WAGENINGEN UR
For quality of life

Postbus 16 | 6700 AA Wageningen

RIVM / SEC / Bureau GGO
Postbus 1
3720 BA Bilthoven

Geachte heer, mevrouw,

- De risicoanalyse voor toegestuurde wijziging van IG 02-127/12.vr.1 volgens bijlage 5: De transposase en de repeats liggen inderdaad op één plasmide. De risicoanalyse volgens bijlage 5 wordt 5.2.f. → **ML-II.**

- De uitbreiding van plasmiden met vectoren: pUTgfp, is van toepassing onder art. 2 lid 2.
Aanvullende opmerkingen:

- De uitbreiding met vector pUTgfp (zonder 2x ,dit is onze interne notatie)) is de laatst vermelde naam in uw brief correct.
- De uitbreiding vector pROBE-AT was reeds in IG 02-127 onder art. 2 lid 2 opgenomen en deze aanvraag komt dus te vervallen.
- Van vector pRL765 (pRL76511p en pRL765rfp) en pGFP zijn de plasmide kaartjes reeds toegestuurd maar als bijlage nog een keer toegevoegd (bijlage 1)
- De gfp (de P11 red shifted mutant van GFP komt eenmaal voor op de betreffende plasmiden.

Additioneel is het verzoek om de volgende kleine wijzigingen in IG 02 127 op te nemen. Het betreft de uitbreiding van gastheren is onder artikel 2 lid 2.
De hierna genoemde stammen zijn/ worden gemerkt met GFP en worden gebruikt in plant kolonisatie experimenten met als doel, ecologisch onderzoek naar gedrag van bacteriën (endofyten) in planten.

Bacteriën volgens klasse 1 (bijlage 1) toevoegen aan art.2 lid 1,5,8 en 9:

- *Pseudomonas fluorescens;*
- *Bacillus licheniformis , T369, T399 ;*
- *Bacillus subtilis;*
- *Bacillus amyloliquefaciens .*
- *Pseudomonas putida KT2440 with phl gene cluster*)*
- *Pseudomonas putida KT2440 with methionine gamma lyase*)*
- *Pseudomonas putida KT2440 with overexpression of pfrI*)*
- *Pseudomonas putida KT2440 with overexpression of chitines*)*

) Plasmid kaartjes en sequences in **bijlage 2.*

*Risicoanalyse volgens voorgestelde inschaling conform 5.2.f. → **ML-I***

Plant Sciences Group

DATUM
28 augustus 2014

ONDERWERP
Beantwoording vragen en uitbreiding van activiteiten

UW KENMERK
IG 02-127/12.vr.1

ONS KENMERK
14/PSG0110

POSTADRES
Postbus 16
6700 AA Wageningen

BEZOEKADRES
Wageningen Campus
Gebouw 107
Droevendaalsesteeg 1
6708 PB Wageningen

INTERNET
www.wageningenUR.nl

CONTACTPERSOON
Dhr. ing R (Reinoud) Bouwer

TELEFOON
+31317480975

FAX
+031317418094

E-MAIL
reinoud.bouwer@wur.nl

Bacteriën volgens klasse 2 , toevoegen aan art.2 lid 2,6, 7, 6, 9:
Agrobacterium tumefaciens ;
Bacillus megaterium;
Bacilus pumilus ;
Bacillus cereus;
Microbacterium testaceum;
Paenibacillus polimyxa;
Pantoea ananatis;
Pseudomonas jessenii;
Staphylococcus epidermis.

Risicoanalyse volgens voorgestelde inschaling conform 5.3.i. → ***ML-II***

Uitbreiding activiteiten m.b.t. het zogenaamde iGEM banana project:

Bijlage 3 Protocol / experimentbeschrijving met voorgestelde/ ongewijzigde inperkingsniveaus.

Uitbreiding van gastheersoort:
Musa spp / Musa sativa (banana) art. 2 lid 9 inschaling ongewijzigd.

Musa spp / Musa sativa (banana) art. 2 lid 14 inschaling ongewijzigd.
Risicoanalyse Conform 5.5.2.c. → PKM-III

Digitale verwijzingen en toelichting op de verwijzing:
The first link is a link to the article that was published for the SEVA plasmids.
1: http://nar.oxfordjournals.org/content/early/2012/11/22/nar.gks1119.full
The second link refers to the website of the SEVA plasmids with a list of all the SEVA plasmids available in their lab. SEVA 254 is however not in their list because it was one I made myself using the SEVA plasmids that they had, I interchanged some of the parts of the SEVA plasmids giving me a plasmid that has:
-Kanamycin resistance
-RSF1010 ori
-lacIq-Ptrc (IPTG inducible promoter coupled with lacIq) lacIq binds to the promoter and inhibits it when IPTG is not around.
2: http://seva.cnb.csic.es/SEVA/Welcome.html

Met vriendelijke groet,

Dhr. ing. R. (Reinoud) Bouwer
Bioveiligheidsfunctionaris Wageningen UR / Plant Sciences Group –Stichting DLO (PRI/PPO)

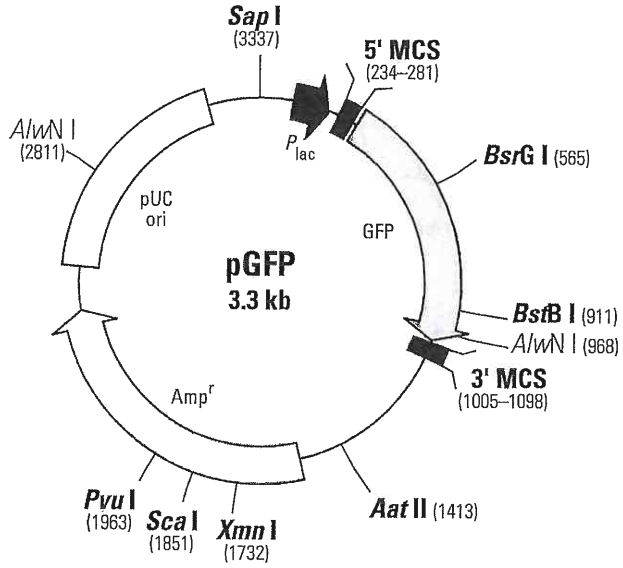
cc. dhr. J. van de Wolf (VM van IG 02-127).

Bilaga 1
(J6 02-127)

Vector Information

pGFP Vector Information PT2039-5

GenBank Accession No.: U17997 Cat. No. 632370



5' MCS
LacZ
ATG ACCATGATTACGCCAAGCTTGCATGCCTGCAGGTCGACTCTAGA
Hind III Sph I Pst I Sal I Xba I
GFP
GGATCCCCGGGTACCGGTAGAAAAA ATG AGT
BamH I Xma I Sma I Kpn I Age I Asp718 I

3' MCS
GFP
stop
TAG CATTCGTAGAATTCCAACTGAGCGCCGGTCGCTACCATTACCAA
Bsm I EcoR I
CTTGTCTGGTGTCAAAAATAATAGGCCTACTAGTCGGCCGTACGGGCCC
Stu I Spe I Eag I BsiW I Bsp120 I Apa I

Restriction Map and Multiple Cloning Site (MCS) of pGFP. Unique restriction sites are in bold.

