

## Supplementary Information

Horizontal gene transfer of a bacterial insect toxin into the *Epichloë* fungal symbionts of grasses

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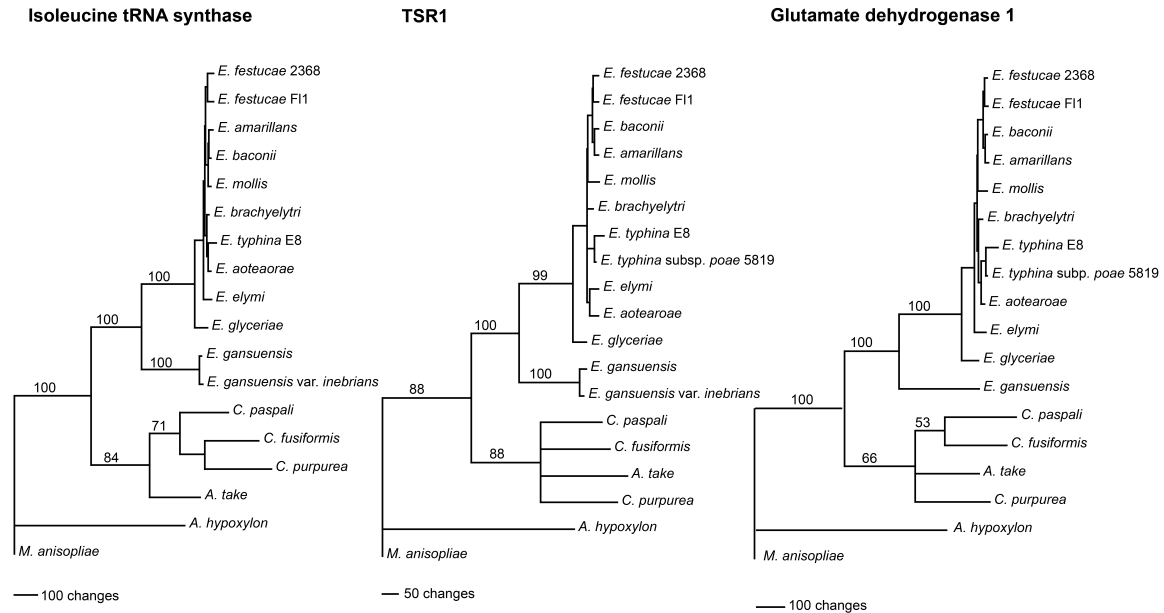
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New Brunswick, NJ 08901

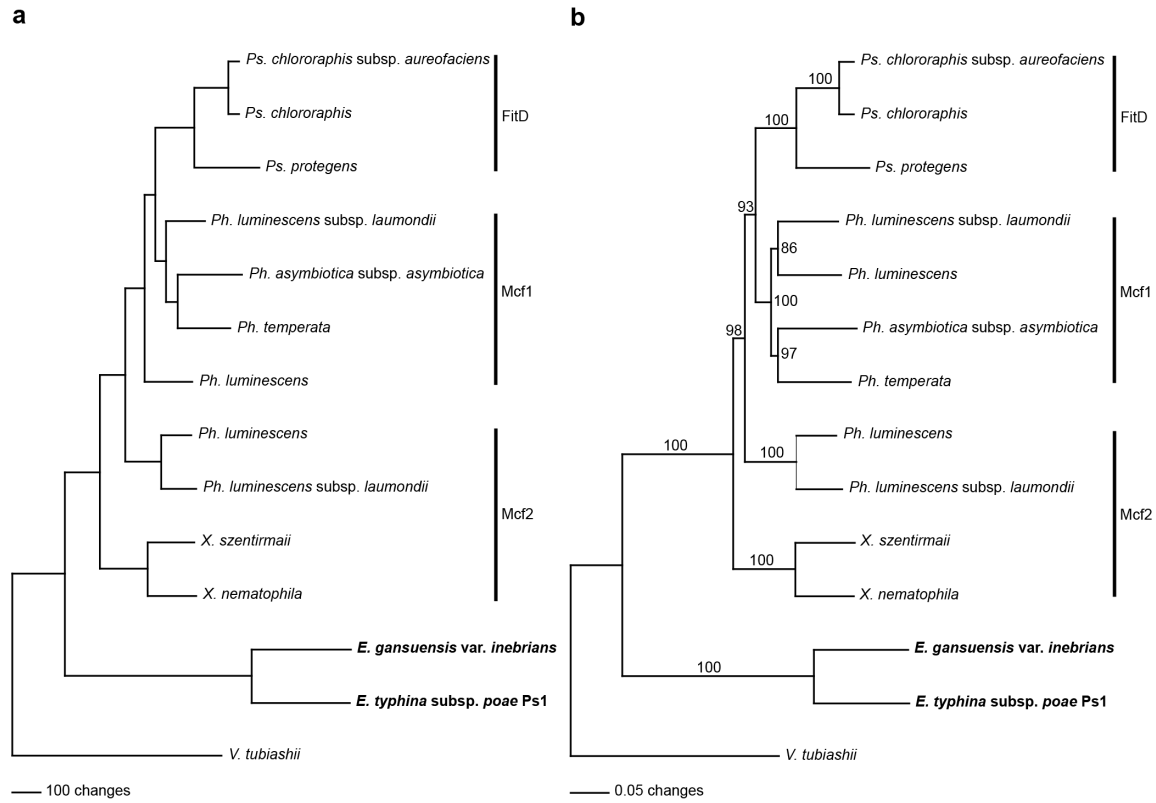
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## Supplementary Figures



**Supplementary Figure 1.** Rooted 50% majority rule maximum parsimony phylogenetic trees of the glutamate dehydrogenase, isoleucine tRNA synthase, and TSR1 DNA coding sequences. The *Metarhizium anisopliae* sequences were designated as the outgroup for rooting the trees. The numbers at the nodes are the bootstrap percentages based on 1,000 replications. The TSR1 tree was based upon 2,538 total characters, of which 1,433 were constant, 478 variable characters were parsimony uninformative, and 627 variable characters were parsimony informative. The unmarked nodes all had bootstrap support of 53 or higher. The glutamate dehydrogenase tree was based upon 3,225 total characters, of which 1,967 were constant, 493 variable characters were parsimony uninformative, and 765 variable characters were parsimony informative. The glutamate dehydrogenase sequence from *E. gansuensis* var. *inebrians* was not included, since the gene sequence was split between two contigs, which introduced a gap into the alignment. The unmarked nodes all had bootstrap support of 66 or higher. The isoleucine tRNA synthase tree was based upon 3,228 total characters, of which 1,884 were constant, 517 variable characters were parsimony uninformative, and 827 variable characters were parsimony informative. The isoleucine tRNA synthase gene from *E. typhina* subsp. *poae* 5819 was not included since the gene was split between two contigs, which introduced a gap into the alignment. The unmarked nodes all had bootstrap support of 70 or higher.



**Supplementary Figure 2.** Mcf phylogenetic trees generated by the branch and bound (a) and, neighbor joining (b) methods. The *Vibrio tubiashii* sequence was designated as the outgroup for rooting the trees. The numbers at the nodes of the neighbor joining tree are the bootstrap percentages based on 1,000 replications. The trees were based upon 1,983 total characters, of which 391 were constant, 404 variable characters were parsimony uninformative, and 1,188 variable characters were parsimony informative.

**Supplementary Figure 3.** Sequence alignment of the Mcf proteins used to generate the phylogenetic trees in Figure 4 and Supplementary Figure 2.

