt cctcttgtca ttcttgaaga gaatgtttaa tacagaatct
 121 cctgtttata aacgtctcag aaatcaagtc tggattggtg gtgcagctgg tctgtttatc
 181 tgtttatgta tcggtgctgc cttcattgcc gtttactaca ctgtccttaa tgacttgtgg
 241 ggaaattctg aagatatctg ggaaggtgtc ttctctctgg ttgctgtgat catgatcact
 301 gccatgggtc ttgctatgct caagactgaa cgtatgcaag aaaagtggaa ggtcaagttg
 361 gctaaagcaa tgcaaaagtc caacagtgaa aagtcatcct ttaaagaaaa acttcaaaaa
 421 tacgcgttct ttgtcttgcc ttttatcacc gttctcagag aaggacttga agctgttgtc
 481 tttattggtg gtgtctcctt gggtatccaa ggaaaatcaa ttcctattgc tgccatcatg
 541 ggtatcatct gtggttgttt ggtcggtttc cttatttacc gtggtggttc cttgattcaa
 601 cttcgttggt tctttgtgtt ctctactgtc gttctttacc ttgtcgctgc tggtttgatg
 661 gctaaaggtg ttggttacct tgaacaaaat gcttggaatc aagtcattgg tggtgaagct
 721 gctgatgtca ttagttacag agtctcaacc gctgtctggc acgtttcttg gggagaccca
 781 gaagccaaca atgatacctc tggtggttgg caaatcttta acgccattct tggttggaac
 841 aatacggcta cttatggttc tatcatcagt tactgtctct actggctctt tgtctgctgt
 901 tatcttgtct ttagttactt taaggaaaag cgtgctgcta tccgtaaagc cgaggctggt
 961 gaatgggatg atggtgatga agctttggag aatgccaaac aatacattgg taatgatggt
1021 gaattcatcg ttgaagacaa agaatctgat gaagaagcca acaatcatcc caaggaaaaa
1081 atcgaatctg atgctattaa ggcttaa
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Figure 1

```
LOCUS       AY344587  1107 bp DNA linear   PLN 25-JUN-2004
DEFINITION  Rhizopus oryzae high-affinity iron permease gene, complete
cds.
ACCESSION   AY344587
VERSION     AY344587.1  GI:33637943
protein_id="AAQ24109.1"

SEQ ID NO:2

   1 MSQDLFNVPI FFILFRETTE AAIIISVLLS FLKRMFNTES PVYKRLRNQV WIGGAAGLFI
  61 CLCIGAAFIA VYYTVLNDLW GNSEDIWEGV FSLVAVIMIT AMGLAMLKTE RMQEKWKVKL