Description:

pGFP carries the complete GFP coding sequence derived from the GFP cDNA by PCR (1, 2). This PCR product was cloned between the two MCSs of the pUC19 derivative pPD16.43 (2, 5). The 5' MCS lies immediately upstream from the GFP start codon; the 3' MCS lies downstream from the GFP stop codon. The GFP gene was inserted in frame with the *lac*Z initiation codon from pUC19 so that in *E. coli,* GFP is expressed from the *lac* promoter as a fusion with several additional amino acids, including the the first five amino acids of the *lacZ* protein. Note, however, that if you excise the GFP coding sequence using a restriction site in the 5' MCS, the resulting fragment will encode the native (i.e., non-fusion) GFP protein. The pUC19 backbone of pGFP provides a high copy number origin of replication and ampicillin resistance gene for propagation in *E. coli* .

GFP excitation maxima = 395 nm, and the emission maxima = 509 nm.

(032113)

Clontech

United States/Canada
800.662.2566
Asia Pacific
+1.650.919.7300
Europe
+33.(0)1.3904.6880
Japan
+81.(0)77.543.6116

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

Location of features:

- lac promoter: 95–178
  CAP binding site: 111–124
  –35 region: 143–148; –10 region: 167–172
  Transcription start point: 179
  lac operator: 179–199
- lacZ–green fluorescent protein (GFP) fusion protein expressed in E. coli
  Ribosome binding site: 206–209
  Start codon (ATG): 217–219; stop codon: 1003–1005
- 5' MCS: 234–281
- Green fluorescent protein gene
  Start codon (ATG): 289–291; stop codon: 1003–1005
  GFP fluorescent chromophore: 481–489
- 3' MCS: 1005–1098
- Ampicillin resistance gene
  Promoter: –35 region: 1474–1479; –10 region: 1497–1502
  Transcription start point: 1509
  Ribosome binding site: 1532–1536
  β-lactamase coding sequences:
  Start codon (ATG): 1544–1546; stop codon: 2402–2404
  β-lactamase signal peptide: 1544–1612
  β-lactamase mature protein: 1613–2401
- pUC plasmid replication origin: 2552–3195

Primer location:

- GFP-N Sequencing Primer (#6476-1): 352–331
- GFP-C Sequencing Primer (#6477-1): 942–964

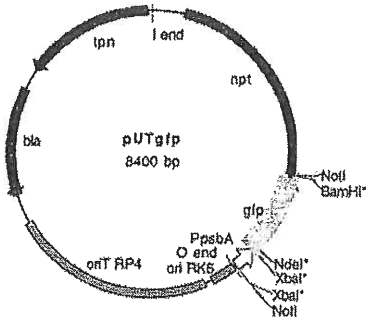
Propagation in E. coli:

- Recommended host strain: JM109
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) to E. coli hosts
- E. coli replication origin: pUC
- Copy number: ≈500
- Plasmid incompatibility group: pMB1/ColE1

References:

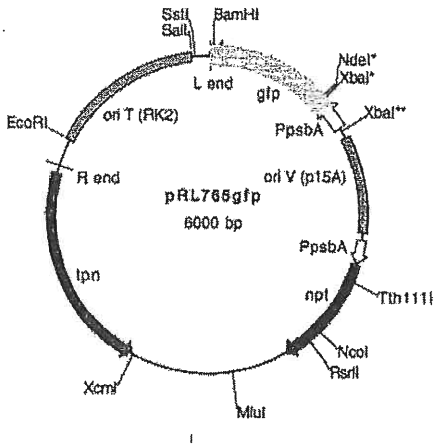
1. Prasher, D. C., et al. (1992) Gene 111:229–233.
2. Chalfie, M., et al. (1994) Science 263:802–805.
3. Inouye, S. & Tsuji, F. I. (1994) FEBS Letters 341:277–280.
4. Wang, S. & Hazelrigg, T. (1994) Nature 369:400–403.
5. Fire, A., et al. (1990) Gene 93:189–198.

pUTgfp2x



mini-Tn5 trasposon, expressing kanamycin resistance and GFP protein.
tnp gene, which is present *in trans* outside the mini-Tn5 element (Elbeltagy *et al.* 2001).
The Tn-5 transposon delivery system contains a PpsbA-RBS gfp cassette.
The *gfp* genes, plus the additional 35 bp region containing the RBS site, are located downstream the constitutive psbA promoter
GFP protein encoded is the red-shifted mutant P11
shift of the maximum excitation wavelength from 396 to 471 nm (emission: 502 compared to 508 nm for wild-type GFP)

pRL76511p/pRL765rfp



The vector pRL76511p is very similar to the pUTgfp. (RFP ex 543 nm ; em 560-600 nm)
tnp gene is inside the mini-Tn5 element (Tombolini *et al.* 1997)

Host: *E. coli* S17.1 λ pir

Ref:

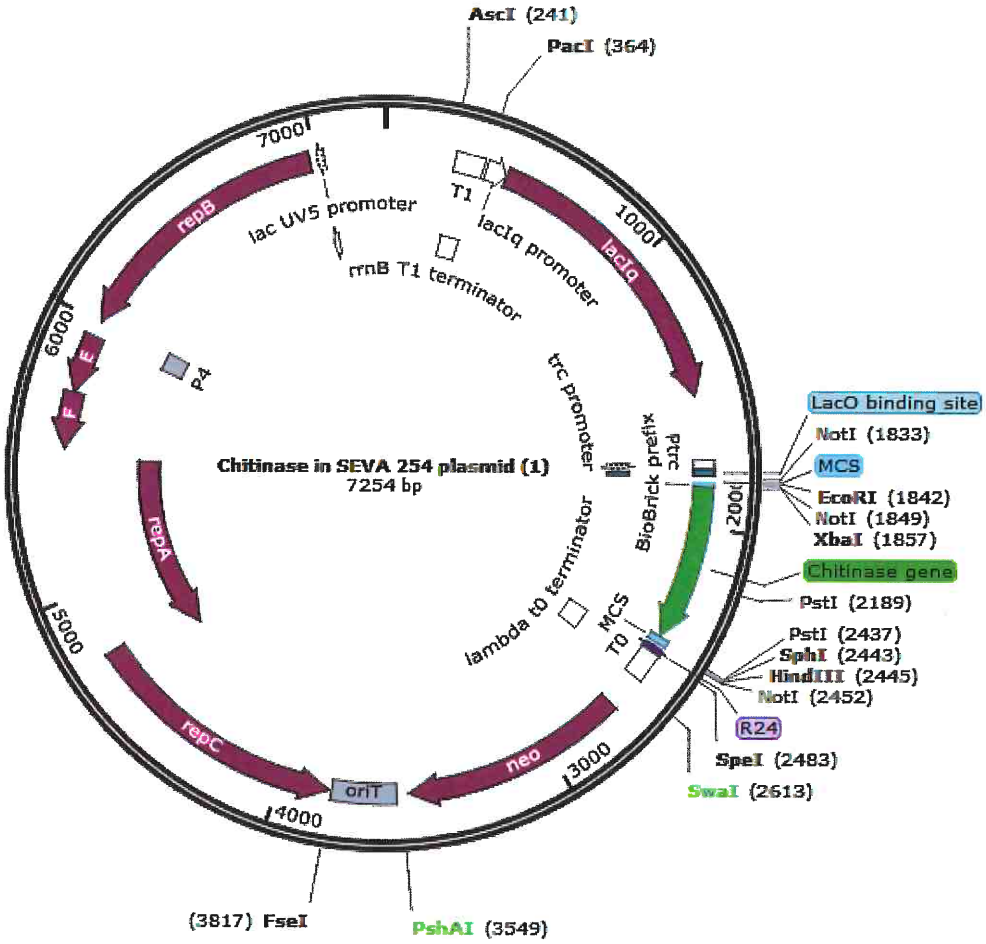
Endophyte Watching: combining molecular and microscopy approaches to isolate, identify, tag, and monitor fungi and bacteria inside plants. Tondello PhD Thesis