CLUSTAL X (1.83) multiple sequence alignment

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Ph.luminescensAAM88787.1      VVTSLDANTFRVYHDGRVNSSL YDNVVMADV KDYQIAGTAEGLAAAYM
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E.t.poe                        IVTDLNDTHYRVFHDGRVGSV L YDNVVMADV ADYQGQGV SDEVAGAYM
V.tubiashiiEIF01430.1        .** *: .**:*.* .**:*:*.*:** *:* .* :**

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Ph.luminescensAAM88787.1      QYVNHQWQLVFQRQEYQRDGG---MLRRLRLD---DEEPLSIQVADSQ
Ph.a.asymbioticaYP_003042199.1 QYVDGEWQLAFQRQEYQDGG---RVWPKLRD---GGEPLSILVADSQ
Ph.luminescensMcf2AAR21118.1  QYVNDQWQLALQRQEYQDGG---TLRRLKLRG---GEEPLSIQIADSQ
Ph.l.laumondiiMcf2NP_930360.1 QYVNDQWQLVFQRQEYQDGG---RLRKLKLRG---DEEPLSIQIADSQ
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X.szentirmaiMcf2CDL84642      QYVDGEWQLVFQRQEYQREGH---MLWPKLRN---GEEPLSIQVADSQ
X.nematophilaMcf2YP_003712268. QYVNGEWQLVFQRQEYQRDGH---TLWPKLRS---NEEPLSIKTADSQ
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E.t.poe                        HYREGRWSLCFQRQTIVAN-ARIVL YEPAPRDGPSILGAEPLIEMVPSY
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G---EAGVNSLVQKFLASADPQA--GSSTLLKNSLIRLHSN-VEAVQAS  
G---EAGVNSLVQKFLASADPQA--GISTLLKNSLIRLHSN-VDAVHAS  
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G---EAGINLLVEKFLFAAADPHA--GSSTLLKNSLIRLHSNLEAVQAS  
G---EAGIDSLVQKFLFASADPQA--GSSTLLKNSLIRLHSN-VEAVQAS  
G---EVGVNSLVEKFLFAAASPQE--GSSTLLKNSLIRLHSN-VEAVQAS  
G---EAGINSLVEKFLFAAASPQE--GSSTLLKNSLIRLHSN-VEAVQAS  
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----NFAIDALCAKLVGLSPNNAADMKNAELKRCLEDLHTS-YPAEAS  
G---EPGANQFASVLFDAAAHPDS--QIATNVYTTVGQLHSN-TDAGLAE  
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IDTGKMAEVPVGNLNVADLSRPEELAGVIGQRRQVEQAVDAQQRVSEDL  
IDTGKMAEVPVGNLNVADLSRPEELAGVIGQRRQVEQAVDAQQRVSEDL

Ps. protegensABY91230.1  
Ph.l.laumondiiNP\_931332.1  
Ph.luminescensAAM88787.1  
Ph.a.asymbioticaYP\_003042199.1  
Ph.luminescensMcf2AAR21118.1  
Ph.l.laumondiiMcf2NP\_930360.1  
Ph.temperataWP\_023045049  
X.szentirmaiiMcf2CDL84642  
X.nematophilaMcf2YP\_003712268.  
E.g.inebrians  
E.t.poeae  
V.tubiashiiEIF01430.1

Ps.c.aureofaciensAFD97973.1  
Ps.chlororaphisAFD97974.1  
Ps.protegensABY91230.1  
Ph.l.laumondiiNP\_931332.1  
Ph.luminescensAAM88787.1  
Ph.a.asymbioticaYP\_003042199.1  
Ph.luminescensMcf2AAR21118.1  
Ph.l.laumondiiMcf2NP\_930360.1  
Ph.temperataWP\_023045049  
X.szentirmaiiMcf2CDL84642  
X.nematophilaMcf2YP\_003712268.  
E.g.inebrians  
E.t.poeae  
V.tubiashiiEIF01430.1

Ps.c.aureofaciensAFD97973.1  
Ps.chlororaphisAFD97974.1  
Ps.protegensABY91230.1  
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Ph.luminescensAAM88787.1  
Ph.a.asymbioticaYP\_003042199.1  
Ph.luminescensMcf2AAR21118.1  
Ph.l.laumondiiMcf2NP\_930360.1  
Ph.temperataWP\_023045049  
X.szentirmaiiMcf2CDL84642  
X.nematophilaMcf2YP\_003712268.  
E.g.inebrians  
E.t.poeae  
V.tubiashiiEIF01430.1

Ps.c.aureofaciensAFD97973.1  
Ps.chlororaphisAFD97974.1  
Ps.protegensABY91230.1  
Ph.l.laumondiiNP\_931332.1  
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Ph.a.asymbioticaYP\_003042199.1  
Ph.luminescensMcf2AAR21118.1  
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Ph.temperataWP\_023045049  
X.szentirmaiiMcf2CDL84642  
X.nematophilaMcf2YP\_003712268.  
E.g.inebrians  
E.t.poeae  
V.tubiashiiEIF01430.1

IDTHKMAEVPVGSGLNVADLSRPEELAGVIGQRRQVEQAVGAQQRVSDQL  
IDTRKMAEVPVGNGLNVADLTRFEELASVIGQRRQVEQVMSAQERITEDL  
IDTGKMAEVPVGNGLNVADLTRFEELSSVIGQRRQVEQVMSAQERITEDL  
IDTGKMAEVPVGNGLNVADLTRFEELASVIGQRRQVEQAVRAPERVTEDEL  
IDTGKMAKVPVGNGLNVADLTRPDELTTVIEQRRQVEQAVNAQVRIAKDA  
IDASKMAKVPVGNGLNVADLTRPDELTTVIEQRRQVEQAVNTQVRIAKDT  
IDTGKMAEVPVSNGLNVADLTRFEELASVIGQRRQVEQTVSTQERVTEDEL  
IDPAKMAKVPVGNGLDVADLTRPDELATVIEQRQWAKQVNTQEKVAEST  
IDPVKMARVPVGNGLDVADLTRPDELATVIEQRQWVKQTINAQEKIADKV  
IDAERVSGIILDYNTLVADLVRPETLLETVASQQQTATFLQDPAQLTASD  
IDAERVSRIGFDHNLTVADLVEPDTLLETITADRKTVTLYHDPAPQLTSSR  
LDTAKMAEVRSLTGLSVQDLASPSDRVANLATADHAARLAQASDLSLSTDL  
:\* . : : . . \* \* \* \* . : . : .

RLGAALTTFDAEQWG-ARFEVASTRLAREHQLGSQWIPPIIANIEEQKE-G  
RLGAALTTFDAEQWG-ARFEVASTRLAQEHQLGSQWIPPIIANTEELKD-G  
RLGAALTTFDAEQWG-ARFDAASTRLAREHQLSSQWIPPIIANterQPE-G  
RLSPALKTFDAEQWG-ARFEVASARLAQEHQLDSRWLPIIATTEDEQGE-G  
QLSTALQAFDAEQWG-ARFEAASTRLAQEHQLDSRWLPIIATTEEQGE-G  
RLSTLLKTLDAEQLG-GRFEVASTQLAQEYQLDSHWLPIIATTEELGE-G  
RLHTALATLDAEQWG-ARFAEASTRLAQENNLQQRWMPVISNTEDQGE-G  
RLHTALATLDAEQWG-SRFAEASTRIAQENNLQQRWMPVIGNTEDEQGE-G  
PLGTALKTFDAEQWG-ARFEAASTRLAQKYQLDNHWLPIIATTKDLGE-G  
QLRTALVTLDAELWG-ERFDNAITELARENGLDQRWMPVIDNIKEQDE-G  
QLHTALVTLDAEQWG-EKFDKAITELAGEHKLQQRWMPVIDNIKEKSE-G  
AFSAQTELLLETLGLGAAWRDARLEESTGMGEHWMPILETMREGDEKG  
TFSAQTELLLETLRLGAAWRDARLEESLGI GEHWMPILETLKEGGEEG  
QLKSALSVLQGANLA-QDFFAAAQDVYRRHRLDHAWVVPFENIEKTPS-G  
: . : : . : \* : : . \* : : . . . \*