 121 AKAMQKSNSE KSSFKEKLQK YAFFVLPFIT VLREGLEAVV FIGGVSLGIQ GKSIPIAAIM
 181 GIICGCLVGF LIYRGGSLIQ LRWFFVFSTV VLYLVAAGLM AKGVGYLEQN AWNQVIGGEA
 241 ADVISYRVST AVWHVSWGDP EANNDTSGGW QIFNAILGWN NTATYGSIIS YCLYWLFVCC
 301 YLVFSYFKEK RAAIRKAEAG EWDDGDEALE NAKQYIGNDG EFIVEDKESD EEANNHPKEK
 361 IESDAIKA
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Figure 2

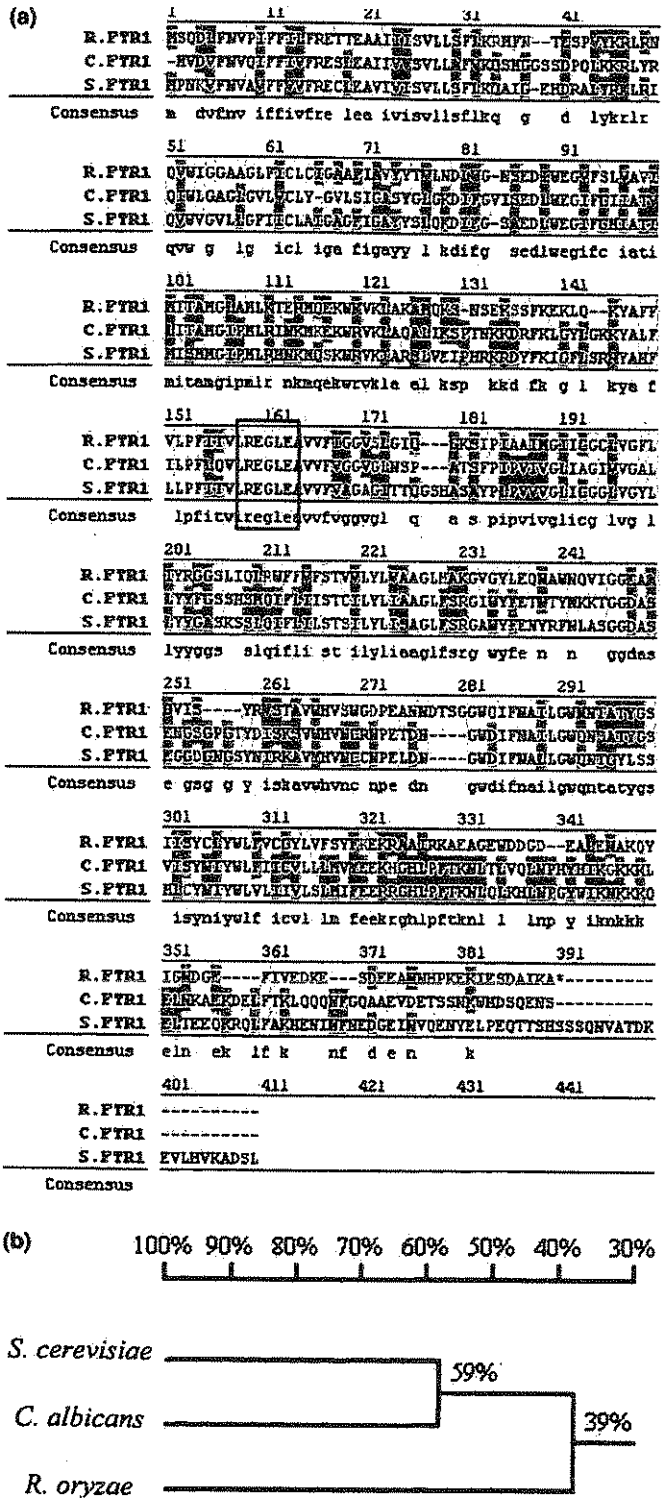

(a)
R.PTR1
C.PTR1
S.PTR1
Consensus
(b)
100% 90% 80% 70% 60% 50% 40% 30%
S. cerevisiae
C. albicans
R. oryzae
59%
39%

Figure 3

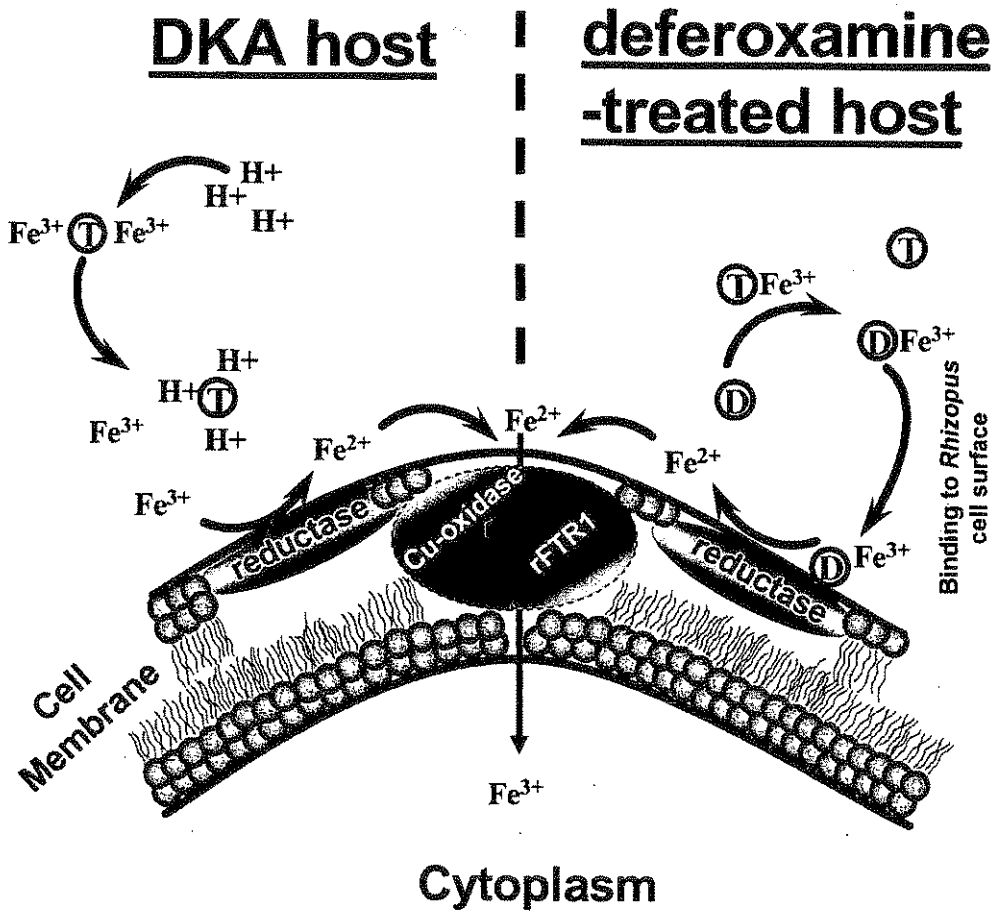

DKA host
deferoxamine -treated host
H+
Fe3+
Fe2+
reductase
Cu-oxidase
rFTR1
Cell Membrane
Binding to Rhizopus cell surface
Cytoplasm

Figure 4

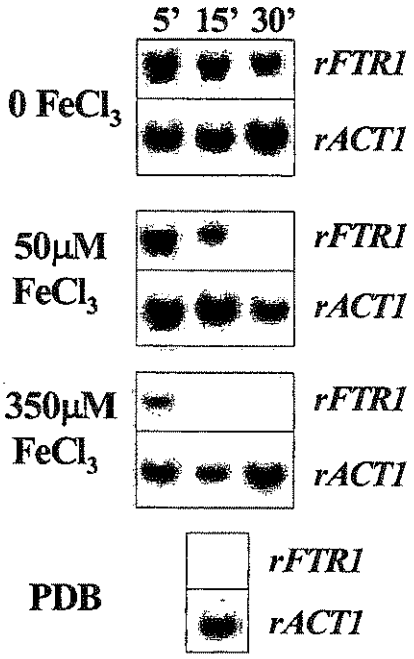


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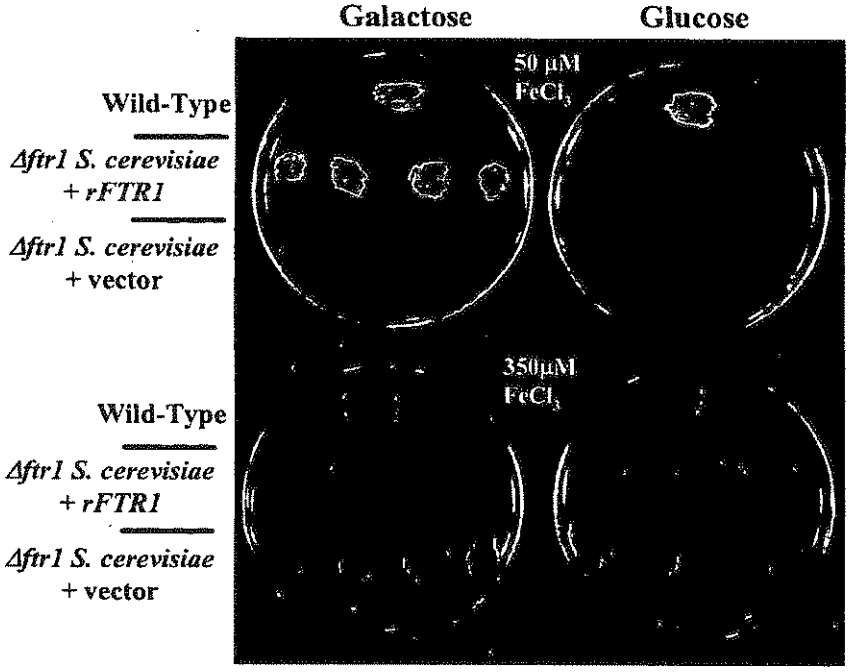


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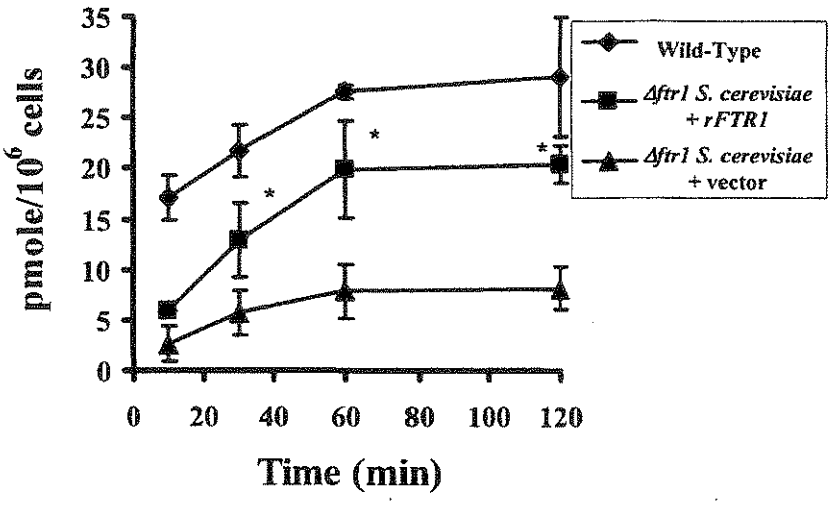


Figure 7

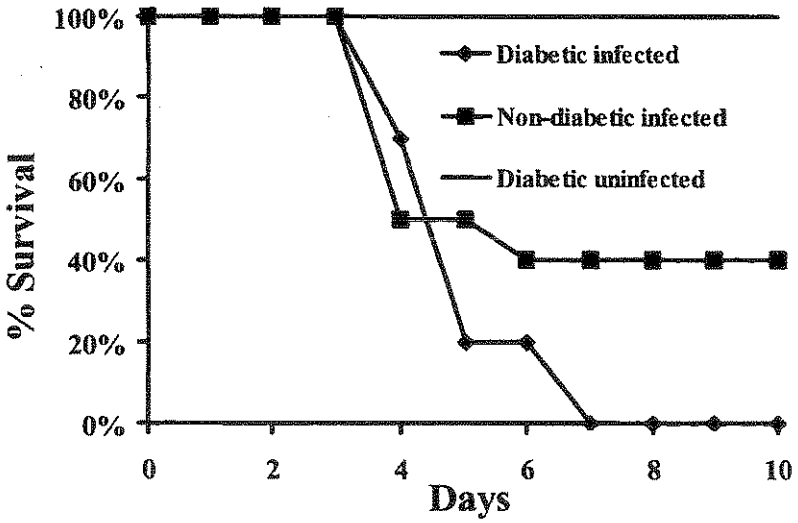


Figure 8

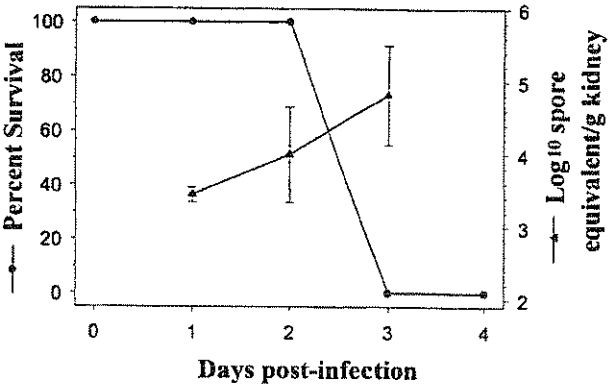


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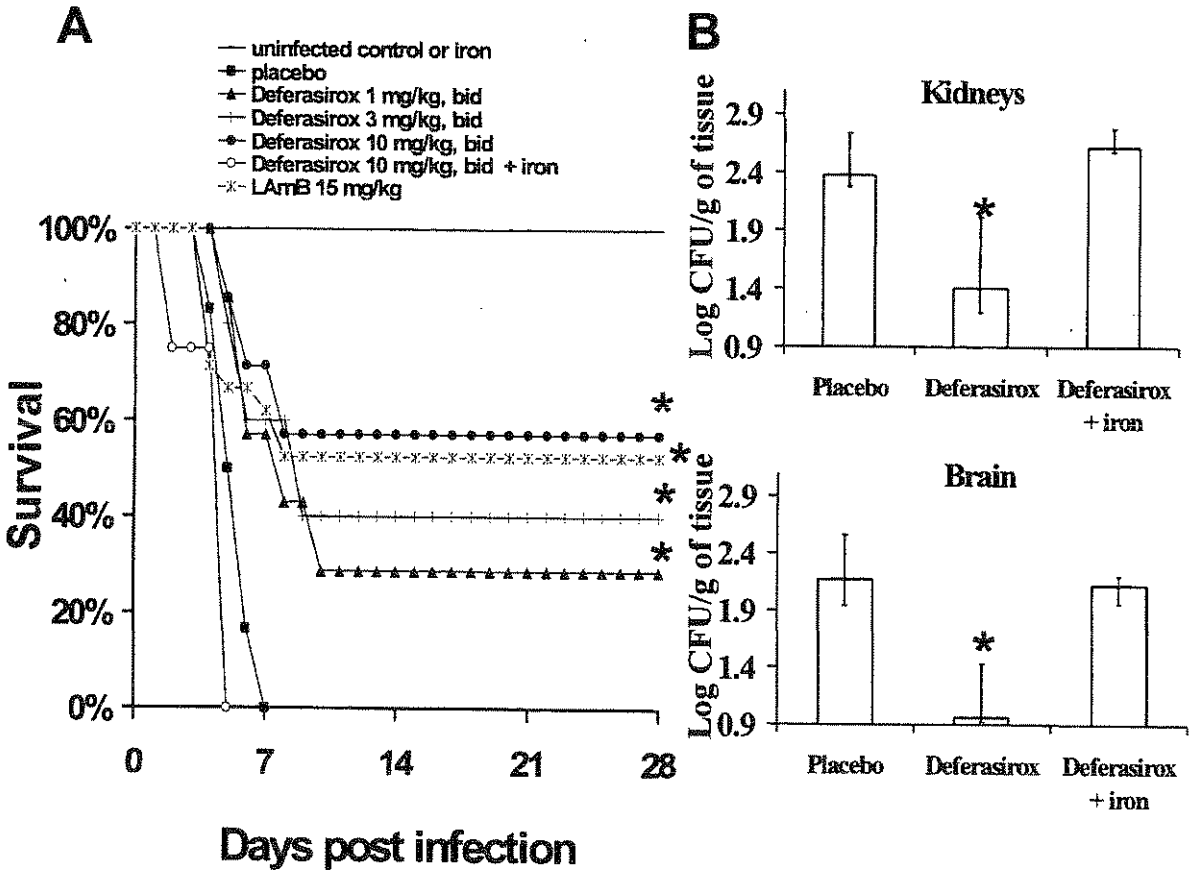


Figure 10

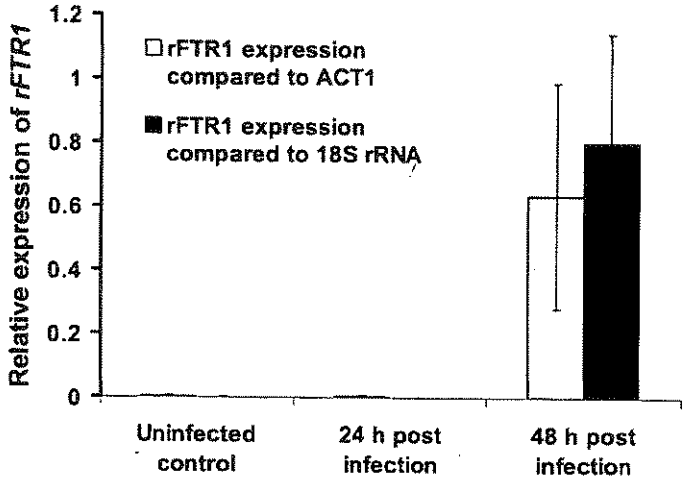


Figure 11

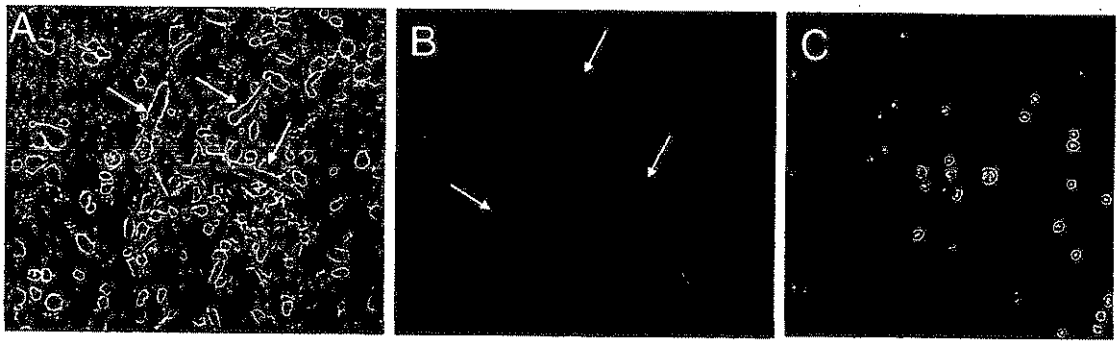


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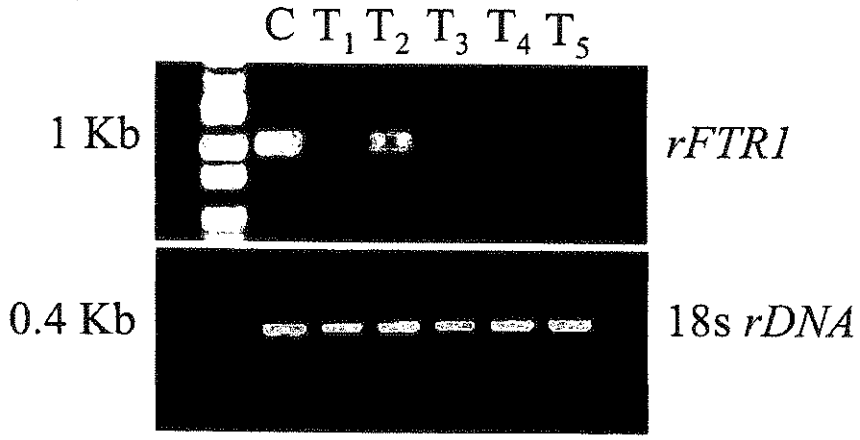


Figure 13

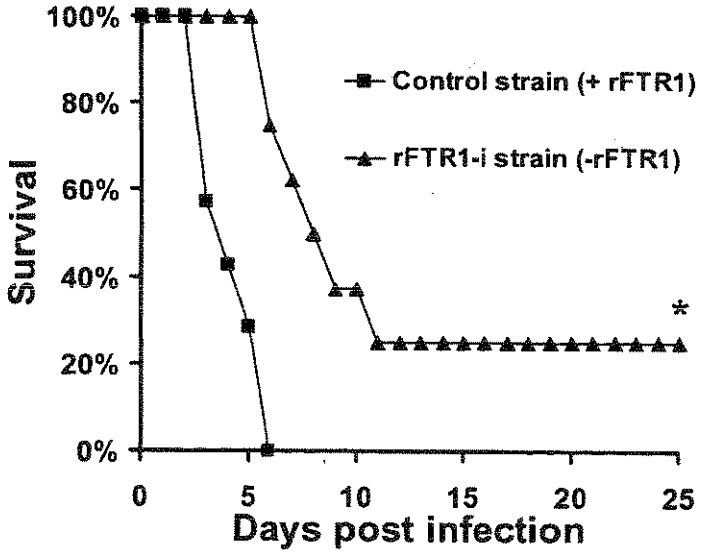


Figure 14

Sequence of high affinity iron permeas from *Aspergillus fumigatus*

```
   1 ATGATCGGAG CGTTCTATGG ATATGGTAAG GATCACTTCG CTAGCACGGA GGACCTGTGG
  61 GAGGGCATCT TCTCCCTGAT CGCCAGTGTC ATCATCACCA TTATGGGTGC TGCCCTGCTT