TONDELLO A., BALDAN B. SQUARTINI A. Co-localizing symbiont and endophytic bacteria in legumes by tagging with different fluorescent proteins . Annals of Microbiology, 59, Special Issue (2009) 29

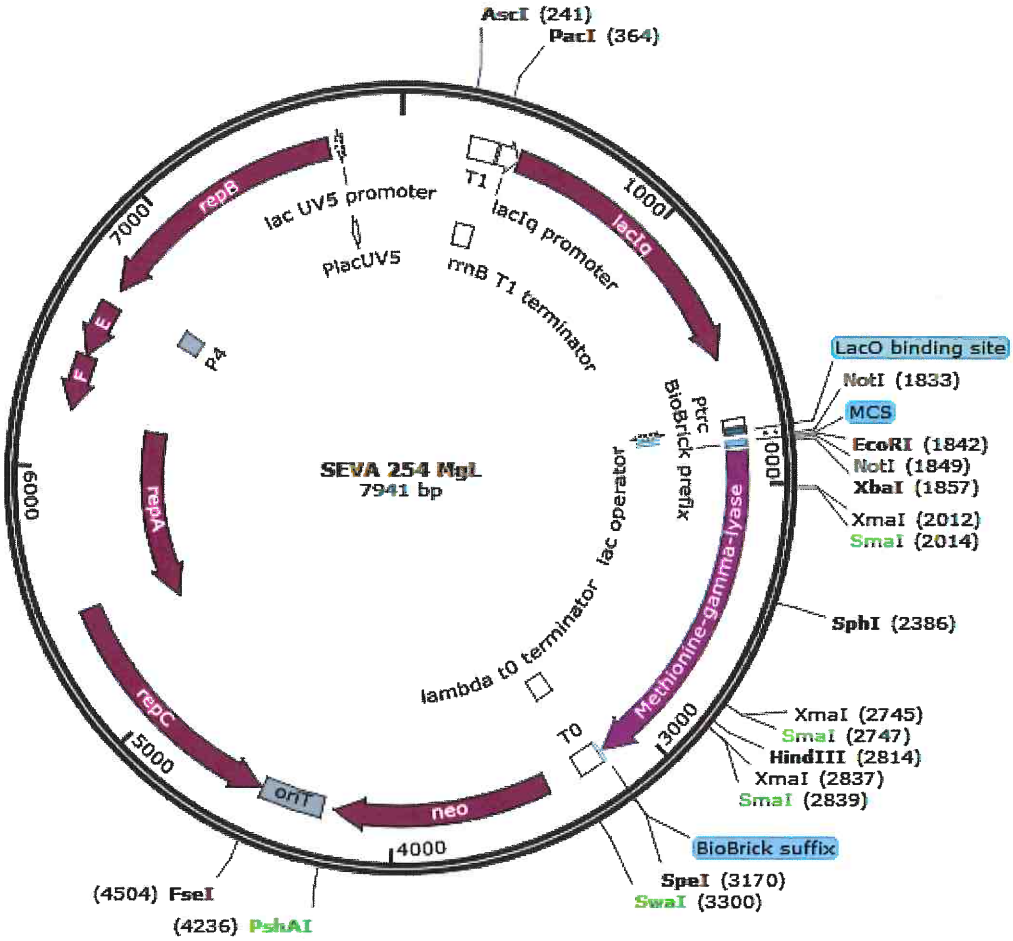
Tombolini R., Unge A., Davey M.E., de Bruijn F. and Jansson J.K. (1997). Flow cytometric and microscopic analysis of GFP-tagged *Pseudomonas fuorescens* bacteria. FEMS Microbiol Ecol, 22:17-28.

Appendix B / Bilaga 2
(GG 02-127)