GYRVQFINREQPE-QTRWLATDDGTFVEFRRFVDEHMRVFNQHTLEHGQ  
GYRVQFINRDQPELQPRWLDTRDGTVEFRRFVDEHMRVNEHFTLEHGQ  
GYRVQFINRDQPE-QTRWLSTDDGTFVEFRRFVDEHMSVLNEHFTLEHGQ  
RYRVQFINRDQPE-QTRWLITSDSTFVEFRRFIDEHMAVLNEHFTLEHGR  
RYRVQFINRDQPE-QTRWLDTDDSTFVEFRRFVDEHMSVLNEHFTLESGR  
HYRVQFINSDQPE-QTLWLTDDSTFVEFRRFVDEHMRVNEHFTLENGR  
RYRVQFINRDQPE-ETRWSSGDSTFIEFRRFVDEHMLTSLQHTFEHGQ  
HYRIQFINRDQPE-ETRWSSGDSTFIEFRRFVDEHMLTSLQHTFEHGQ  
RYQIQFINRDHPE-QTRSLDTNDSTFVEFRRFVDKHMSVLNEHFTLEHGR  
PYRIQFINRDNLE-EM-WINSDDSTFIEFRRFIDEHMRVNEHFTLERGQ  
GYRVQFINRDNLE-EK-WVNSNDGTFVKFRHFIDEHMRVNEHFTLERGQ  
SYEVQFINLKNKN-ETKWLSTKSPAIKDFKARLDKHLKSLSEYEFESGS  
SYEVQFINLKNMN-ETKWISTESSAIKDFKARLDEHLETLSKTHEFESGS  
QYRIPLIHREDNR-EISVLTSD-EK-WVNSNDGTFVKFRHFIDEHMRVNEHFTLERGQ  
\* : : \* : . . : : . . : \* : : : : . . . \*

IRPRAGVSEASVPDGLNAGFAVQTLIQWFADKNRKDTAQGTVPDLALAL  
IRPRAGVSEASVPDGLNAGFAVQTLIQWFADKNRKDTAQGTVPDLALAL  
IRPRGGVGEVAHVVDGLNAGFAVQTLIQWFADKNRKDAAGVSPDLALAL  
MRLRGGVGEAAPVDGLNAGFAVQTLIQWFADKNRHDAASGVSPDLALAL  
MQLRRGVSEVAPVDGLNAGFTVQTLIQWFADKNRHDAASGVSPDLALAL  
IRPKSGISEAAPVDGLNAGFAIQTLIQWFADKNRNDAARGIASPDLALAL  
IQPKGGIGEAPVDGLNAGFAIQSLIQWFTDKNRNDAARGIASPDLALAL  
MRLRGGVSEAMPVDGLNAGFAVQTLIQWFADKNRHNVASGVSPDLALAL  
IQHNGGVGEAAPVDGLNAGFAVQTLIQWFANKNRNDAAGVSPDLALAL  
MRHKGGVGEAAPVDGLNAGFAVQTLIQWFANKNRNDAAGVSPDLALAL  
FMRKENLPHAEADGLNAMSIVQTLIEHFSSK---ETEESKTNVQLADAL  
FMREENLAHAEADGLNAMFIMRTLIEHFAGK---KTEESKTNVQLADAL  
LTRHASITDVEHVDGLNAFAVQAILSWFNNHSRTAAAGDPLPQGLNTAL  
: . . : : \* : : : : . . . \* : : . . \* \* \*







E.g.inebrians  
E.t.poa  
V.tubiashiiEIF01430.1

EYNIHGVGANYTIGLNPVAMTLSS-----NKSSTRWVLDGK  
EYTIHGAGANYTIGLNPVAILSS-----GWSNTCWVLDGK  
HYAFNGKGGYHISLQSGASLSLQV-----TSRRDRS----TRWILDARQ  
\* ::\* \*..\* :.\*: .. : \* : :\*: \* :

Ps.c.aureofaciensAFD97973.1  
Ps.chlororaphisAFD97974.1  
Ps.protegensABY91230.1  
Ph.l.laumondiiNP\_931332.1  
Ph.luminescensAAM88787.1  
Ph.a.asymbioticaYP\_003042199.1  
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Ph.temperataWP\_023045049  
X.szentirmaiMcf2CDL84642  
X.nematophilaMcf2YP\_003712268.  
E.g.inebrians  
E.t.poa  
V.tubiashiiEIF01430.1

LESDSISVSKD----QMVG---VVVKLDPASNGQVLLVNAKGEVREV  
LASESISVSKG----QLVVG---VVVKLDPEPNGVLLVNAKGEVREV  
LESDSITVAKD----HLLVGG---VKVRLDPAQSGQVLLVNAKGEVRAA  
LASDSISVSKN----QLVVG---VVVELDPTQNGQVLLVNGKGEVREV  
LASDSISVSKD----QLLVGEGKGEVVVKLYLAQNSQVLLVNGKGEVRKV  
LASNGISVSKN----QLVFG---VVVELDPVKNKSVSVVN--KGMHV  
LASDSISVSKN----RLVVG---MAVELSDWKEQVLLVNRKGEVREV  
LASDSISVSKN----RLVVG---VVVELSDRKEQVLLVNCCKGEVREV  
LDNDNIKVSKNPLENQLIEIG---VVVKLDLTQNRQVSVVNGKGEVRKV  
LSDSTIKVTKN----RLEIG---VVVELDPSQNGQVLLVNSNNEVREI  
LDNGTIKVLN----RLEIG---VVVEIDPSRNGQVLLVNSKNEVHEI  
LDGEDIMVEAQ----AVSILG---FRVNLADRNFESMFICKPNGEILQV  
LDGENIVIEAR----AVSISG---FRVNLADTLFSSMLIYKPNGEILEV  
LSSDHLVTRD----HLNIG---VRVNLPLGLSWGSIIVINKHNEVLVV  
\* . : : : . : . \*.: : : : :\*:

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Ps.chlororaphisAFD97974.1  
Ps.protegensABY91230.1  
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Ph.temperataWP\_023045049  
X.szentirmaiMcf2CDL84642  
X.nematophilaMcf2YP\_003712268.  
E.g.inebrians  
E.t.poa  
V.tubiashiiEIF01430.1

DFA---GMSARVVEEDASKWQLPGQRIEQHSELAKAHQLHGQYVVVDNY  
DFA---GMSARVVEEDASKWQLPGQRIEQHSELAKAHQLHGQYVVVDNY  
DFA---GQTTYVVKEDASQWQVSGQRIEQHSELAKAHQLHGQYVVVYNY  
DFA---GLTTQVVEDAGKWQVPGQIEQHLSDLAKAHQLHGQYVVVYNY  
DFT---SLTAQVISEDASKWQVPGQIEQHLSDLAKAHQLHGQYVVVYNY  
DFS---NSQPIVNLVSDADGWQPDQIEQHLKLNKAHQLHGQYVVVYNY  
DFA---SLTAQVVEDASKWQVPGQIEQHLNDLAKAHQLHGQYVVVYNY  
DFS---NLTTQVVEDASKWQVPGYSIEQHLSDLAKAHQLHGQYVVVYNY  
DFTGQLNQTPKVVAVDAS----KQQIEQHLSELAKAHQLHGQYVVVYNY  
NFS---TLTADVikedASKWQVPGQIEQHLSDLAKAHQLHGQYVVVYNY  
NFS---AQTADVVEDASKWQVSGQIEQHLKDLAKAHQLHGQYVVVYNY  
DFD---KKTTFPIEEDGAKYQGGSQLKEHLDDLNDKHLAAELIVVNEY  
DFA---NKTTFPIKEDGKYQGGSQLTDHLNDLSDKHLGTALIVVDKY  
DPE---NSRYQITSEDASSWQN-AEQLHQHIQSEVAKHHLDSAFVAVENF  
: \* . : :\*.. \* \* :\*::