 121 CGTGTCACCA AGTTGCAGGA GAAGTGGCGC GTCAAGCTAG CTCAAGCCCT GGAAGCAAAG
 181 CCGTTGACTG GCGGCACATT CAAAAACAAC CTCAAACTTT GGGCGGAGAA ATACGCCATG
 241 TTTCTCCTCC CCTTCATCAC CGTTCTCCGA GAAGGCCTGG AAGCAGTGGT GTTCATTGGA
 301 GGCGTCAGTC TCAGTTTTCC TGCAACTGCC TTCCCTCTAC CTGTTTTTAC TGGCATTCTC
 361 GCAGGAGTGG CCATTGGGTA CCTACTGTAT CGGTATGTTG AAACCCCTGA ATCAGCTGTC
 421 TTTCTCAAAT AGACCACCAT GCTGATGGTC TGCAGAGGAG GAAACCAAGC CTCCCTCCAG
 481 ATCTTCCTGA TCATCTCCAC TTGCATCCTC TACCTGGTTG CTGCCGGCCT CTTCTCCCGA
 541 GGCGTCTGGT ATCTGGAGAA CAATACTTGG AACCACGTAA TTGGTGGTGA TGCTGCCGAG
 601 ACAGGTGCCG GTCCGGGATC GTATGACATC CGACAGAGCG TCTGGCATGT CAACTGCTGT
 661 AGTCCTCTCG TTAATGGTGG CGGGGGATGG GGTATCTTCA ACGCCATCCT TGGCTGGACA
 721 AACTCGGCAA CCTATGGCTC CGTTCTTTCA TACAACCTTT ACTGGATTGC GGTGATCGTC
 781 TGGTTTGTGG CTATGCGTCA CAAGGAACGC CATGGACGAT TGCCTGTGGT CGACCCTCTG
 841 CTGAATCGGC TGCGAGGCCG AAAGTCTGCC GAACCTGGGA ATGGAGAGCA AGATGTCGAG
 901 GTCAGCACGA TACCATCTGA TTTGCAGACG GAGTCCAAAA TACCGAAAAG CGGAGCATCC
 961 CTTGTCTGA
```

Figure 15

Sequence of high affinity iron permeas from *Candida guilliermondii*

```
   1 ATGAACTTTG AAGACTACTT CTCGGTTCAA ATCTTCTTTA TAATCCTCCG AGAAACGTTG
  61 GAGACCGCTA TAGTGATTTC GGTTCTTCTT TCGTTCATCA ATCAAAGAAG CCAAGAAGCA
 121 AATGACCGAG GAAATTCTGC TAATGAAGCT GCTCATACTC GAGGATTGCG GGTCCAGGTA
 181 TGGGCCGGGG CCTTAATGGG ATTTGTTGTG TGTTTTGCCA TCGGAGTGGC ATTTGTGGTT
 241 GCATTTTATG TCATTGGAGA GAACTACTGG CTGTATGCTG AAAGACTCTG GGAGGGTATT
 301 TTCTCGCTTC TTTCGAGTAT AATAATCACA GTGATGGGTA TCGGATTGCT ACGTATAAAC
 361 CGCGTGATGA AAGTTAAGTG GTTTGCTAAG TTGGGTGATG CCTTTGATCT GCATTCGCAC
 421 GGTAGAGGCC ATAAAAAGAA GTACTTTCTT GCATTATTAC CATTTATAAC CACACTCAGA