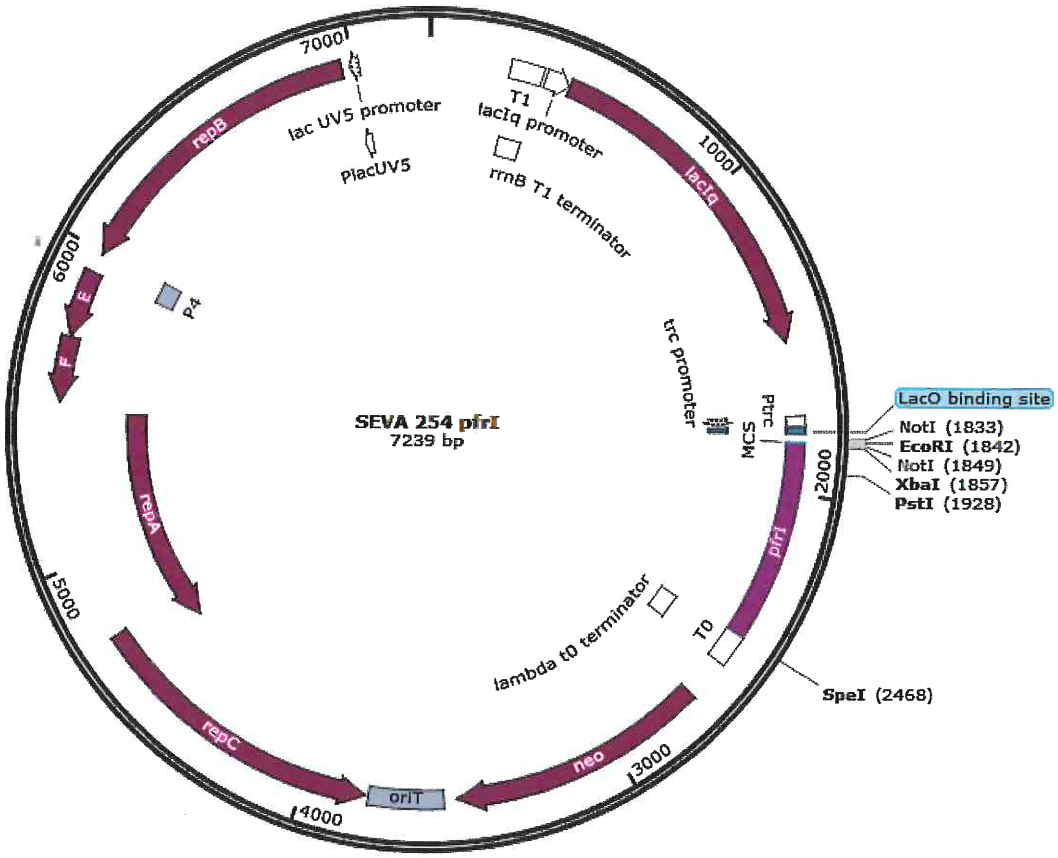
Chitinase



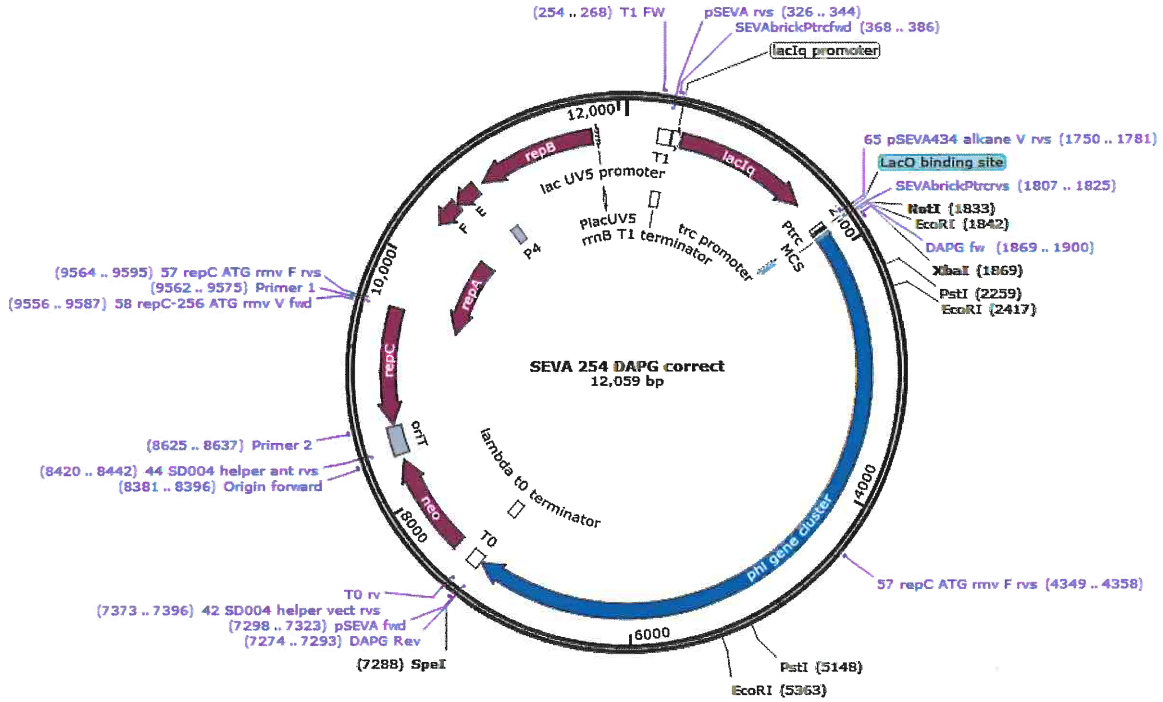
MgL


AscI (241)
PacI (364)
1000
lacIq
lacIq promoter
T1
rrnB T1 terminator
PlacUV5
lac UV5 promoter
7000
repB
E
F
P4
6000
repA
repC
5000
oriT
neo
4000
(4504) FseI
(4236) PshAI
SEVA 254 MgL
7941 bp
lambda t0 terminator
lac operator
BioBrick prefix
Ptrc
1000
LacO binding site
NotI (1833)
MCS
EcoRI (1842)
NotI (1849)
XbaI (1857)
XmaI (2012)
SmaI (2014)
SphI (2386)
Methionine-gamma-lyase
3000
T0
XmaI (2745)
SmaI (2747)
HindIII (2814)
XmaI (2837)
SmaI (2839)
BioBrick suffix
SpeI (3170)
SwaI (3300)

PfrI


7000
lac UV5 promoter
PlacUV5
T1
lacIq promoter
rrnB T1 terminator
1000
lacIq
repB
6000
E
F
P4
repA
5000
repC
oriT
4000
neo
3000
lambda t0 terminator
T0
pfrI
2000
trc promoter
Ptrc
MCS
LacO binding site
NotI (1833)
EcoRI (1842)
NotI (1849)
XbaI (1857)
PstI (1928)
SpeI (2468)
SEVA 254 pfrI
7239 bp

DAPG


(254 .. 268) T1 FW
pSEVA rvs (326 .. 344)
SEVAbrickPtrcfwd (368 .. 386)
lacIq promoter
12,000
repB
T1
lacIq
65 pSEVA434 alkane V rvs (1750 .. 1781)
LacO binding site
SEVAbrickPtrcrvs (1807 .. 1825)
NotI (1833)
EcoRI (1842)
DAPG fw (1869 .. 1900)
XbaI (1869)
PstI (2259)
EcoRI (2417)
lac UV5 promoter
PlacUV5
rrnB T1 terminator
trc promoter
Ptrc
MCS
2,000
u
t
P4
repA
(9564 .. 9595) 57 repC ATG rmv F rvs
(9562 .. 9575) Primer 1
(9556 .. 9587) 58 repC-256 ATG rmv V fwd
10,000
repC
SEVA 254 DAPG correct
12,059 bp
oriT
(8625 .. 8637) Primer 2
(8420 .. 8442) 44 SD004 helper ant rvs
(8381 .. 8396) Origin forward
lambda t0 terminator
4000
8000
neo
T0
phl gene cluster
57 repC ATG rmv F rvs (4349 .. 4358)
T0 rv
(7373 .. 7396) 42 SD004 helper vect rvs
(7298 .. 7323) pSEVA fwd
(7274 .. 7293) DAPG Rev
(7288) SpeI
6000
PstI (5148)
EcoRI (5363)

Fernando Garcia Bastidas (WUR-PRI), Wen Wu (WUR-SSB), and Marlene Birk (WUR-SSB)

Introduction

In the iGEM program at Wageningen University and Research Center genetically modified *Pseudomonas putida* are evaluated for their inhibitory effects on wildtype *Fusarium oxysporum* f.sp *cubense* (Foc). Four fungal growth inhibitors (DAPG, DMDS, Siderophores and Chitinase) will be expressed by the organism. The genetically modified biological control agent will be tested for its functionality by adding the transformed *Pseudomonas* to the soil of pot grown banana plants, inoculated with Wild type *Fusarium oxysporum*. Additionally, beneficial or adverse effects of the active compound on the banana plant will be investigated.