Ps.c.aureofaciensAFD97973.1  
Ps.chlororaphisAFD97974.1  
Ps.protegensABY91230.1  
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Ph.temperataWP\_023045049  
X.szentirmaiMcf2CDL84642  
X.nematophilaMcf2YP\_003712268.  
E.g.inebrians  
E.t.poa  
V.tubiashiiEIF01430.1

RHEGR--NVGRAFYDVANKRMLFTDT-QVEQAR-NAQLGAVIGEHAYFVD  
SHEGR--NVGRAFYDVAKDRMLFTDT-QVEQAR-NAQLGAVIGEHAYFVD  
KHGER--NVGRAYEYVAKDRMLFTDQVQ--ARNAQFGAVIGEHAYFVD  
SHNGR--DVGRAFYDVGKERMLFTNT-THEQTR-HAQLGAVMGDHYFYD  
RHQGR--DVGRAFYDVTKDRMLFTDT-TNEQAK-RAQLGAVMGDYAYFYD  
RHNGR--DVGRAFYDVVKDRMLFTDT-SQKQAR-HAQLGAVIGDYAYFYD  
SHNGR--DVGRAFYDVTNKRMLFTDT-THEQTK-HAQLGAVMGNHAYFYD  
RYNER--NVGRAFYDVAKDRMLFTDT-PHKQAR-HAQLGAVMGDHYFYD  
RHYG-----RAFYDVAKDRMLFTDT-SQEAK-HAQLGAVIGDHYFYD  
SHNGH--DVQAFYDVAKDRMLFTDT-TQEAK-HAQLGAVMGDHYFYD  
KHNGR--DVQAFYDVAKERMIFDT-TQEAKHHAQLGAVMGDHYFYD  
TTPAG-QSVGRAFYEPTNKRLLYTIDAPEELTKSVQVGVLTAGGKVFYFN  
TTPAG-QSVGRAFYEPTSKRLLYTIDAPEELTNSAQVGVLTAGGKVFYFN  
VPQDQDHSVGLAYYQVDQQRFIYTPSPAHSDFLSDAKLVAQTGDLAWFNK  
\*:\*: ..\*::\* . :\* .

Ps.c.aureofaciensAFD97973.1  
Ps.chlororaphisAFD97974.1  
Ps.protegensABY91230.1  
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Ph.luminescensAAM88787.1  
Ph.a.asymbioticaYP\_003042199.1  
Ph.luminescensMcf2AAR21118.1  
Ph.l.laumondiiMcf2NP\_930360.1  
Ph.temperataWP\_023045049

AENAAWRVEIATGKVDQFAPS-LNQSAG--RISRFWQEGE-AVYLARR  
AENAAWRVEIATGKVDQFAPS-FNQSAG--RISRFWQEGD-AVYLARR  
AENAAWRVDIASGKVDQFAPA-FNQSAG--QISRFWQEGD-AVYLARR  
ADNAAWRVDIATGQVDAQFEPW-FNQAG--QISRLWQEGD-AVYLARR  
ADNAAWRVDIATGQVDAQFEPW-FNQAG--HISRFWQEGD-VVYLARR  
ADNAVAVRVDIATGQVDAQFELW-FNQCDG--KISRLWQEGD-AVYLARR  
ADNAAWRVDIATGQIDAQFEPW-FNQSAG--QISRLWQEGD-AVYLARR  
ADNAAWRVDIATGQVDAQFEPW-FNQSAG--QICRLWQEGN-AVYLARR  
ADNAVAVRVDIATGQVDAQFEPW-FNRNAG--NISRFWQEGD-VVYLARR

X.szentirmaiMcf2CDL84642  
X.nematophilaMcf2YP\_003712268.  
E.g.inebrians  
E.t.poae  
V.tubiashiiEIF01430.1

Ps.c.aureofaciensAFD97973.1  
Ps.chlororaphisAFD97974.1  
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Ph.l.laumondiiNP\_931332.1  
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X.szentirmaiMcf2CDL84642  
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E.g.inebrians  
E.t.poae  
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Ps.chlororaphisAFD97974.1  
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Ph.a.asymbioticaYP\_003042199.1  
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Ph.temperataWP\_023045049  
X.szentirmaiMcf2CDL84642  
X.nematophilaMcf2YP\_003712268.  
E.g.inebrians  
E.t.poae  
V.tubiashiiEIF01430.1

Ps.c.aureofaciensAFD97973.1  
Ps.chlororaphisAFD97974.1  
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X.nematophilaMcf2YP\_003712268.  
E.g.inebrians  
E.t.poae  
V.tubiashiiEIF01430.1

Ps.c.aureofaciensAFD97973.1  
Ps.chlororaphisAFD97974.1  
Ps.protegensABY91230.1  
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Ph.luminescensAAM88787.1  
Ph.a.asymbioticaYP\_003042199.1  
Ph.luminescensMcf2AAR21118.1

ADNAAAWRVDIATGQVDAQFMPW-FNHAG--KISRLWQEGN-AVYLARH  
ANNAVAWRVEIATGQVDAQFMPW-FNYNAG--KISRLWQEGD-AVYLARH  
TEHCAIWRVDVSTGVCEVKYHALCPSNKI---TLQRVWADADNHIHAVFL  
TEHSAIWRVDVSTGVCEAKYHALCPFSKR---TLQRVWVDADNQIHALFR  
DN--QVWLVNIAESRIVAQYRAFQWDYAHG-KAASRVWQENQ-RLYWSIE  
: \* \*::: . .:: \*.\* : : ::

YQLKERE-----AELNFRILGDRME  
YQLKERE-----AELNFRIGDRME  
YQLKE-R-----EAELSFIRILGDRME  
YRLKEKE-----AELEKEAELCYRILSDRME  
YRLKERE-----AELGYRIIGDRME  
YWLKVKQEPEKRLLEEQLKDWPEEQAEELLKQRPEKRGAEELGYKILGDRME  
YQLKGKE-----AELSYRLLGDRME  
YQLKGKE-----AELSYRLLGDRME  
YQLKERE-----SELSYQILGDRME  
YQLKERA-----AELGYRIVGDRME  
YQLKERE-----AELSYRIVGDRME  
HQLSEDFYFG-----HLNYILTADSMN  
HQLSENHFG-----TLNYILTADSMN  
QQTNAGH-----PAKWTVLDENSMK  
: : : \*:

LVGVVGGDALLQ-LSARTDQH---GDALKTLLQGYQSQATQRATPVYS-  
LVGVVGGDALLQ-LSARTGQH---GDALKMLQGYESQATQRATPVYS-  
LVGVVGGDESLQ-LSASNSQHGDAKTLLNLTLLKGYEQATQRATPVYS-  
LVSavgdDALLQ-LLARTSQH---SDVLKAILQGYESKSIQRETPMYT-  
LVSavgdDALLQ-LSARIGRH---GDELEAILQGYRSNSTQRGTLMYT-  
LVSavgdDALLQ-LSAGTNRH---NDELQVIRKGYESDGIKRPKTPTYT-  
LVSavgdDALLQ-LSARTGRH---GDELKTLQGYESNSTQRETPTYM-  
LVSavgdDALLQ-LSARTGLH---SDELKTLQGYENNSIQRETPMYT-  
LVSavgdDALLR-FSARTDRH---NDELKVMLQDYESNSTQRTVPMYT-  
LVSavgdHELLQ-QLANTDRH---GDVLGAIKQSYESNSTRGEISTYT-  
LVSavgSDEVLQ-HLASTDQH---ADALDTLLESYENNSTQGVISAYT-  
LVSMAGSPALLEKLNGLDKWTG----EATLLED-YTDTREVMMLHNP  
LVGMVGGPALLAKLHQEDKWTG----EVRLLLEDYTGPESELLPKLKL  
LVDVNGDLQLDNLMADTAPN----RATTNYFFQSFQAG-VEGEIDLR--  
\*\*.\*.: : : .