 481 GAAGGCTTGG AGGCCGTGGT ATTCGTGGGT GGAATTGGTC TTTCGCTGCC AGTTTCTTCC
 541 ATCCCATTTT CCATTCTCAG TGGAATCTGT GTGGGATCCA CAATCGGTTA CACTTTGTAC
 601 AAAGGAGGCA ACAAGCTTTC TCTCCAATAC TTCCTCATCT TGCTGACCTG CTTTTTGTAC
 661 ATAGTTCTGG CAGGGCTTAT GAGTAGAGGA GTATGGTTTT TGGAACTCGA GCTGTATGTG
 721 AGAAAATGTG GAGGACTCGA CGTTAGCGAA ACAGGTAGTG GACCTGGATC TTATGATCCT
 781 GCTACAAGCG TGTGGCATGT CAATTGCTGT AACGGACTCA CAGATGGCTG GTGGATGGTT
 841 TTAAATGCTC TTGTTGGCTG GACCAATTCC GCAACTTATG GCAGCGTAGG TGCTTATTGT
 901 GCCTATTGGA TATTGGTCAT TTCATGGCTT GAGATCCGCT TGTACGAAGA GCATCATGGA
 961 CTCTTACCCT TTGTTCCTGT GCGTTGGCAG TTGAAGCGTA TCAGAAAAAA AATCAAATTA
1021 TACGAAGCCC GGGCCAAACA TGGCGCTGCA ATAGAGGCTG AAACAGAGGC AGAATTACTC
1081 ATGGAATAA
```

Figure 16

Sequence of high affinity iron permeas from *Aspergillus flavus*

Gene 1

```
   1 AGCCCTCTTT CATTCCTTTC TGAAGCCTCT ACCTTCCACT CAACATGGCA ACCGATGTAT
  61 TTGCAGTTCC CAGTATGCAT TCTTTTCTTG TTATGCCCCA TCTATTAATC AGGAACTAAC
 121 TTAGTTTGAC TAACTTATCG AAGTATTCTT CATCTGCTTT CGAGAATGCG TTGAGACCAG
 181 CATCATTGTT TCGGTATTAC TTGCCTTCAT CAAGCAGACG CTGGGGTCGG ACACGGATGC
 241 CTTTACTCGC AAAAGGCTTA TCAAACAGGT TACTACCATC TTCTCATTCC GTCCCCACCC
 301 TATAGAGACA ACATTGACGA TCAATAGGTC TGGTGGGGAG TTGCGGTCGG GCTGTTTATA
 361 TGCCTCTGTA TTGGAGGTGG TATGATTGGG GCCTTCTACG GGTATGGCAA GGACCATTTT
 421 GCCAGCACGG AGGATTTATG GGAAGGCATC TTTGCTTTAG TCGCCGCCGT CATCATCACA
 481 GTCATGGGAG CAGCCCTTCT GCGGGTGAAT AAACTGCAGG AGAAATGGCG TGTCAAGCTG
 541 GCCCAGGCAT TGGCGGCAAA ACCTCAACCT CAAGGGAGAA TGACAGACAA GATCAAGCAA
 601 TGGTCACAGA AGTATTTCAT GTTCATCTTA CCCTTCATTA CGGTACTTCG GGAGGGTCTA
 661 GAGGCTGTGG TGTTTATCGG GGGTGTTAGT CTCAGCTTCC CCGCAAGCGC GTTCCCTCTC