All experiments concerning genetically modified organisms are conducted at the Wageningen UR premises and under strict quarantine conditions (**ML-I, ML-II lab or PKM-III** greenhouse).

Materials & Methodology

Materials

- Wild type of *Fusarium oxysporum* f.sp *cubense* II5 (TR4)
- Wild type *Pseudomonas putida KT2440*

1. *Gm Pseudomonas putida KT2440 with phl gene cluster*
2. *Gm Pseudomonas putida* KT2440 with methionine-gamma-lyase
3. *Gm Pseudomonas putida* KT2440 with overexpression of pfrI
4. *Gm Pseudomonas putida* KT2440 with overexpression of chitinase

(Sequences can be found in Appendix and Plasmid map in Appendix A+B (Bijlage 2))

Additional descriptions of the gm strains of *P. putida:*

1. Pseudomonas putida KT2440 with phl gene cluster

2,4-DAPG or 2,4-Diacetylphloroglucinol is a widely known antibiotic that has antifungal activities. It is often used against plant pathogens.

This P. putida will contain a plasmid containing phlACBDE gene clusters obtained from Pseudomonas protgens pf-5 (also a soil bacteria). PhlABCD genes are found to be synthesis genes needed for 2,4-DAPG synthesis and phlE is found to be an efflux pump for 2,4-DAPG. These genes are found behind a IPTG inducible promoter (Ptrc). Also on the plasmid is a lacIq which suppresses the promoter when no IPTG is present. The plasmid that it is is known as SEVA 254 with kanamycin resistance and RSF1010 ori.

Plasmids were cloned using E.coli Dh5-α, a popular E.coli strain that is often used for plasmid cloning. Genes were first obtained by PCR and then digested and ligated into SEVA 254.

2. Pseudomonas putida KT2440 with methionine-gamma-lyase

This *P. putida* will contain the SEVA 254 plasmid harbouring a methionine-gamma-lyase gene from Brevibacterium linens, a micro-organism that is found in cheese. This gene will be synthetically made and will be codon optimized in order to work better in *P. putida* KT2440. The reason for this gene is due to the fact that it increase the production of DMDS and DMTS. Where DMDS has shown to promote plant growth and DMTS has up to 80% inhibition of *Fusarium oxysporum cubense*.

3. Pseudomonas putida KT2440 with overexpression of pfrI

This *P. putida* KT2440 will contain the SEVA 254 plasmid harbouring a pfrI gene. It will be an overexpression of pfrI because *P. putida* KT2440 already contains this gene and it was obtained from KT2440 itself. This gene is

overexpressed in order to increase the pyoverdine production of *P. putida*. Pyoverdine binds to iron in the soil leading to iron competition and pfrI was found to be a transcription factor for the pyoverdine synthesis genes.

4. Pseudomonas putida KT2440 with overexpression of chitinase

This *P. putida* KT2440 will contain the **SEVA 254 plasmid** harbouring a chitinase gene obtained from *P. putida* KT2440. This gene is used in order to overexpress chitinase so that it van break down fungal cell walls. To increase its ability of being a bio control.

The transgenic *Pseudomonas putida* isolates are stored in **ML-I** labs, at the Wageningen University and Research Center department of Systems and synthetic biology (SSB) according to the regulations. Transport of transgenic *Pseudomonas putida* will be conducted in in petri-plates , which are sealed with parafilm and placed in a double plastic bag in a plastic box container.

The Foc isolates are stored in **ML-II** labs at the Wageningen University and Research center Business unit Biointeractions & Plant Health according to the regulations. Transport of Foc is conducted in Petri-plates, which are sealed with parafilm.

Media

Foc strains are grown on sterilized maize kernels. *Pseudomonas putida* will be grown in LB medium containing IPTG in order to induce gene expression.

Methods

Five different organisms will be tested for their functionality as a biological control agent. Each organism will produce a different active compound and one will produce all four active compounds. Degree of wilt and fungal biomass of plants inoculated with *Fusarium* and the different biological control agents will be determined. Non-inoculated plants and plants solely inoculated with the biological control agent but not with *Fusarium* will serve as control. Plants treated with the wild type *Pseudomonas putida* strain will serve as control to confirm that the effects are based on the new or over expressed active compounds.
Possible positive or negative effects on growth will be tested by determining the above ground biomass of the banana plant after 4 weeks. Accumulation within the plant will be tested using plants treated with the biological control agent producing all four growth inhibitors. Non-inoculated plants will serve as control. Three plants will be used per treatment. Number of plants and the general set up of the experiment can be found below.

Pseudomonas putida strain / Plant treatment	Producing Chitinase	Producing DMDS	Producing Siderophores	Producing DAPG	Producing all growth inhibitors	Water (Control)	Wild Type
Inoculated	3	3	3	3	3	3	3
Mock	3	3	3	3	3+3*	3+3*	3

*Plants for accumulation experiment

- **GM *Pseudomonas putida* inoculum preparation**

- Erlenmeyer flasks are labelled with code for organism, GMO number, Construct, Name of researcher, and the date of inoculation.

- Petri dishes containing transformed *Pseudomonas putida* strain(s), sealed with parafilm are taken from the 4°C refrigerator at the SSB **ML- I** lab and placed in a double plastic bag in a plastic container (triple containment principle). The container will be transported to the down-flow hood at the Biointeractions & Plant Health department.
- Petri dishes containing transformed *Pseudomonas putida* strain(s) are opened under the down-flow hood and one colony of cells is taken out using a disposable pipet tip, and transferred in Erlenmeyer flasks with 10 ml medium.
- All used disposable utensils and the used petri dishes are placed in plastic waste bag in **BSC class II** down-flow hood after use.
- Inoculated Erlenmeyer flasks are transferred to a rotary shaker for incubation at 30°C overnight.
- Waste materials including biological waste and disposable utensils are placed in garbage container for autoclaving.
- At the day of inoculation, the flasks will be placed in double contained plastic boxes and blue containers with lid for transgenic strains and then transferred to the Wageningen UR **PKM-III** facilities for inoculations using an air-wheel trolley in this contained compartment.

- ***Pseudomonas putida* inoculation**

- Bioassays will be conducted in the Wageningen UR **PKM-III** facilities.
- All individuals involved in activities during inoculation will use disposable clothing (coats, gloves, hairnets, shoe covers) that after completion of the activities will be discarded in double red plastic bags for autoclaving.
- Inoculum, pots, sand, dishes, cutlery, containers, trays, will be brought into the facility.
- Two to four months old banana plants will be taken from greenhouse.
- The pots are placed on dishes on plastic in tabletops. The plastic will be removed for autoclaving after the experiment and the table tops will be disinfected by greenhouse personnel.
- Banana plants will be uprooted and their roots will be cut at 2/3 or 1/3 lengths outside the greenhouse space where the inoculum is placed.
- The plants will be transferred to the pots, placed on the layer of sand and potted with additional sterile sand.
- The inoculum will be added to the soil by using syringes close to the roots to avoid splashing.
- The disposable contaminated materials (including coats etc.) will be discarded in red plastic bags for autoclaving.