----LGAPMMEPSAAALVTVFGLDSAK--VAHRYWVRTSDG-----  
----LGAPMMEPSAAALVTVFGLDNAK--VAHRYWVRTSDG-----  
----LGAPVLEPTAAELITVFGDNAK--VAHRYWVRTSDG-----  
----LGTRLLQPTSAALVTVFGVDAAG--VAHRYWVRTSDG-----  
----LGARLIQPTSAALVTVFGVDAAG--VPHRYWVRTSDG-----  
----LGARLIQPTSAELITVFGDAAN--VPHRYWVRTSDR-----  
----LGARLIQPTSAALVTVFGVDTAG--VAHRYWVRTSDG-----  
----LGARLIQPTSAALVTVFGVDAAG--VAHRYWVRTSDG-----  
----LSARLIKPTSAALVTVFGVDAAN--VPHRYWVRTSDG-----  
----LGTQQVQPTGADLITVFGVDAAN--VKHRYWVRTSDG-----  
----LGTQRIKPTSAALITVFGIDAND--VKHRYWVRTSDG-----  
GS-LAGADIDAIDSHMILVLGPKPDESIIHHRFWIRMSDR-----  
ES-LAGADIDAIDPHLIFVLGPKGKDHYFYHQFWIRMSDR-----  
----ADRWIEASLSDVISVS-ANKQG--QKHRYWVLAEQ-----  
. . . : \* . \*::: :

---TVIKPNLAPPAGQPPVDAQGKQSAWIPADLVFAGSMPQPAG--Q  
---TVIKPNLAPPAGQPPVDAQGKQSAWIPADLVFAGSMPQPAG--Q  
---IVIKPNLAPPAGQAPRADAPGQAQSAWIPADLVLAGSQAQPGG--Q  
---TLIKPNLAPPADQTPHFKEQTRSAWIPADLVLAGSMPQPAG--K  
---TLIKPNLAPPADQTLHFEAHEQTRSAWIPADLVLAGSMPPLGG--K  
---TLIKPNLAPPADQAWNLKEGKQTRSAWIPADLVLAGSMPQPAG--K  
---TLIKPNLAPPADWTLHFEAHEQTRSAWIPADLVLAGSIPRAG--T

Ph.l.laumondiiMcf2NP\_930360.1  
Ph.temperataWP\_023045049  
X.szentirmaiMcf2CDL84642  
X.nematophilaMcf2YP\_003712268.  
E.g.inebrians  
E.t.poeae  
V.tubiashiiEIF01430.1

---TLIKPNLAPPADRTLHFEAHEQAHSAWPIPADLVLGSMPPQGGKEV  
---TLIKPNLALPADKPRYFKEHEQTRSASWEIPVDLVLGSIIPQPGD--K  
---TLIKPNLAPPADNTLSLEELKQARSHWQIPADLVLGSIITPAG--K  
---TLIKPNLAPPADNTLYFEAHKQTRSNNWQIPADLVLGSIIFDLG--K  
---TVIKAHP-----DIHLDYLLVGLNSTDGS-G  
---TVIKPHP-----DIHLNLYLVGTIASTGDS-G  
-----RGDLG-----VIHANLNTQ-----PDDLILALVQNKGET--D

: . : ::.

Ps.c.aureofaciensAFD97973.1  
Ps.chlororaphisAFD97974.1  
Ps.protegensABY91230.1  
Ph.l.laumondiiNP\_931332.1  
Ph.luminescensAAM88787.1  
Ph.a.asymbioticaYP\_003042199.1  
Ph.luminescensMcf2AAR21118.1  
Ph.l.laumondiiMcf2NP\_930360.1  
Ph.temperataWP\_023045049  
X.szentirmaiMcf2CDL84642  
X.nematophilaMcf2YP\_003712268.  
E.g.inebrians  
E.t.poeae  
V.tubiashiiEIF01430.1

EVFFFYSQKQKVLFRQEGPGQKVLGDSQPSALRLATPTLANVLELNGHLL  
EVFFFYSQKEKVLFRQEGPGQKVLGDSQPSALRLATPTLANVLELNGHLV  
EVFFFYSKAQQVLFQEGPGQKVLDAQGPSALRLSTPPLANVNLNGHLL  
EVFFFYSKEQKVLFRQEGPGQAVLDASHPTALRISTPALANLVNLSGSL  
EVFFFYSKEQKTLFRQEGPGQEVLDANQPSALRVTPALTNVINLNGHLV  
EVFFFYSKKEKALFRQEGPGQAVLDASQPSALRVTPALSNVINVNGHLI  
EVFFFYSKEQKALFRQEGPGQTVLDANHPSALRISTPELANLVNLSGSL  
EVFFFYSKEQKVLFRQEGPGQVLDANHPSALRINTPALANLVNLSGSL  
EVFFFYSREQKALFRQEGPGQAVLNANQPSALRVTPALANVINVNGHLI  
EVFFFYSHEQKVLFRQEGPGQTELDASHPTALRVSTPPLANVVNLSGSL  
EVFFFYSNELKLLFRQEGSGQTVLDASHPTALRVSTPSLANVVNLSGSL  
EQFCFFSHKEHKLVIQQGNGKQAKE-PRPVTMSSEFGTISNFFCVNNSLL  
LIFCFFSYNHKLVVQRNGTQAEK-PRPVTISPGLTISNFFSANNLNF  
QHYFFYSHSQQRLYYQASNRSQARSVSLP----EFSGLLRNVLDNQHLF

: : \* : \* \* . . \* : \* . : \*

Ps.c.aureofaciensAFD97973.1  
Ps.chlororaphisAFD97974.1  
Ps.protegensABY91230.1  
Ph.l.laumondiiNP\_931332.1  
Ph.luminescensAAM88787.1  
Ph.a.asymbioticaYP\_003042199.1  
Ph.luminescensMcf2AAR21118.1  
Ph.l.laumondiiMcf2NP\_930360.1  
Ph.temperataWP\_023045049  
X.szentirmaiMcf2CDL84642  
X.nematophilaMcf2YP\_003712268.  
E.g.inebrians  
E.t.poeae  
V.tubiashiiEIF01430.1

AVTEDGRVARIATGRLSYEAVNEHWLKGRST-----WWQDLAS-VNGS  
AVTEDGRVARIATGRLSYEAVNEHWLKGRST-----WWKDLAS-VNGS  
AVTNDGRMARIATGRLSYEAVNEHWLKAHSN-----WWKNLAE-VAGS  
AITEDGRVARLDALGQLNYEAVNEHWLKGRTH-----WWQDLAS-VADG  
VVTEDGRVARLDALGQLSYAAVNEHWLKGRIH-----WWQDLTS-VTDG  
AITEDGYVARLDAQQLNYAAVNEHWLKKHIH-----WWQDLAG-VAGF  
AITEDGRVARLDAQQLNYEAVNEHWLKGRTH-----WWRDLAN-ITD-  
AITEDGRVARLDAQQLNYEAVNEHWLKGRTH-----WWQDLAN-ITDS  
AMTEDGCAQLDALGQLSYGAVNEHWLKRTH-----WWKDLAD-VTGF  
AITEDGRVAQLNASGQLNYEAVNEHWLKERH-----WWQDLAG-VAGS  
AITKDGRAQLNAQGQLNYEAVNEHWLKGHPQ-----WWQDLAS-VTGS  
SITTTGFI LRLTTHYTLALEAVNHQWLEHFEKDVDG-PWWTALPKLAKDH  
SITDAGFILRLTTHCTLALAVNHQWLEHREKVDVGGPWWTALSKLAKDH  
AIANDQTLWLADQTPRL--AGVTSHWLQSHHQVLP--QLKALSEKAAAK