 721 CCTGTATTCA CGGGACTCTT GGCTGGTGTG GTAGTTGGTT ATATCATTTA CCGGTGAGTG
 781 TGATATGGGC GGCATTGAAA CTGAATCCCA ATGCTGATAG TAGTCTTTGC GATTTATCAT
 841 AGGGGCGGGA ATCAAACCTC ACTTCAGATA TTCATGGTCA TCTCGACATG TCTGCTCTAC
 901 TTGGTCGCTG CTGGACTTTT GTCCCGAGGC GTCTGGTTCT TGGAGAACAA CACTTGGAGC
 961 AACCTCATCG GTGGCGATGC CTCAGAAACG GGAGCT
```

Gene 2

```
   1 ATGGCAAATC AAGTCTTTGC AGTCACTGGT AACTCTCATT ATACTCTCTG AGTCTGCTTT
  61 TGTACAAGAC GACCGTTGCT GACCGTGTAT AGTCTTTTTC ATTTGCTTCC GAGAATGTCT
 121 CGAAAGCAGT ATCATTGTAT CGGTGCTTCT TGCCTTCCTC ACCCAAACTT TGGGTGCTGA
 181 AGGAGACAAG GCAGCTCTGA AGAGATTGCG AATACAGGTG AGTGTCCTTC CCTATTTCTT
 241 TATACCTTTG TTTATCATGA ACATCATTCG ATAATGAGCT GCTAACAATA ACAGGTATGG
 301 TGTGGAGTAG GTTTAGGTCT ATTCTTGTGC CTATGTATCG GTGCAGGTAT GATCGGAGCT
 361 TTCTACGGGT TGGAAAAAGA TACCTTCACG AACACAGAGG ATATCTGGGA AGGCATTTTT
 421 GGCTTTATAG CATCCATCAT TATTTCTATC ATGGGGGCGG GCCTTTTGCG TGTGAACAAA
 481 ATGCGCGAGA AATGGCGCGT CAAGCTCTCC CGTGCCTTGG AGAAAAAGGA AAAGTCTACG
 541 ACCATAATGG GTCGTCTGAA GGACTGGTCC GAGAAATATG TCATGTTCAT CTTGCCATTC
 601 GTTACCGTTC TTCGAGAGGG ACTTGAGGCT ATCGTTTATG TCGGTGGTGT GGGACTGGGA
 661 TTGCCAGCGT CTTCATTCCC CTTGGCCGTG TTCTGTGGCC TTTTGGCTGG TGTTGCAGTA
 721 GGCTATGTGA TGTATCGGTA AGTCTTGTGC GTTACATTCT GTCTACTTTA AGAATCATTA
 781 TCTGCACAGT ATCGTTCGAC GGTGGCTAAC GAATCTTGAT GCAGGGGTGG AAGCTCAACT
 841 TCCTTACAGT ACTTTCTGAT TATATCTACT TGCTTCCTCT ACTTGGTCGC CGCAGGCTTG
 901 TTTTCCCGCG CTGTCTGGTA CCTGGAGAAT AATACCTGGA ACCATGTCAT TGGTGGAGAT
 961 GCTTCTGAGA CCGGCTCAGG CCCTGGATCT TACGATATTC GCCAGAGTGT CTGGCATGTA
1021 AACTGCTGTA ACCCAGAGCT TGGCGGTGGT GGTGGTTGGG GTATCTTCAA CGCTCTGTTT