- **Foc inoculum preparation**

- Inoculation with Foc will be done 3 days after inoculation with gm and Wild type *Pseudomonas putida*.
- Petri dish(es) with Foc strain are taken from incubator in **ML-II** lab to down-flow hood.
- Petri dish with Foc is opened and 5-6 plugs are taken from the Petri plate, using a pipet tip, and transferred in Erlenmeyer flasks containing the sterilized maize kernels.
- Petri dish is closed, sealed and returned to incubator or placed in plastic waste bag in down-flow hood.
- All used disposable utensils are after use paced in plastic waste bag in down-flow hood.
- Inoculated Erlenmeyer flasks are transferred to an incubator at 25°C for 5 days in darkness.
- Plastic bag with waste materials (biological and utensils) is placed in garbage container for autoclaving.
- On the day of inoculation the inoculated maize kernels in Erlenmeyer flasks will be placed in double contained plastic boxes and be transferred to the Wageningen UR **PKM-III** facilities for inoculations using an air-wheel trolley.

- **Foc inoculation**

- Bioassays will be conducted in the Wageningen UR **PKM-III** facilities.
- All individuals involved in activities during inoculation will use disposable clothing (coats, gloves, hairnets, shoe covers) that after completion of the activities will be discarded in double red plastic bags for autoclaving.
- With disposable sterile forceps 5-7 maize kernels colonized with FOC will be transferred from the MKM to the double pots. Through force will the kernels be placed in the soil close to the roots.
- The disposable contaminated materials (including coats etc.) will be discarded in red plastic bags for autoclaving.

- **Plant maintenance and scoring**

- All individuals involved in activities during plant maintenance and scoring will use disposable clothing (coats, gloves, hairnets, shoe covers), which after completion of activities will be discarded in double red plastic bags for autoclaving.
- All plants will be watered at daily intervals until scoring.
- All plants will be observed regarding disease progress using a wilting scale.
- Internal symptoms will be scored using Assess 2.0. Each plant will be lifted in double red plastic bags. The plastic pot will be removed, the adherent soil will be shaken off and the corm of the plants will be cut vertical with a knife and transported in a double plastic bag in a plastic box container to the Wageningen UR ML-II facilities for evaluation.
- The foliage (upper part) will be placed on a table covered with red plastic, and it will be dissected using a knife to be weighted. The scale will be placed on a table covered with red plastic. The foliage will be placed in a disposable dish before placed on the scale.
- Remaining plant parts will be put in a double red plastic bag along with the disposable utensils and table covers for autoclaving.
- The pots , soil, stems and foliage's will be discarded in double red plastic bags and inactivated by autoclaving.
- Used hardware/tools will be placed in a double plastic bag in a plastic box container and transported to the **ML-II** facility for autoclaving.
- The red plastic bags will be autoclaved and surface of the working space will be cleaned with 70% ethanol
- The water used for root washing will be autoclaved after use.
- The disposable contaminated materials (including coats etc.) will be discarded in red plastic bags for autoclaving.

Appendix A: Sequences

DAPG gene cluster, phlABCDE (*Pseudomonas protegens*)

TGGAATCAAGAGGAAAATGAACGTGAAAAAGATAGGTATTGTCAGCTatggcgcgggtattccggtatgccgcctgaaagtccaggaag
tgatcaacgtctggaaaaacaccgacctcaagctggtggaggaaaacctcggcgtcacggaaagagccgtgctgcaaccggatgaagatgtcatcaccctcgg
ggtgctggcggcgcaacgggccctggataaagtccccggtcatcagatcgaagccctgtacctgggcacctgcaccaatccctatgactcacgggcctcggcttc
gatcatcctggaaatgctcggcagcggctacgacgcctattgcgccgacgtgcagttcgccggcaagtccggaacttctgccctgcagatctgccaggcgctggt
ggcttccggcatgacgggcagcgccctggccatcggcgccgacaccatcaaccgcaacaccgcgccgggcgacctgaccgaatcctatgccggggccggagcc
gctgcgctgctgatcggcagccaggatgtcatcgccgaattcgacgccagcttctcctgcgccgccgatgtcgccgacaacatccgcccccagggcgatcgctac
attcgttcgggcatgggcctgggctcggacaagaacagcatcggcctggaagaccagacccgccgcgccgccgaaggcctgatggccaagctgcacaccagcc
ccgccgactatgactatgtggtgttccagcaaaacctggtgtccacgccctactccctggccaagcacctgggcttcaaccccaagcaggtggaaccgggcatct
atgccggcaacgttggcgatgccggatcggccagcccgctgctcggcctgatcaacgtactggaccaggcacgcccggggcagaaaattcttttggtgtcctacg
gtttcggcgccggcagcgacgccatcgccctgaccgtcaccgacgccatcgagcagtaccagaagcacaacaagcctctgcgcgaactgctggaatccaagat
ctacgtcgactacggcacgtccatcaagtacgagttcaagtacctgcgggctgactacgccctgaccgcctatctctgattacgcctgtctacgcaaggagcatgc
acatgtgcgcacgtcgtgttgcaatcgtttcggccgcctatacgccgaaacccggaagttcacgagttcggcagacgttcaaggaaatgatcgtcgagtccgcct
acaaggcactcaaggacgccaagatgcacccccgggaaatccaggccgtggcctatggttatcacggtgaaggcatttccgaatatggcggcctgggcccgac
catttccgacgccctgggcatcagcccggcgccgaccttcatgagcaccgccaactgcaccagcagctcggtgtcgttccagatgggccaccagatggtggcgtc
cggggagtacgacatcgtcctatgcggcggcttcgagaagatgaccgatcacttcaactacgccgaatacatcggctccagcactgaatgtgaatacgattattt
cctcggcatttcccacaccgatgccttcgccctggccaccgccgagtacttccagaagttcggctatgccggtcgcgaggccgacgtactggccaccttcggccgg
cagatgcgcatctacgcacagaacacccccaccgccacccgttatggccagccgattccatccctggaagtgttgaagaacagcgaagcctgcggctcgatgct
ggcctggggcgaagccagtggctgcgcgatcctggtggccgaacacctggcccacaagtacaccgataaaccggtgttcgtacgcggttgcgcctataccgggg
tctcccactatttcggtacgcgctttcacaacccgaccctgcatcatccgggcctgcccaaggatgtgggcatggcggtttcggccaactccatcgcctgtgcgga
gatcgcctacaagaaagccgggatcaccgccaaggacatcgacgtggcccaggtctacgacctgctcggcgccgggctgatccagatggagtccatgggcatc
tgtggcaagggccaggccggcgacttcgtgctcgaaggcggcatcgccctggatggccagctgccgctcaataccgacggcggcaacatcggccgcggccatg
cctccggctgcgacggcatcctgcacatcaccgagctgttccggcagctgaggggtgaatccgacaaccaggtcaagggcgcgcgcatcggcgtgtcgcagaa
ccttggcggttacgcggcgcacaactcagtgattgtcctctcgaacgactaaggagcccggaccatgtccatgtacccagaacagatccacagaatgaccaccg
ccagcatgcttcgcgaatggcgcgagcatggcggcaagtaccgcctcgaaggcagccagtgcgaagaatgcaacgaaatcttcttcccccggcgcaccgtctgc
ggcgcttgcaactccctgagtgtgaagccgtatcgctgcgcgcgcagtggcaagatcgaggtcatggccccggccgagaacccgatcctggccgccatgggcta
tggcgaaaccgtgccgcgcatcatggccatggtgcgcctggacgacggcctggtgatcgcttcggaaatcgtcgatgtgtgcgatcagcaacagctgaaagtcg
gtgcgccggtgcgcatggtgatccgcaagcatgtgcgcgaaagcaacctggcctggcaatacgcttataagttcgtactcgatatataagttgttattcaaggtgc
ctttatataaggcaccttgaagttagccattaaaataaaatccaacttaaaagttacatcaacggcaacttcacgccccgaagttgccttaaggccctgatttcctt
tagggcctacagattcctgtgcctataaacggggactatccccacccctaacttgttggcttacttaaagccaaggacccatcatgtctacactttgccttccaca
cgtcatgtttccgcaacacaagatcacccagcaacagatggtcgatcacctggaaaacctgcacgccgaccatccacgcatggccctggccaagcgcatgatcg
ccaacaccgaagtcaacgagcgccacctggtgttgccgatcgacgaactggcagtgcacaccggtttcacccaccgcagcatcgtctacgagcgtgaagcccgg
cagatgtcgtcggccgcggcgcgccaggccatcgagaatgccgggctgcagatcagcgacattcgcatggtgatcgtcacttcctgcaccggcttcatgatgccg
tcgctgaccgcgcacctgatcaacgacctggccctgccaacctccaccgtgcagttgccgatcgcccagctgggctgcgtggccggtgccgcggccatcaaccgc
gccaacgacttcgcccggctcgatgcccgcaaccacgtactgatcgtgtccctggaattctcctcgctgtgctaccagccggacgacaccaagctgcacgccttca
tctccgcggcgctgttcggcgatgcggtatccgcctgcgtgctgcgcgccgatgaccaggccggcggcttcaagatcaagaagaccgagtcgtacttcctgccca
agagcgagcactacatcaagtacgacgtgaaggacaccggctttcacttcaccctcgacaaggcggtgatgaactccatcaaggacgtggcaccggtcatgga
gcggctcaactacgagagcttcgaacagaactgtgcgcacaacgacttcttcatcttccacaccggtggtcgcaagatcctcgacgagctggtgatgcacctgga
cctggcatccaaccgggtctcgcaatcgcgcagcagcctgtcggaagccggcaacattgccagcgtggtggtgttcgacgtactcaagcggcagttcgattccaa
cctcaatcgcggcgacatcggcctgctggcagccttcggcccggggttcaccgcggaaatggcggtgggcgagtggaccgcctgatgtgactgcctgtgcctgtc
ccacctgacgttgccgggctgcatggcaacgtcgggtggcctcccacggcgcttgcgccgggttcatggtttcacaaggaagctgagcgatgaacactggaatg
ccaaccacggctgccaacagccgttacgaaagatccatggtgctgttgctgtcactgagtttcgggctggtggggctggatcgcttcatcatcctgccgctgtttcc
ggtgatcatgcacgacctgcaactggactatcaggacctgggctacctgtccgccgcgctggccttcacctgggggctgtccgccctgggcatgggcagcgtgat
cgaacgcctgggcacccgccgcgtgctggtgacctcgattgccgtgctttcgctactgtccggtttttccgcgctggccaccggcgtgctggggctggtgattctgc
gcgggctgatgggcatctgcgagggtgccttcacccccaccagcatcattgtcaccaatgaagtctcgcgccccgaccggcgcgggctgaacctgggtatccagc