: : \* . \* . : \* : \*

Ps.c.aureofaciensAFD97973.1  
Ps.chlororaphisAFD97974.1  
Ps.protegensABY91230.1  
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Ph.a.asymbioticaYP\_003042199.1  
Ph.luminescensMcf2AAR21118.1  
Ph.l.laumondiiMcf2NP\_930360.1  
Ph.temperataWP\_023045049  
X.szentirmaiMcf2CDL84642  
X.nematophilaMcf2YP\_003712268.  
E.g.inebrians  
E.t.poeae  
V.tubiashiiEIF01430.1

N-ATLAVFGAKAADGKSALPVWYHN--GQVVVAASSLQGKPLQFLGFDSA  
N-ATLAVFGAKAADGKSALPVWYHN--GQVIVAASSLQGKPLQFLGFDSA  
N-ATLAVFGVKAADGKSALPVWYHN--GQVVVASSLQGKPLQFLGFDSA  
R-ATLAVFGIKGTDGKSLLPVWYHK--GQVIVASAPLQDKRLQFLGFQVD  
R-ATLAVFGVKDTDGKSLLPVWYHN--GQVVVASAALQDKHPQFLGFQVD  
R-ATLAVFGVKGTDGKSLLPVWYHN--GQVVVASAALQDKHLQFLGFQVD  
T-TTLAVFGIKGSDGKSLLPVWYHN--GQVVVASAALSDKSLQFLGFQVD  
T-TTLAVFGVKGTDGKSLLPVWYHN--GQVVVASAALQGKSLQFLGFQVD  
S-ATLAVFGVKGADGKSLLPVWYHN--GQVVVAS--LQDKHLQFLGFQVD  
G-ATLAVFGVKGTDGKSLLPVWYHN--GQVVVASAALQGKPLQFLGFQVD  
G-ATLAVFGVKGTDGKSLLPVWYHN--GQVVVASAALQGKSLQFLGFQVD  
AAATVSIIVGLCDAGSPVQAWLCS---GRFVVAGPSLRGKPRHIAAGLTEG  
GAAIVSIIVGLSDAEGSPVQAWLCS---DRFVVAGPSLRGKPRHMAAGLTEG  
L-AQLVLLGMTDSQGE-TVNLWYDNQSATLIRAGSNLNGQHLNGLTAD

: : : \* : . . . : \* . \* : : \*

Ps.c.aureofaciensAFD97973.1  
Ps.chlororaphisAFD97974.1  
Ps.protegensABY91230.1  
Ph.l.laumondiiNP\_931332.1  
Ph.luminescensAAM88787.1

SSSARLFEPESG-----KL YLQAPMTSEALATA  
SSSARLFEPESG-----KL YLQAPMTPEALAAA  
SASARLFEPESG-----KL YLQPPMTAQALATA  
GSGARLFEPASG-----KL YRQPPMTADALAAA  
GSSARLFEPASG-----KL YRQPPMTADALAAA

Ph.a.asymbioticaYP\_003042199.1  
Ph.luminescensMcf2AAR21118.1  
Ph.l.laumondiiMcf2NP\_930360.1  
Ph.temperataWP\_023045049  
X.szentirmaiMcf2CDL84642  
X.nematophilaMcf2YP\_003712268.  
E.g.inebrians  
E.t.poae  
V.tubiashiiEIF01430.1

Ps.c.aureofaciensAFD97973.1  
Ps.chlororaphisAFD97974.1  
Ps.protegensABY91230.1  
Ph.l.laumondiiNP\_931332.1  
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Ph.luminescensMcf2AAR21118.1  
Ph.l.laumondiiMcf2NP\_930360.1  
Ph.temperataWP\_023045049  
X.szentirmaiMcf2CDL84642  
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E.g.inebrians  
E.t.poae  
V.tubiashiiEIF01430.1

Ps.c.aureofaciensAFD97973.1  
Ps.chlororaphisAFD97974.1  
Ps.protegensABY91230.1

GSSARLFELANG-----KL YRQPPMTADALAAA  
GTSARLFEPESG-----KL YRQPAMTANALAAA  
GASARLFEPESG-----KL YRQPAMTANALAAA  
GSSARLFEPASG-----KL YLQPPMTADALAAA  
STSARFFEPESG-----KL YYQPAMTASELAAA  
GTSARLFEPESG-----KL YHQPAMTANALAVA  
GTKAWLWYIESKGSGLHLEVEPIVRVQLETVDSGHL YAQPTVRDEELETV  
GSKAWLWHMETE-----DSGHL YAQPTVRGKELETV  
QQWAWLYNHDDA-----SL YRQAVVNDLIL--  
\* :: \*\* \* . : . :

FG-GDEVLEASQAQLPAATLPMPEV-QLRSVQVDAGLRLTTVQGEILLRA  
FG-GDEVLEASQAQLPAATLPMPEV-QLRSVQVDAGLRLTTVQGEVLLRT  
FG-NDEVLEASQAQLPAAIDWMPKL-QLRSVQVDAGLRLTTVQGEVLLRS  
FG-TDEVLEVSSQLPAAGELASGL-HLKAVEQVDVGLRLTTVKGEILLQT  
FG-TDEVLEASQAQLPAANELEPEL-HLKAAEQVDAGLRLTTVKGEILLRT  
FG-SEAVLKASAQLPAVSELMPEL-HLKAAEQVDAGLRLTTVKGEILLRT  
FG-ADQVLDSSAQLPAASELTPEL-HLKAAQVDVGMRLITVKGEILLRT  
FGGTGQILSASAQLPSASELTPEL-HLKAAQVDTGMRLITVKGEILLRT  
FG-TDEVLDASAQLPAASELPEL-HLKAAEQVDAGLRLTTVKGEILLRT  
FG-SDQVLDTSQAQIPVASELTPEL-SLKEVQLVDVGMRLITTKGEILLRA  
FG-SDHVLDASAQLPVAASELTPEL-SLKEVQVDVGMRLITTKGEILLRS  
FCPKAPFVKAALPDGRSVPPELHP-FKTVALMTDGGRLRYTTKDGVLTLTSL  
FRLKAPFVNAEAVPDGRGVLVKHP-FKTVALT-EGGLRYTTKEGVLLILA  
----DDKLVLQGEVADAEQFATLAGEALTHAAWQGSLLQLSTRDGLVVELS  
: . . . . : : \* . \* : : :

NKG----DVQLVALDKGWQQHLGNLSQALAQVAEQWRAGVLTQGAES  
NSG----DVQLVAVDKGWQQHLGNLPQALAQVTAQWGAQVLTQGAES  
NNG----DVQLVAVDKGWQQARLGNLPQALATVAGQWGAQVLSLQDGD  
NDG----KLQLVAVDKGWQDNFARLPQALAEVADKWRKGVLTQGGDT  
HDG----KLQLVAVDKDWQDNVRLSQALAEVAGQWRVKGVLTQGGDT  
NDG----KLQLVAVDKDWQVNLARLPQALAEVAGQWPTKGVLTQGDDES  
NNG----ELQLVGVDKGWQNNLAQLSQALTKLADQWQAKGILTQDNKE  
NNG----ELQLVGVDKGWRQNNLTHLPQALAKLADQWQAKGILTQDNKE  
NGG----KLQLVAVDKGWQDNRTHLPQALAKVAGQWHTKGVLTQDDGI  
NNG----KLQLTGVDEVWQSNKTDQQAQALSELASQWHAQALTLQSHNT  
NNG----KLQLVGVDKAWLQNNKTDQIQALTKLADQWHAEGTLTQSSSI  
SER----SARLYGVDKVWQ-QNQSDVNAALATLVNTWSHGIEVVMGSEP  
SER----SARLYGVDKVWQ-QNRSDLSGEIALTKTWDHGESVVMGSEP  
ANAGPMDKPILRVAVTMEWQTHHQ-YRHQAIDALESEYQLPKQVRLLG-SV  
. \* . : \* . : : : : : : :