1081 GGGTGGACCA ATTCCGCCAC CTATGGATCA GTGCTATCTT ACAACCTTTA CTGGGTAGTG
1141 ATCATTACCT CATACGTCTG CATGAGGTAC AATGAGAAGC ACGGCTACAT TCCCGTCTTA
1201 ACACCGATCG CAAGGAAGCT GAAGCTTGGT CGATTCAAGA AGGGCTCTGA GGAAGAACAT
1261 GTCCCCGAGG TTGTCGAAGA GAGGAAGGAG GTTAACCATC TGGCCCGGCA AATTGTTACC
1321 AGAACAATGA GTGAAGCATA G
```

Figure 17

Sequence of high affinity iron permeas from *Candida tropicalis*

```
   1 ATGGCACAGA TCGAGGAGTA CTTTTCTGTT CAGATATTCT TTATCATTCT CAGAGAGACT
  61 CTTGAAACAG CTATCATCAT CTCGGTGTTG CTATCCTTCA TCAACCAACG TTCGCACAAG
 121 CATGGCTCCC AGTCTCAGAA CATAGCAGTA TCATCGTCAT CACCATCGAC ATCATCACCT
 181 GGTTCAGTGG ACCCCGCCGA TGAGCCACTA CCTTCGAGTT CAACCACTGT CACCCCGGGA
 241 CAGACATCCC ATTTGGAGAA AGTGCATCGT AAGCTTAAAC TTCAAGTATG GGTGGGCGCC
 301 CTTCTTGGGC TTTTCATTTG TTTTGTCATC GGGATGGTAT TTACGTTGAT GTTTTATTTT
 361 GTGGGACAGG ACTACTGGGC ATACACGGAA CGAGTATGGG AAGGTGTGTT CTGCATATTG
 421 TCAAGTGTGA TCATCACTGT GATGGGGATT GGGCTTTTGA GAATCAACAA AGTTATGAAG
 481 ATCAAATGGT GGATCAAGTT GGGAGATGCG TACAATAATG AAGAATACGC AGAAGACGAA
 541 GAGGCAGAAG GCGAGGAAGA GATTGCCAAG TTAGGAGACG ACGATGTGAT GTATGAGGAT
 601 AGTATGGCTA ATTATGGAGG CACCAGGTCG AGCTCAGAGT CAAACACCGT GGAGGAGAAC
 661 ATCCCATTAA CCGGTACGCC TGCTACTCCT GCAACAGCTA GAACCACAAC CACAAAGAAG
 721 AACACACCAA GGAAACAGGG GTTCACCAAG AAATACTACC TTGCTATTTT ACCATTGGTC
 781 ACCACATTGA GAGAAGGGTT AGAAGCGGTT GTGTTCATTG GTGGCTCAGC CATGACGTCC
 841 ACGGTATTTT CGATCATTGT GTCTGTTGTC TGCGGAATTG CATTTGGTTC CTTGATTGGG
 901 TACTTGCTTT ACCAAGGGGG GAACAAACTC TCCCTTCAGT ATTTTCTTAT TTGCTCAACA
 961 TGTTTTCTTT ACATGGTCAG CGCTGGGTTG ATCAGTCGTG GTGTCTGGTT TATGGAGCTT