aggcgctgttcccgattctcggcctgtgcctggggccgctgatcgccgcttacctcctgcaaatcactggctcgtggcgtgcggtgttcgccatcatttccctgccgg
gcctgctgctggccggctacctgtggaaaatctaccgaccgctgccggcgccccaggccgaccgcaccgcaggcacctggctggcggctttcaagagcggcaac
gtgagcctgaacatcctgatcatgttctgcatactcacctgccagttcgtcctctgcgccatgctgcccagctacctgaccgaccatatgcacctggaaaccctgtc
catggccttcgtcatctcggccatcggcgtgggcggcttcatcggccaattgatcatccccggcatgtccgaccgtctcgggcgcaagccggtggtgtcggtgtgc
ttcatgaccagcagcaccctggtgggcctgctgatcgtcagcccgccccttccctggctgctgttcctgctgctgttcctgctgtcgttcttcaacttcagcctgatct
gcatgacggtcggcccgctgaccagcgaatcggtcaccccggccctgctgccgacggcgaccggaatcgtggtggggttcggcgagatcctgggtggcgggtta
tcaccggccgtggccgggttcgccgccagccatttcggcctgccatcgatcctgtacgtggccctgagcggcagcctggccggcttgctgctgtcattcctgctcaa
ggaacaagccctcgaaccgcgccgttcgcgcgtgcgcgaagcgctgcccatcctccccgcccccttgcccctgaaggactag

Methyl-γ-lyase (original sequence)

>Brevibacterium linens L-methionine-gamma-lyase (mgl) gene, complete cds

ATGAGTATCACCCAGAACGGAATCTCGACCCGCTCTGTGCATTCGGGGGCCAACCCCGAATCGCATACCGGTTCGGTTGTT
GCCCCTATCTTCCAGACCTCGACCTTCATGATGGACACCCCGGGCCAGACCCGCGCCGGCTTCGACTACGCGCGCACCGGC
ACCCCGAACCGCAGCGACCTCGAAGACGTGCTGTGCGAGCTGGAGAACGCCTCCTTCGCAGCGGCGGTGAACTCGGGGA
CTTCCGCCGAGGTCGCGGTCTTCTCGGCACTGCTGGGTCCCGGCGATGAGATCATCATCCCGCGCGACATCTACGGCGGC
ACGTACCGGCTGCTGAAGAATGAATACGAGCGCTGGGGCATCAGCATCCGCACCGTCGATCTCACCGACACCGAGGCGCT
GGCGGCCGCGATCTCGGCCAAGACCGCGATCGTGTGGGTCGAGACTCCGAGCAATCCCGGGCTCGACATCGTCGACATC
GCCGAGACTGCCAAGCTCGCCCACGCCGCAAACGCCATCCTGGCCGTCGACTCCACCTTCGCCACCCCGATTCTGCAGCGC
CCCATCGAGCTCGGCGCCGATGTCGTCATCCACTCGACGACGAAGTTCATCAACGGCCACTCCGATGTCATCGGCGGTGCC
GTTCTCGCCGGTGACGGTTCGACCTGTCCCCGTGCCGCCAAGGTTGTCGAACGTCTCGAGTCCTACCTCGCATCCGTCGGG
TTGGGCATCGCCCCCTTCGATGCATGGCTGACTCGCCGCGGCATCAAAACCCTGCCGGTGCGGATGGCTAAGCACTGTGA
GAACGCGCAGGCCGTGGCGCAGTGGTTGGAGAGCCGTCCTGAGATCGCTGAGGTGTATTACCCAGGTCTGCCATCTCACC
CGGGTCATGAGGTCGCGAAGAAGCAGATGAGCGGCTTCGGGGGAGTGGTCTCATTCCGCACCGACACCGAGGCAAGAG
CTCTGTCGCTGGTGAAGAGCACGAAGCTCATCACTCTGGCCGAATCGCTCGGTGGTGTTGAGTCACTGATTGACCATCCTG
CGACCATGACCCACCTGGCCGTCGCAGACTGCGAACTCAGCGTCTCGCCGACATTCATCCGCCTGTCCCTGGGCATCGAAG
ACATCGCCGACATCCTCGCCGACCTCGACGCGGCGCTCGCTTCGACGGTGCAGACTCCGGACGGCGTGAGCTCCGACGCG
GAGGCACTGGGTGCGGAGGTTCCAATTGCGGACCCCTCACCGTCAACTCTTCAACACCCTGTAGCAACGGTATGAA