LGWFDVASGRMFASTGIP-AASDLRFIGVAAESQRSAYVVSPTQVLYKV  
QGWFDVASGQMFASNGIP-AASDLRFIGVAAESQRSAYVVSPTQALYKV  
RGWFDIASGQMFASNGIP-GGSDLRFIGVAAAGTPNSAYVVSPTAQALYQV  
QGWFDVSGQVFSYGSIQ-AKDNLRFIGIAAG-KKGAYVVSPTDQALYRV  
QGWFDVSGQVFSIGGIP-ATDNLRFIGIAGV-KKGAYVYNPTDQMLYQV  
RGWFDVDSGQLFSVGGILAAADNLRVFGVDAG-KKGAYVVSPTDQALYRI  
PGWFDIGSGQIFSGNGIP-VSDNLRVFGIAAG-EKAAYVVSPTTQALYQI  
LGWFDIGSGQVFSNGIP-VSHNLRVFGIAAG-EKAAYVVSPTTQALYQI  
QGWFDVSGQVFSLGGIP-AADNLRVFGIAGV-KKGAYVVSPTDQTYLWI  
PSWFDVSGQVFSVNGIR-TSDHLSFVIGADAK-NKTAYVYDQTNQKLYCL  
PSWFDIGSGQIFAGNEIP-ASDHLFVGTNAK-NKTAYVYNQTTQELYQL  
PQWYLTSSGKILAAATNATFTWLDAPTWLGADLSGAKGYAYVSAHRKIYEL  
PQWYLTSSGNIVSAAKATFTWLDAPTWLGADPSGARGYAYVAGQGRIYEL  
PSWYLVHESGRVTAPGLD-VQHNLTYLQSVDDGD--YVFDVAQTYLQQ  
\* : \*\* . . . \* : : \*

KEGAAQKLGHYANVERIG-SSLLLQGG--DGRQDELAP---PLIAGLDSV  
KQGAAQKLGHYGDVERIG-SSLLLQGGVGGRRQDELAP---PLIAGVDSV  
KDGKALQLGHYANVERIG-SSLLLQGASGNARQDDLAP---PLIAGVDSV

Ph.l.laumondiiNP\_931332.1  
Ph.luminescensAAM88787.1  
Ph.a.asymbioticaYP\_003042199.1  
Ph.luminescensMcf2AAR21118.1  
Ph.l.laumondiiMcf2NP\_930360.1  
Ph.temperataWP\_023045049  
X.szentirmaiiMcf2CDL84642  
X.nematophilaMcf2YP\_003712268.  
E.g.inebrians  
E.t.poe  
V.tubiashiiEIF01430.1

KDSVQKLNHYTSVERIG-SSLLIRGGGTGRRDDLTP---PLITGVDSV  
KESGAQKLNHYADVERIG-SSLLLQDG----GKGDLSP---MLIAGVDSV  
QGGGV---HYTSVERIG-SSLLLQGGEMSGRQDELTP---PLIAGVDSV  
KDGNAQMLNKFTAVERIG-SSLLLQGGVGGSKDDLTP---PIIAGVDSV  
KDGNAQMLNKFTGVERIG-SSLLLQGGVGGSKDDLTP---PLIDGVDSV  
KDGGVQKINHYTSVERIG-SSLLLQGG---WGQDDLTP---PLIVGVDSV  
NDDKTLTINKLNDVERIN-SSLLLQGG---EADDNLTP---PLIDGVDSL  
NNYKDKILNKINGVERVN-SSLLLQGG---RSADTLMP---PIIDGVDSL  
RQSSAEET-SAASREDAFASRLHDVLAPEVSPGASFRP---FALENVLYT  
GEGSAEEKKSAASQEVAFASRFQDVLAVKPSPGASFRQ---FALENVHYT  
HLTDHSPLGHFSMALTEGGTHLVLQGS---EGSKIQT---LPRKGVRSL  
: : . : :

Ps.c.aureofaciensAFD97973.1  
Ps.chlororaphisAFD97974.1  
Ps.protegensABY91230.1  
Ph.l.laumondiiNP\_931332.1  
Ph.luminescensAAM88787.1  
Ph.a.asymbioticaYP\_003042199.1  
Ph.luminescensMcf2AAR21118.1  
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Ph.temperataWP\_023045049  
X.szentirmaiiMcf2CDL84642  
X.nematophilaMcf2YP\_003712268.  
E.g.inebrians  
E.t.poe  
V.tubiashiiEIF01430.1

VLH---GGGASDITYRFSAMWAHYRTVVIDNDDPG-QALDRLILPVADGK  
VLH---GGGASDITYRFSAMWAHYRTVVIDNDDPG-QALDRLILPVADGK  
VLH---GGAGDDTYRFSAMWAHYRSVVIDNDDPG-LALDRVILPVADGK  
VLH---GGADNDTYRLSQEMWSHYRTVVIDNDDPG-QALDRLIMPVDAE  
VLH---GGAGSDTYRLSQTMWSYRTVVIDNDDPN-QVLDRLIILAVDAE  
VLH---GGADSDTYRLEAMWSHYRTVVIDNDDL- QALDHLIIPVDAE  
VLH---GGADDDTYRLNKAMWSHYRTVVIDNDDRG-QALDRLILPLADPQ  
VLH---GGADNDIYRLSKAMWSHYHTVVIDNDDRS-QALDRLILPLADPE  
VLH---GGADDDTYRLSQEMWSHYRTIVIDNDDPG-QVLDRLIMLVTDAAE  
LLY---GGANHDSYNFTREAWDHYRTVVIDNNDPA-QKLDRLILPIANPE  
VLY---GGANHDYKFTKEMWDHYRTVVIDNNDPA-QALDRLILPIANPQ  
ILSQVEGDTSFMEYVIPSSSWESLDGLVVKWNDQGRVEIEGSTRHPRRAG  
ILSQVEGDTSFMDYSVPSASWESLSGLVIEWKDQGRVEMEGSTMHPGLG  
LWS---GNSRDYHSLDENDLAHYQQVLIQSHRVG-ETLK---LALENPN  
: \* . \* . : : . : :

Ps.c.aureofaciensAFD97973.1  
Ps.chlororaphisAFD97974.1  
Ps.protegensABY91230.1  
Ph.l.laumondiiNP\_931332.1  
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Ph.l.laumondiiMcf2NP\_930360.1  
Ph.temperataWP\_023045049  
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X.nematophilaMcf2YP\_003712268.  
E.g.inebrians  
E.t.poe  
V.tubiashiiEIF01430.1

NILVSRGEDVLL  
NILVSRQGENVLL  
NILVSRGEDVQL  
KILVSRHEDNML  
KIFVSRHEDDLML  
KILVSRHEDDLIL  
SILVNRHDDLVL  
SILVNRDDDLML  
KILVSRHEDDLML  
SILVSHNDLML  
SILVSHNDLML  
SFLGNRLDDDLIM  
WYLAKRLGDDLII  
ALLQRRGDDLAL  
: : : : :

## Supplementary Tables

**Supplementary Table 1:** Comparison of *Epichloë* Mcf-like amino acid sequences with those of the bacterial Mcf1, FitD, and Mcf2 sequences.