1021 GAAAGATATG TCCGTGCATG TGGGGGCATG GACGTTAGTG AAACGGGAAG TGGGCCAGGT
1081 TCATATGATA TCGCAACCAG TGTGTGGCAT GTCAACTGTT GTAACGGACT AACTGACGGT
1141 TGGTGGATGG TGTTGAACGC AATTGTCGGC TGGACCAATT CGGCTACATA TGGCAGTGTG
1201 ATTAGTTATA TGGCATACTG GTTGTTGGTA ATTGTGTGGT TGAAAGTTAA GTTGTATGAA
1261 GAGAGGGAAG GTGTGTTGCC TTGGATTCCC GTAAGATGGC AACTCAAGAG AATTAGAAAG
1321 AAGATTAGAT TGTATGAATT GAGGACCCGT CAGCAAGAGC AACAGGAGCA ACAGAGAGGT
1381 GGTAGTGGTA GTGGTAATGA ATTGCCAGAA TCGCAAGGAT TGTTGCAACA GGATTGA
```

Figure 18

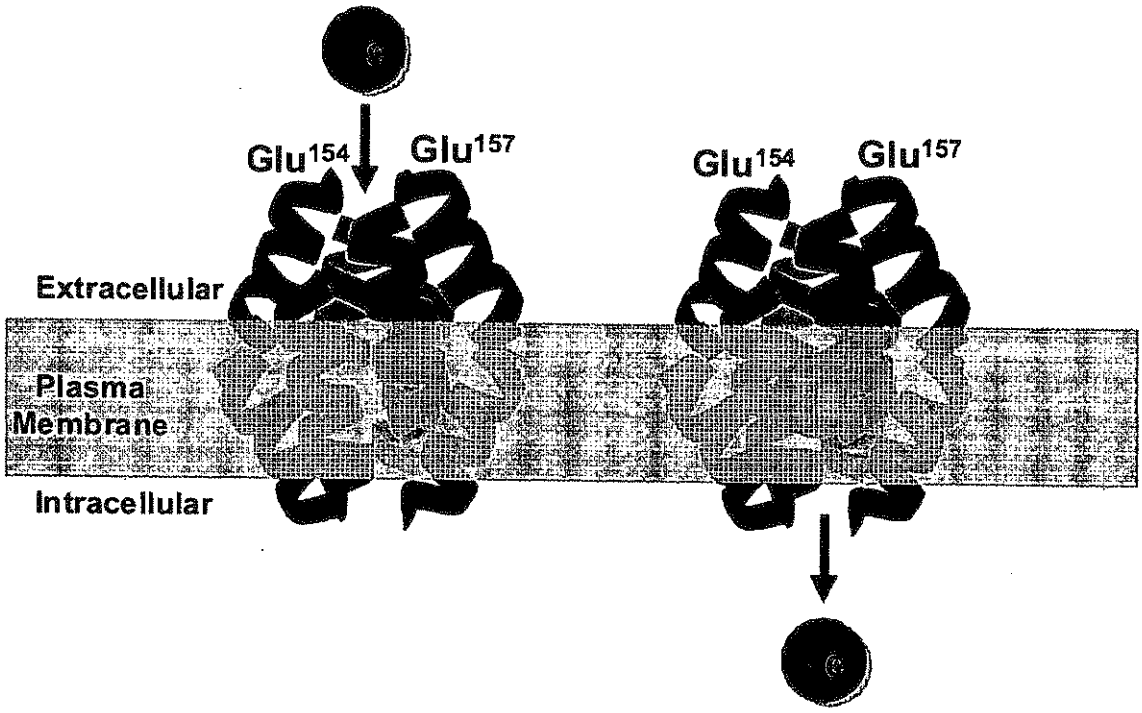

Glu154
Glu157
Glu154
Glu157
Extracellular
Plasma
Membrane
Intracellular

Figure 19

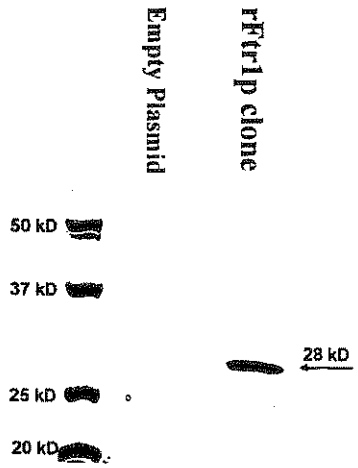


Figure 20

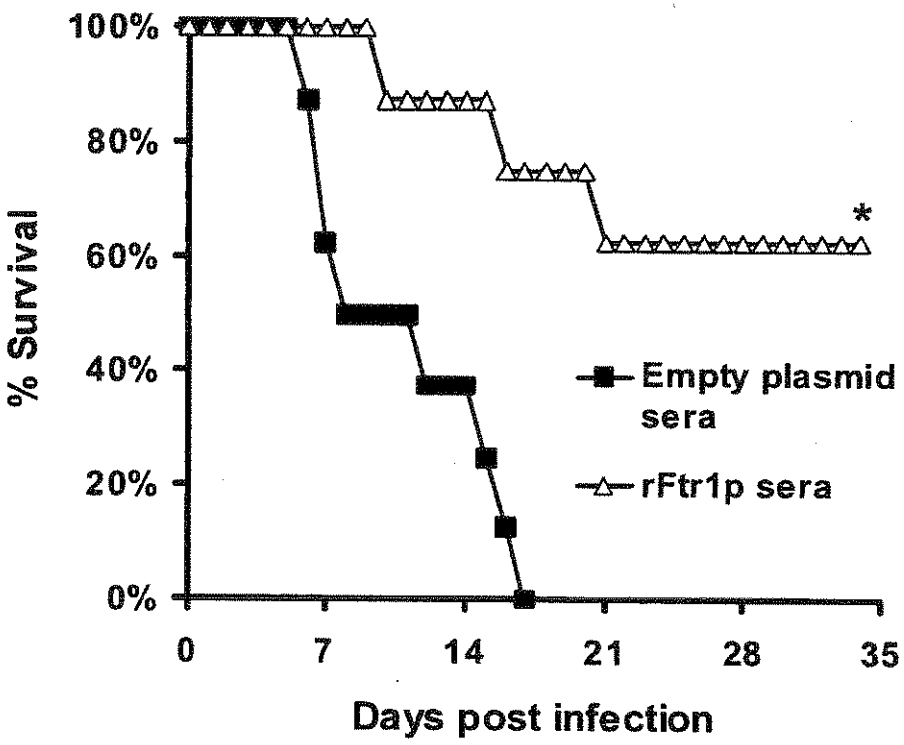

100%
80%
60%
40%
20%
0%
% Survival
0
7
14
21
28
35
Days post infection
Empty plasmid sera
rFtr1p sera
*

Figure 21

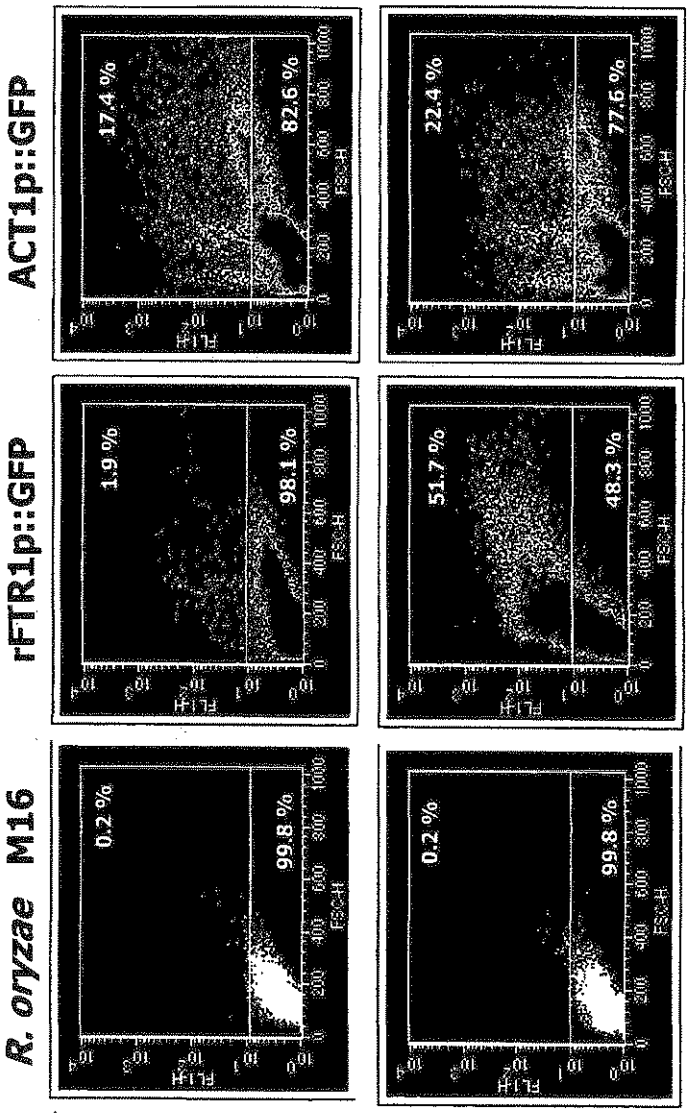

R. oryzae M16
rFTR1p::GFP
ACT1p::GFP
Iron-replete
Iron-depleted
0.2 %
99.8 %
1.9 %
98.1 %
17.4 %
82.6 %
51.7 %
48.3 %
22.4 %
77.6 %
FL1-H
FSC-H

FIGURE 22A

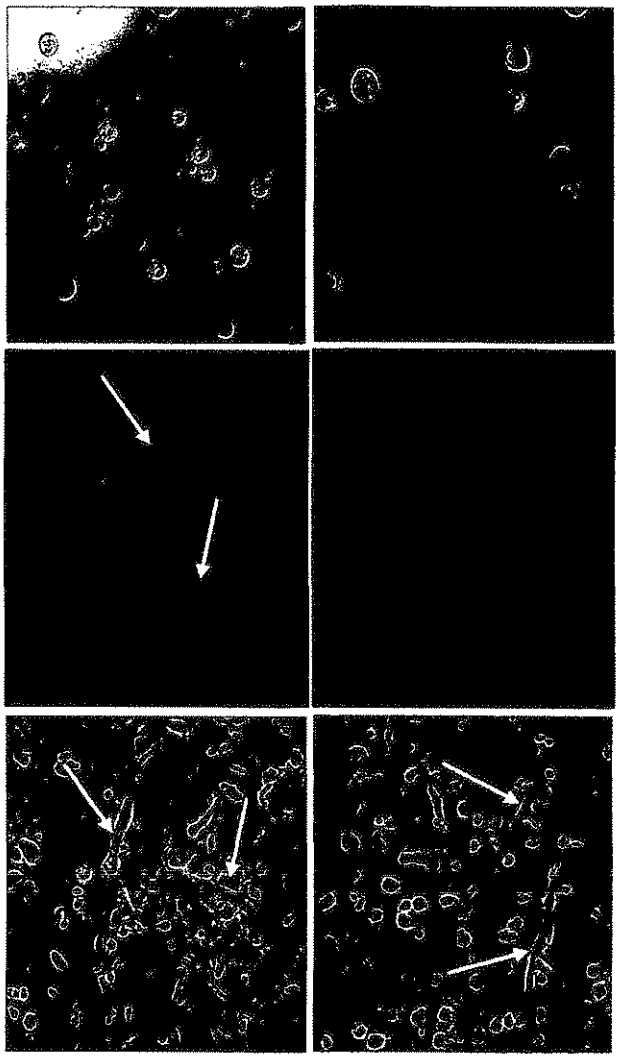

R. oryzae expressing GFP
R. oryzae PyrF-complemented
H & E
Anti-GFP Ab
DIC

FIGURE 22B

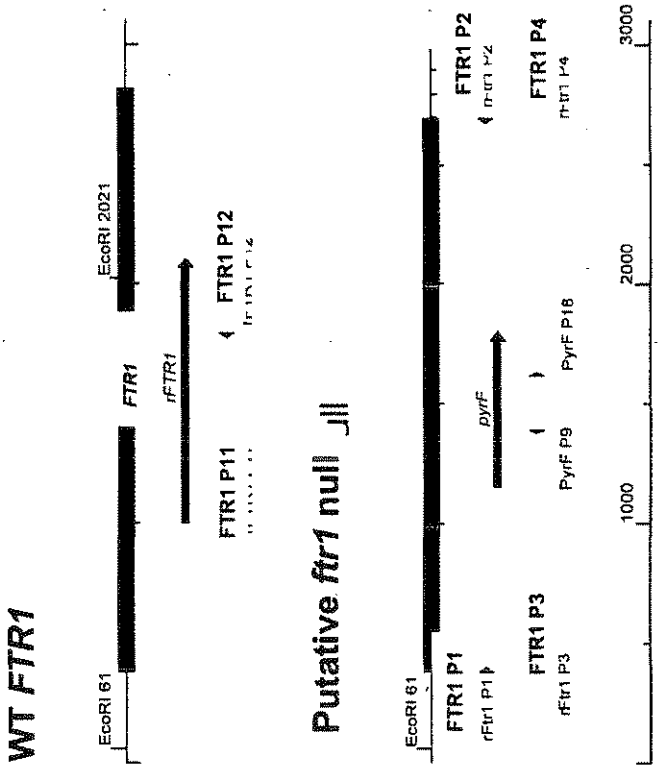

WT FTR1
EcoRI 61
FTR1
EcoRI 2021
rFTR1
FTR1 P11
FTR1 P12
Putative ftr1 null
EcoRI 61
FTR1 P1
rFtr1 P1
pyrF
FTR1 P2
FTR1 P3
rFtr1 P3
PyrF P9
PyrF P18
FTR1 P4
1000
2000
3000

Figure 23A

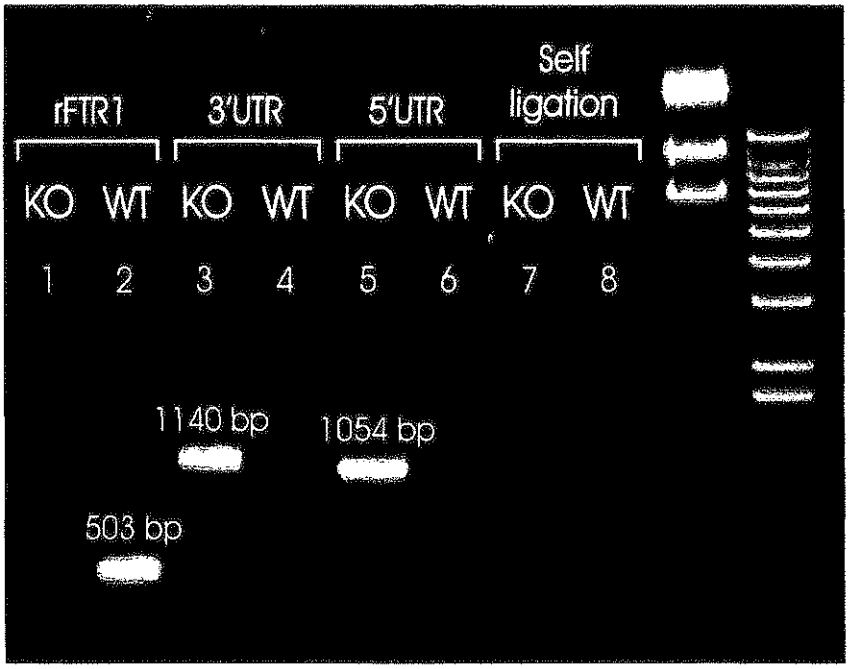


Figure 23B

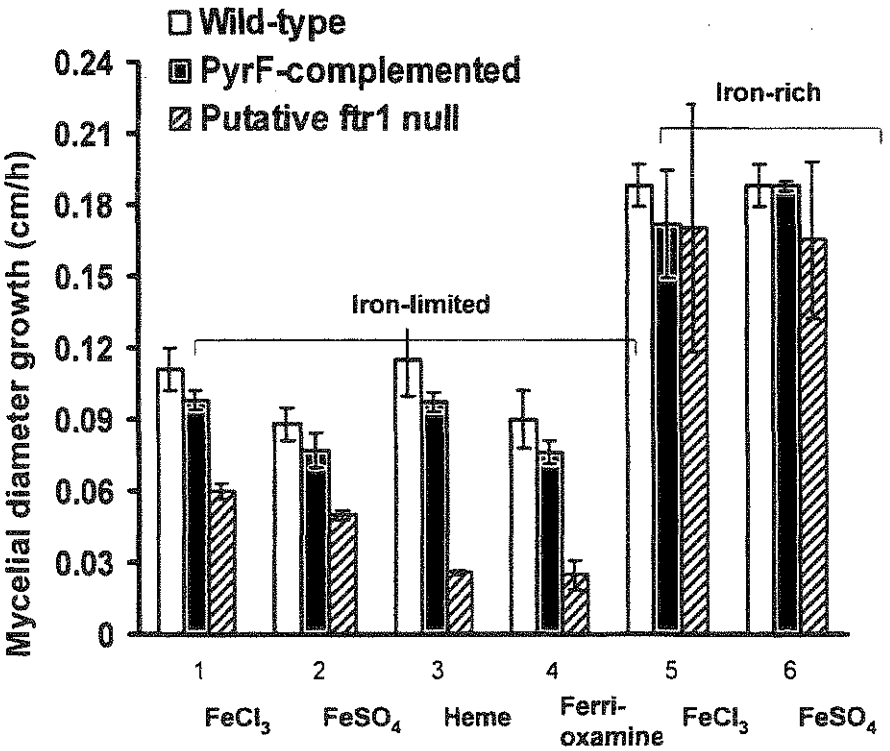


Figure 23C

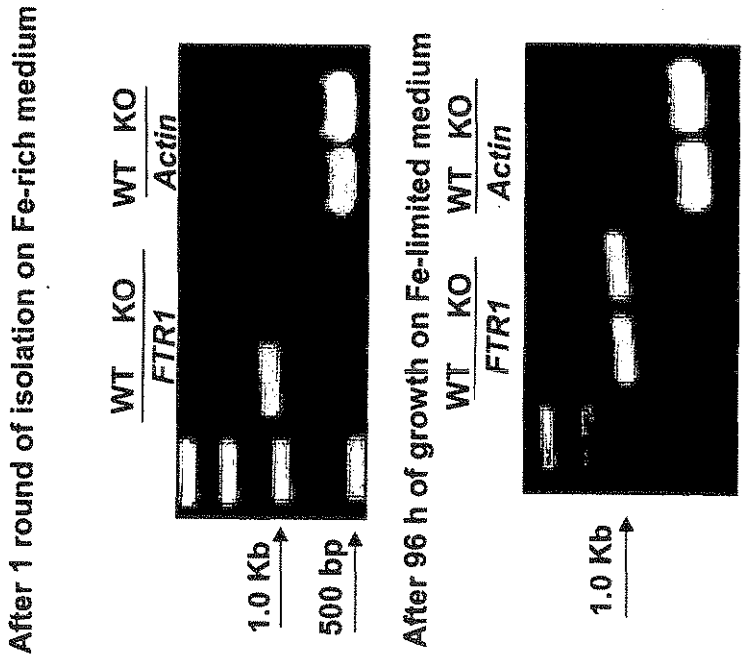

After 1 round of isolation on Fe-rich medium
WT KO WT KO
FTR1 Actin
1.0 Kb
500 bp
After 96 h of growth on Fe-limited medium