Methyl-γ-lyase codon optimized

ATGAGCATTACCCAGAACGGCATCTCCACCCGCAGCGTCCACTCGGGCGCGAATCCGGAGAGCCACACGGGTTCGGTCGT
GGCCCCGATTTTCCAGACCAGCACCTTTATGATGGATACCCCTGGCCAGACCCGGGCCGGCTTCGACTACGCCCGCACCGG
CACGCCGAACCGTTCCGACCTGGAAGACGTGCTGTGTGAGCTGGAAAACGCGAGCTTCGCCGCAGCCGTGAACTCGGGT
ACCTCCGCAGAAGTCGCGGTGTTCAGCGCCCTGCTCGGTCCAGGCGATGAGATTATCATTCCGCGCGATATCTACGGCGGT
ACGTATCGCTTGCTGAAGAACGAATACGAGCGTTGGGGCATCTCCATCCGTACCGTCGACCTGACCGACACCGAGGCCCT
CGCCGCCGCCATCAGCGCCAAGACCGCGATCGTGTGGGTCGAGACCCCATCGAATCCCGGTCTGGACATCGTAGACATCG
CGGAGACGGCAAAGCTGGCGCATGCCGCTAACGCTATCCTGGCTGTGGATTCGACCTTCGCCACGCCCATCTTGCAGCGC
CCAATTGAGCTGGGCGCAGACGTGGTGATCCACTCCACGACCAAGTTCATCAACGGTCACAGCGACGTGATCGGCGGCGC
GGTACTGGCCGGCGACGGCAGCACGTGTCCGCGCGCTGCCAAGGTGGTGGAGCGCCTCGAAAGCTACCTGGCTTCCGTG
GGCTTGGGCATTGCCCCGTTCGATGCCTGGTTGACGCGCCGCGGCATCAAGACCCTGCCGGTCCGTATGGCAAAGCATTG
CGAGAACGCCCAGGCGGTGGCTCAGTGGCTGGAGTCGCGCCCGGAGATTGCTGAAGTCTACTACCCGGGCCTGCCTTCCC
ATCCGGGCCACGAAGTCGCAAAGAAGCAAATGAGCGGGTTCGGCGGCGTGGTAAGCTTCCGGACCGACACGGAAGCCCG
GGCGTTGTCCCTGGTGAAAAGCACCAAGCTGATCACGCTGGCAGAGTCGCTGGGCGGCGTGGAATCCCTGATCGACCACC
CCGCCACCATGACGCATCTGGCCGTGGCAGATTGCGAGCTGAGCGTGTCCCCAACGTTCATCCGCCTGTCGTTGGGTATCG
AAGACATTGCAGACATTCTGGCCGACCTGGACGCTGCCCTGGCGTCGACCGTACAAACCCCGGACGGCGTGTCGTCCGAC
GCTGAGGCGCTGGGTGCCGAGGTGCCTATCGCTGACCCGAGCCCAAGCACCCTGCAACACCCCGTCGCGACCGTGTAATC
C

pfrI *P. putida* KT2400

>ENA|AAN69824|AAN69824.1 *Pseudomonas putida* KT2440 ferric regulator PfrI : Location:1..531
ATGGCGGAACAACTATCCACAAGTAAGTGCGATTCACCATTACTGCAGGCCTTCGTCGAC
AACCGCAGCATCCTGGTCAAGATCGCCGCCCGTATCACAGGCTGCCGTTCACGCGCTGAG
GACGTGGTACAGGATGCCTTTTTCCGGCTCAGCACCGCCCCGCAGATCACCTCATCGTTC
AAGGCCCAGCTGAGCTACCTGTTCCAGATCGTGCGCAACCTGGCGATCGACCACTATCGC
AAGCAGGCGATGGAGCTGAAGTACTCCGGTAGTGAAGAGGAAGGCATGAACGTGGTGGTGCAGAACGCCTCGCCCGAA
GCCACCCACATAAACCGCGCCGCGCTGAATGACATCGCGGAGGCCTTGAACGAACTGCCGCCGCGCACCCGCTATGCTTT
CGAGATGTACCGCCTTAATGGCGTGCCGCAGAAGGACATTGCCAAGGAACTGGGCGTATCGCCGACCTTGGTCAACTTCA
TGATCCGCGATGCGCTGGTGCATTGCCGCAAGACGGCCAGTCGCCAGGCCTGA

Chitinase (*P. putida*)

>ENA|AAN68674|AAN68674.1 *Pseudomonas putida* KT2440 pyocin R2_PP, lytic enzyme : Location:1..552
ATGGCTATCTCAGTTCAACAGCTGCAACAGATCCTCCCCAACGCCGGCCGCAAAGCCGGC
GTTTATGTTCCCGGACTCAACGCTACTATGGGGAAGTTCGCCATCATCACGCGCCTGCGT
ATGGCCGCGTTTCTCGCCCAGATCGGGCATGAGTCGGGTCAGTTGCAGTACGTGCGTGAG
CTTGGCAATGACAAGTACCTGTCGAAGTACGACACCGGCCGGCTGGCTGAGCGCCTGGGC
AACACGCCCGAAGCAGATGGCGATGGTCAGCTCTATCGTGGTCGTGGGCTTATCCAGGTG
ACGGGCCGCTTCAATTACGAGGCCTGCAGCGAGGCTCTGTTCGGTGACGGTCGCCTGCTC
AATACCCCAGAGCTGCTCGAGCATCCGGTCTACGCATCGATGTCCGCCGGCTGGTTCTGG
CAGAAGGAGGGCCTGAACAGCTTGGCCGACAAGGGCGACATCCTGGCGATCACCAAACGT
ATCAATGGTGGTAGCAACGGTCTGGAGGATCGCAAGGCCCTCTACAAGCGAGCGCTCGAGGTGCTGCAGTGA