WT KO WT KO
FTR1 Actin
1.0 Kb

Figure 23 D

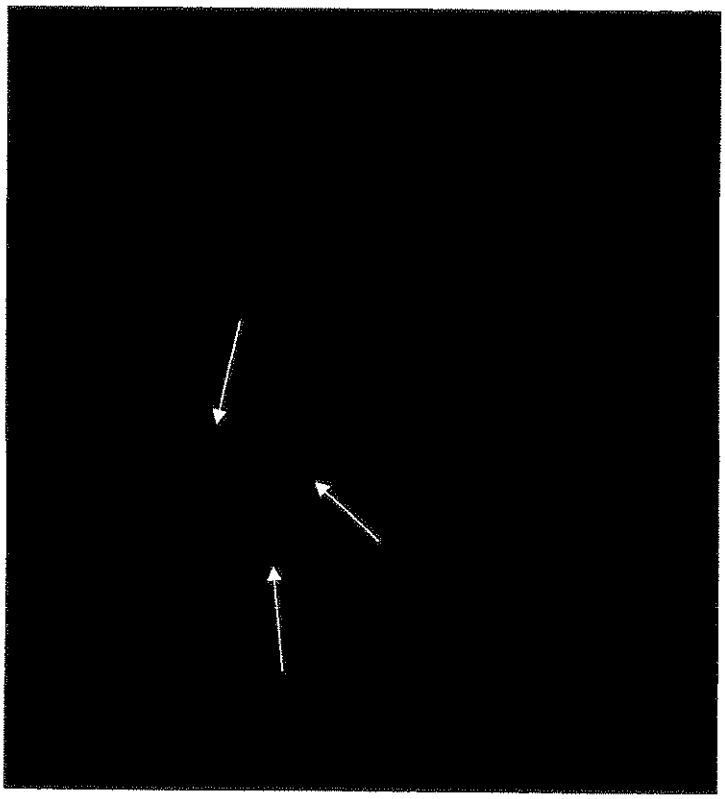


Figure 24A

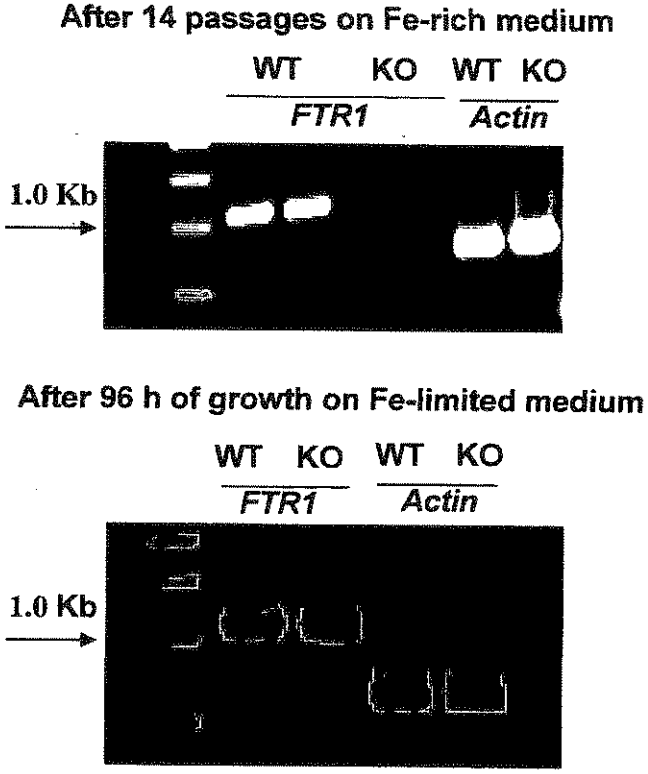

After 14 passages on Fe-rich medium
WT KO WT KO
FTR1 Actin
1.0 Kb
After 96 h of growth on Fe-limited medium
WT KO WT KO
FTR1 Actin
1.0 Kb

Figure 24B

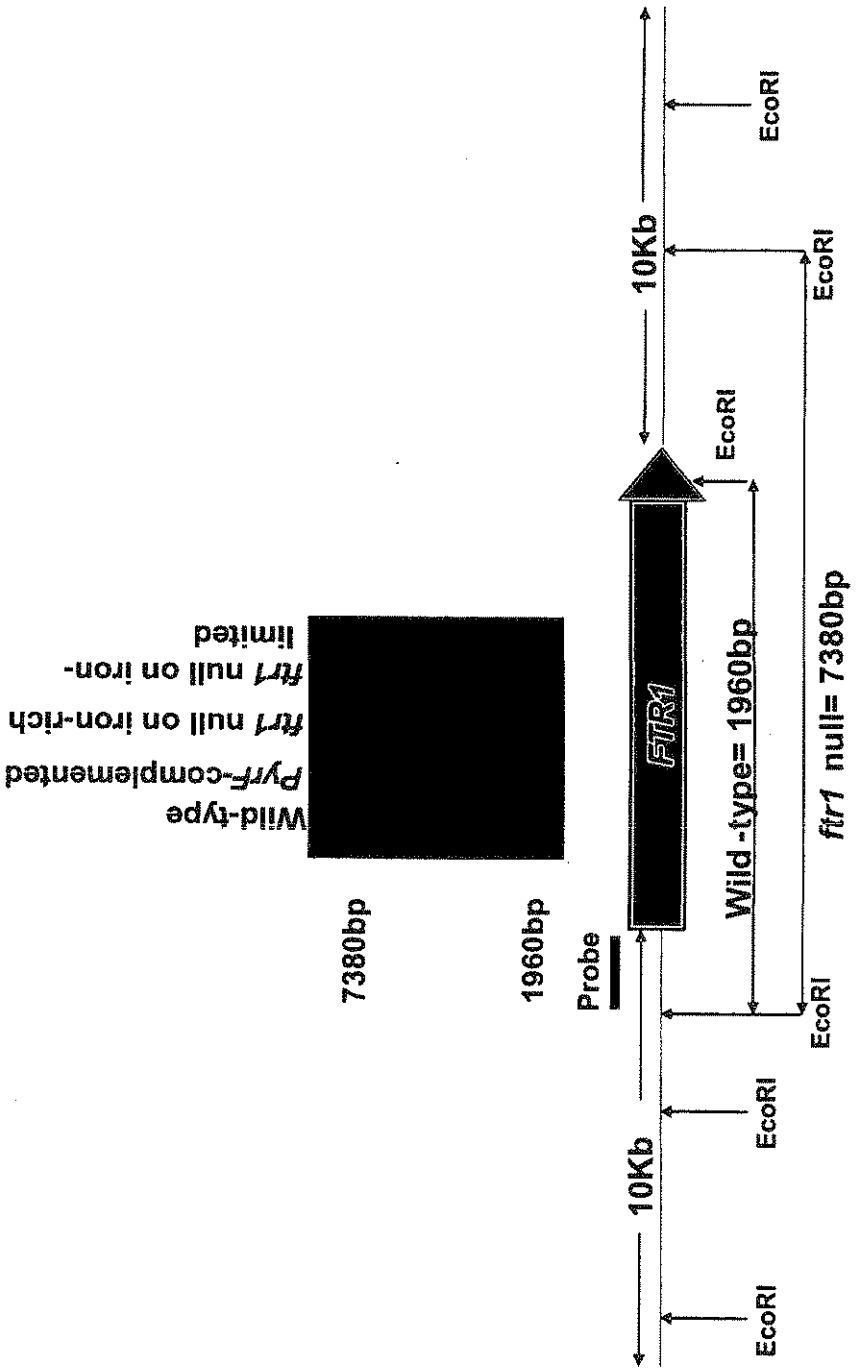

EcoRI
10Kb
EcoRI
EcoRI
FTR1
Wild -type= 1960bp
ftr1 null= 7380bp
EcoRI
EcoRI
10Kb
EcoRI
Probe
1960bp
7380bp
Wild-type
Pyrf-complemented
ftr1 null on iron-rich
ftr1 null on iron-limited

Figure 24C

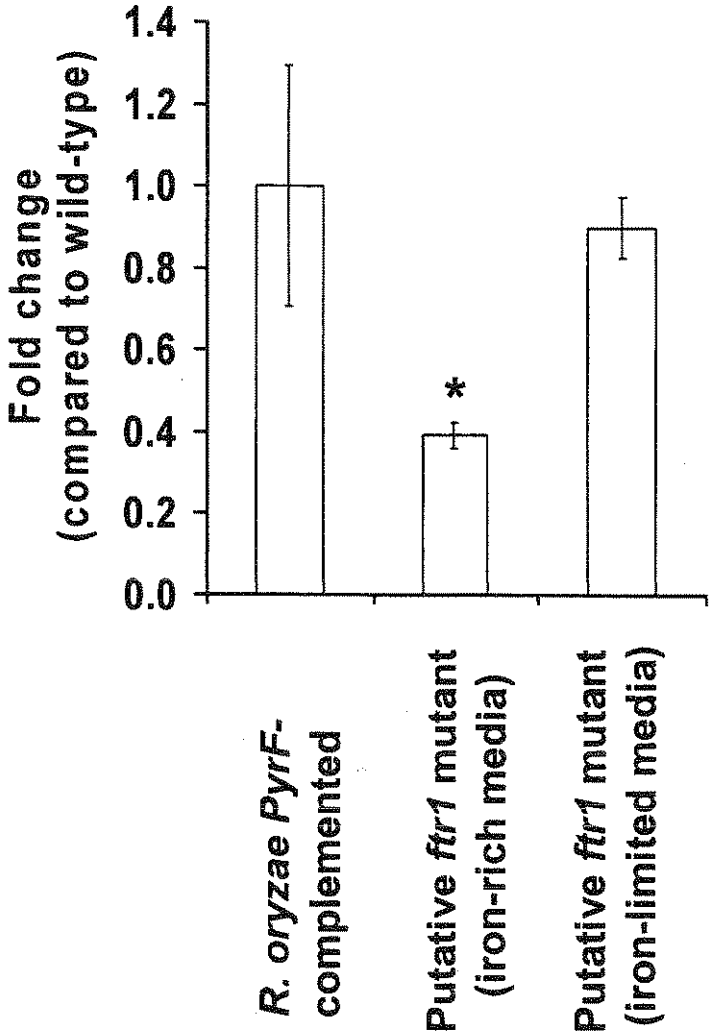

Fold change
(compared to wild-type)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
R. oryzae PyrF-
complemented
Putative ftr1 mutant
(iron-rich media)
Putative ftr1 mutant
(iron-limited media)

Figure 25A

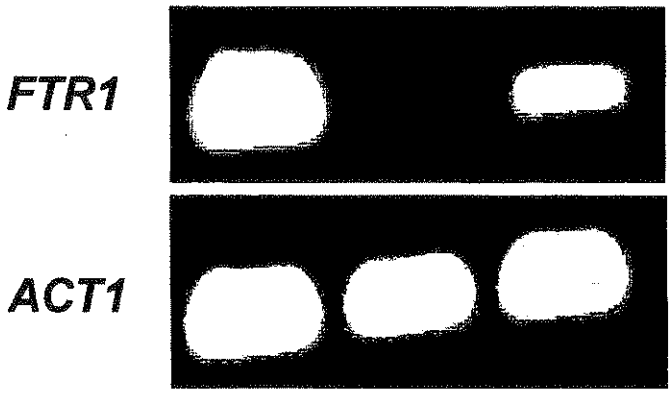


Figure 25B

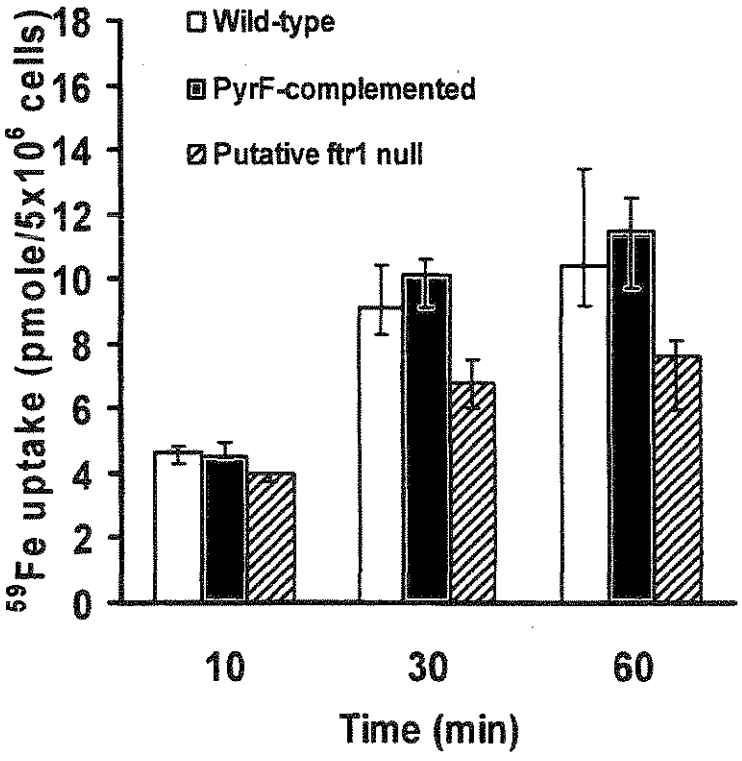


Figure 25C

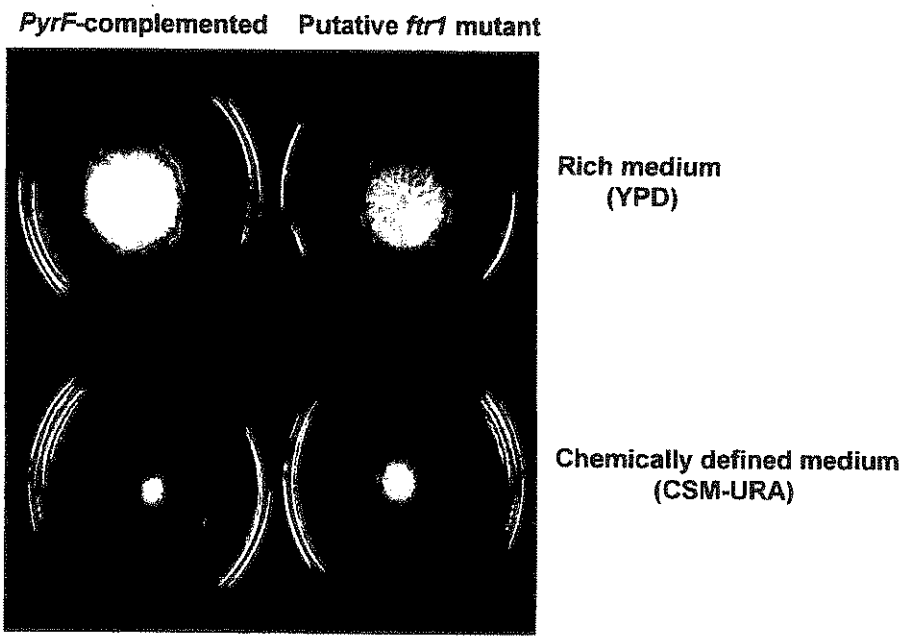

PyrF-complemented
Putative *ftr1* mutant
Rich medium
(YPD)
Chemically defined medium
(CSM-URA)

Figure 26A

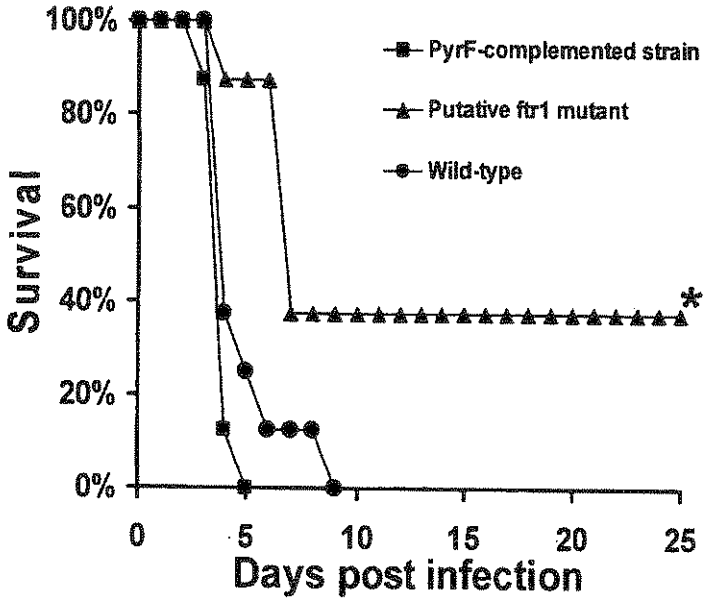


Figure 26B

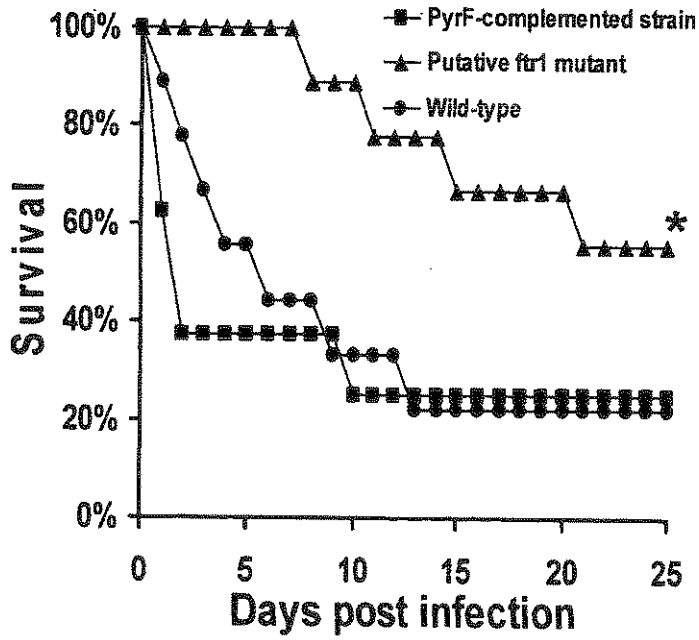


Figure 26C

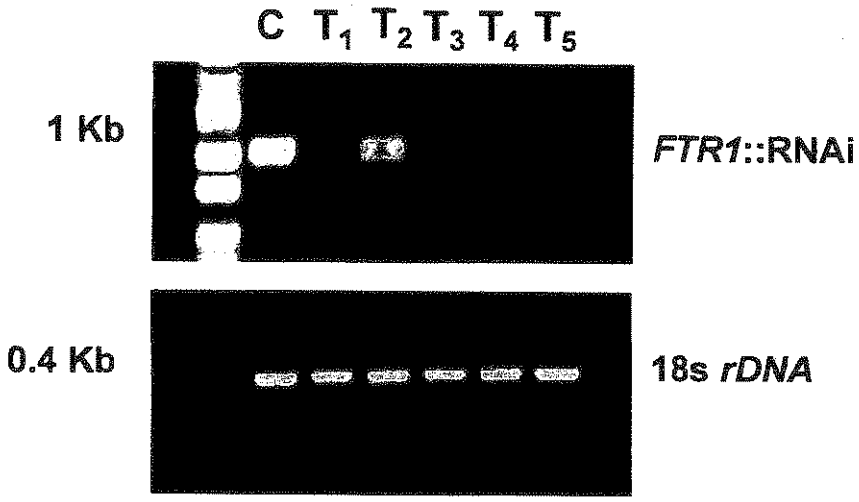


Figure 27A

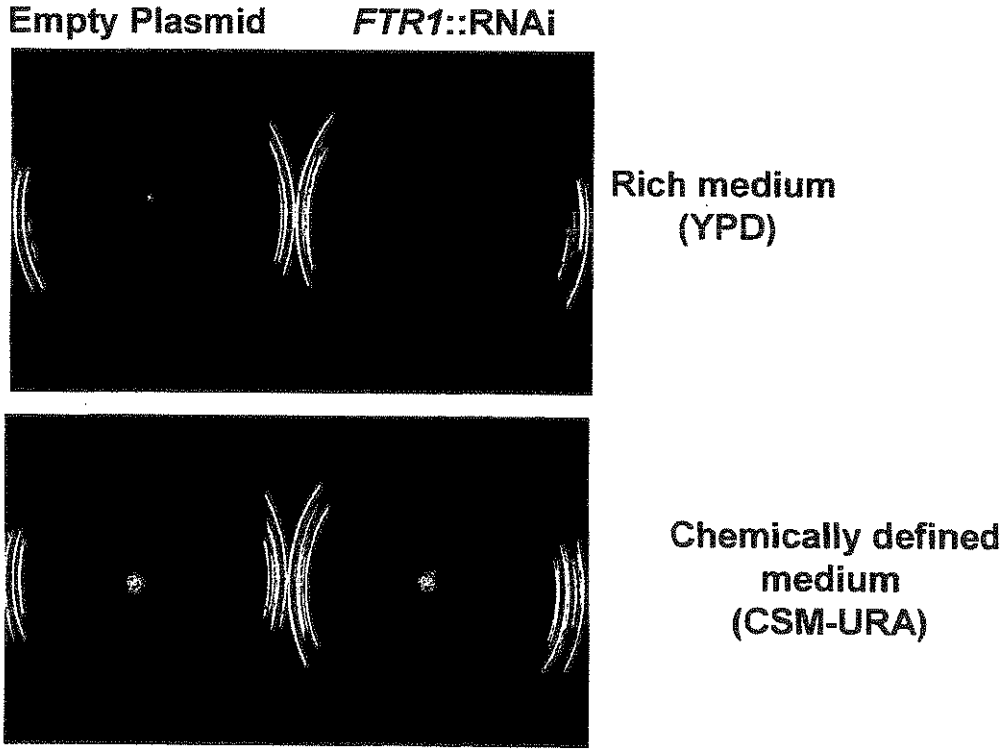


Figure 27B

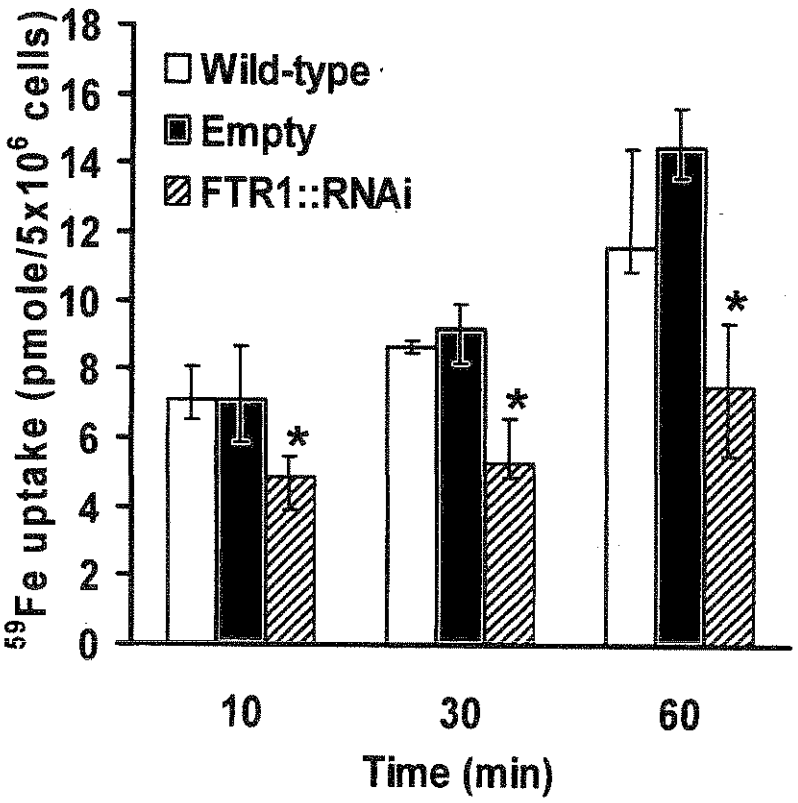

^{59}Fe uptake (pmole/5x10^6 cells)
18
16
14
12
10
8
6
4
2
0
Wild-type
Empty
FTR1::RNAi
*
*
*
10
30
60
Time (min)

Figure 27C

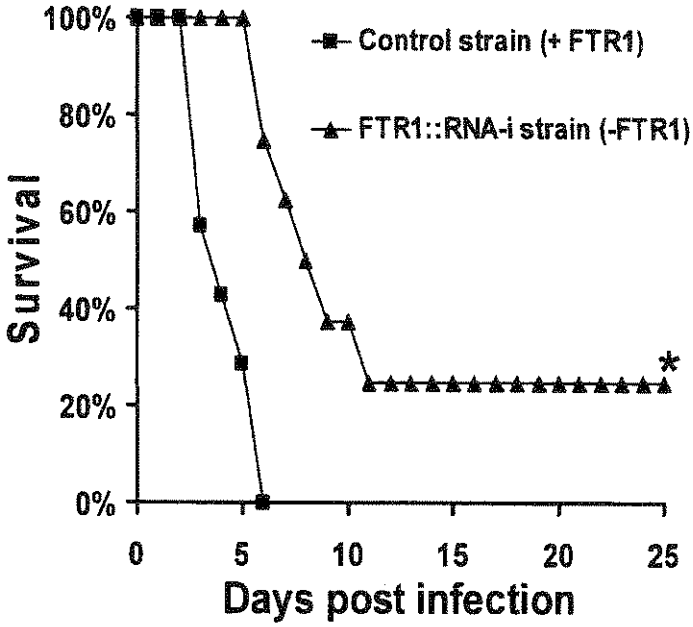


Figure 28A

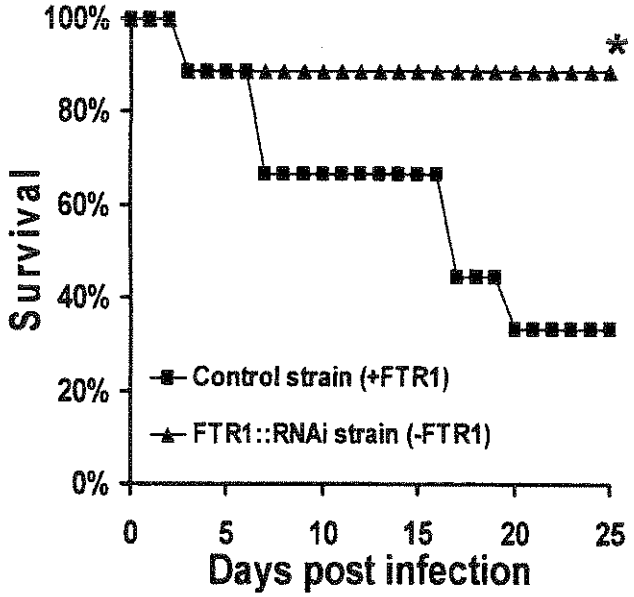


Figure 28B

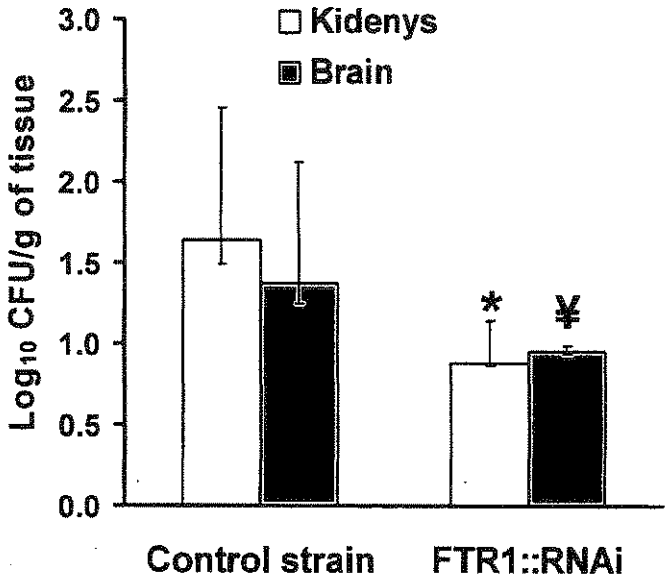


Figure 28C

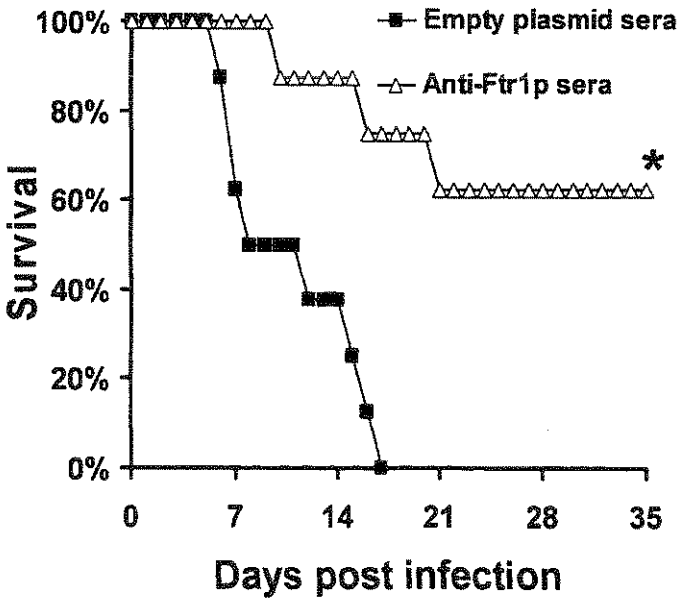


Figure 28D