Species	Identity		
	Mcf 1 (AAM88787.1) 2,929 aa	fitD (AFD97974.1) 2,996 aa	Mcf2 (AAR21118.1) 2,388 aa
<i>E. amarillans</i> 1,994 aa	673/2,032 (33%)	673/1,922 (35%)	649/1,856 (35%)
<i>E. aotearoae</i> 1,986 aa	676/2,037 (33%)	660/1,909 (35%)	629/1,846 (34%)
<i>E. baconii</i> 2,044 aa	662/1,924 (34%)	672/1,915 (35%)	652/1,861 (35%)
<i>E. brachyelytri</i> 1,988 aa	636/1,839 (35%)	658/1,912 (34%)	638/1,836 (35%)
<i>E. festucae</i> 2368 1,844 aa	619/1,883 (33%)	611/1,766 (35%)	631/1,881 (34%)
<i>E. festucae</i> F11 1,844 aa	617/1,883 (33%)	611/1,766 (35%)	631/1,881 (34%)
<i>E. gansuensis</i> var. <i>inebrians</i> 1,983 aa	669/1,991 (34%)	671/1,883 (36%)	677/1,997 (34%)
<i>E. glyceriae</i> 2,009 aa	655/1,924 (34%)	663/1,865 (36%)	639/1,842 (35%)
<i>E. mollis</i> 2,012 aa	644/1,870 (34%)	682/1,922 (35%)	657/1,850 (36%)
<i>E. typhina</i> subsp. <i>poae</i> Ps1 1,997 aa	652/1,899 (34%)	669/1,921 (35%)	673/1,985 (34%)
<i>E. typhina</i> E8 2,004 aa	673/2,023 (33%)	667/1,944 (34%)	660/1,955 (34%)

**Supplementary Table 2:** Sources of DNA sequences used in this study.

<b>Species</b>	<b>Source</b>
<i>Aciculosporium take</i>	AFQZ00000000.1
<i>Atkinsonella hypoxylon</i>	<a href="http://www.endophyte.uky.edu/">http://www.endophyte.uky.edu/</a>
<i>Claviceps fusiformis</i>	AFRA00000000.1
<i>C. paspali</i>	AFRC00000000.1
<i>C. purpurea</i>	CAGA00000000
<i>Metarhizium anisopliae</i>	ADNJ00000000.1
<i>Epichloë amarillans</i>	AFRF00000000.1
<i>E. aotearoae</i>	<a href="http://www.endophyte.uky.edu/">http://www.endophyte.uky.edu/</a>
<i>E. baconii</i>	<a href="http://www.endophyte.uky.edu/">http://www.endophyte.uky.edu/</a>
<i>E. brachyelytri</i>	AFRB00000000.1
<i>E. elymi</i>	AMDJ00000000.1
<i>E. festucae</i> E2368	ADFL00000000.2
<i>E. festucae</i> F11	AFRX00000000.1
<i>E. gansuensis</i>	AFRE00000000.1
<i>E. gansuensis</i> var. <i>inebrians</i>	AMDK00000000.1
<i>E. glyceriae</i> E277	AFRG00000000.1
<i>E. mollis</i>	<a href="http://www.endophyte.uky.edu/">http://www.endophyte.uky.edu/</a>
<i>E. typhina</i> E8	AMDJ00000000.1
<i>E. typhina</i> subsp. <i>poae</i> E5819	AFSE00000000.1
<i>E. typhina</i> subsp. <i>poae</i> Ps.1	KJ502561 (This study)
<i>Photorhabdus asymbiotica</i> subsp. <i>asymbiotica</i> Mcf1	YP_003042199
<i>Ph. luminescens</i> Mcf1	AAM88787
<i>Ph. luminescens</i> Mcf2	AAR21118
<i>Ph. luminescens</i> subsp. <i>laumondii</i> Mcf1	NP_931332
<i>Ph. luminescens</i> subsp. <i>laumondii</i> Mcf2	NP_930360
<i>Ph. temperata</i> Mcf1	WP_023045049
<i>Pseudomonas chlororaphis</i> subsp. <i>aureofaciens</i> FitD	AFD97973
<i>Ps. chlororaphis</i> FitD	AFD97974
<i>Ps. protegens</i> FitD	ABY91230
<i>Xenorhabdus szentirmaii</i> Mcf2	CDL84642
<i>X. nematophila</i> Mcf2	YP_003712268
<i>Vibrio tubiashii</i>	EIF01430



**Supplementary Table 3:** Estimation of the divergence rate of the basal *Epichloë* spp. relative to *Atkinsonella hypoxylon*.

<b>Gene</b>	<b>Length, nt</b>	<b>Species</b>	<b><math>K_s</math></b>	<b><math>K_a</math></b>	<b>Average <math>K_s</math></b>	<b>Divergence rate</b>																																			
Glutamate dehydrogenase	3,204	<i>E. gansuensis</i>	1.290	0.042	1.251	$7.72 \times 10^{-9}$																																			
		<i>E. glyceriae</i>	1.211	0.042			Isoleucine tRNA synthase	3,216	<i>E. gansuensis</i>	1.236	0.048	1.243	$7.67 \times 10^{-9}$	<i>E. gansuensis</i> var <i>inebrians</i>	1.243	0.049	<i>E. glyceriae</i>	1.249	0.050	MCM7	2,441	<i>E. gansuensis</i>	0.957	0.040	0.961	$5.93 \times 10^{-9}$	<i>E. gansuensis</i> var <i>inebrians</i>	0.943	0.040	<i>E. glyceriae</i>	0.983	0.040	TSR1	2,487	<i>E. gansuensis</i>	1.080	0.075	1.050	$6.48 \times 10^{-9}$	<i>E. gansuensis</i> var <i>inebrians</i>	1.037
Isoleucine tRNA synthase	3,216	<i>E. gansuensis</i>	1.236	0.048	1.243	$7.67 \times 10^{-9}$																																			
		<i>E. gansuensis</i> var <i>inebrians</i>	1.243	0.049																																					
		<i>E. glyceriae</i>	1.249	0.050			MCM7	2,441	<i>E. gansuensis</i>	0.957	0.040	0.961	$5.93 \times 10^{-9}$	<i>E. gansuensis</i> var <i>inebrians</i>	0.943	0.040	<i>E. glyceriae</i>	0.983	0.040	TSR1	2,487	<i>E. gansuensis</i>	1.080	0.075	1.050	$6.48 \times 10^{-9}$	<i>E. gansuensis</i> var <i>inebrians</i>	1.037	0.073	<i>E. glyceriae</i>	1.033	0.070									
MCM7	2,441	<i>E. gansuensis</i>	0.957	0.040	0.961	$5.93 \times 10^{-9}$																																			
		<i>E. gansuensis</i> var <i>inebrians</i>	0.943	0.040																																					
		<i>E. glyceriae</i>	0.983	0.040			TSR1	2,487	<i>E. gansuensis</i>	1.080	0.075	1.050	$6.48 \times 10^{-9}$	<i>E. gansuensis</i> var <i>inebrians</i>	1.037	0.073	<i>E. glyceriae</i>	1.033	0.070																						
TSR1	2,487	<i>E. gansuensis</i>	1.080	0.075	1.050	$6.48 \times 10^{-9}$																																			
		<i>E. gansuensis</i> var <i>inebrians</i>	1.037	0.073																																					
		<i>E. glyceriae</i>	1.033	0.070																																					

**Supplementary Table 4:** Estimation of the divergence times between the basal *Epichloë* spp. and *Claviceps purpurea*.

Gene	Species	$K_s$	$K_a$	Average $K_s$	Divergence time, Mya
Glutamate dehydrogenase	<i>E. gansuensis</i>	0.841	0.048	0.830	53.7
	<i>E. glyceriae</i>	0.818	0.049		
Isoleucine tRNA synthase	<i>E. gansuensis</i>	0.918	0.058	0.939	61.2
	<i>E. gansuensis</i> var <i>inebrians</i>	0.927	0.061		
	<i>E. glyceriae</i>	0.971	0.059		
MCM7	<i>E. gansuensis</i>	0.749	0.052	0.766	64.6
	<i>E. gansuensis</i> var <i>inebrians</i>	0.786	0.051		
	<i>E. glyceriae</i>	0.764	0.052		
TSR1	<i>E. gansuensis</i>	0.735	0.073	0.720	55.6
	<i>E. gansuensis</i> var <i>inebrians</i>	0.705	0.074		
	<i>E. glyceriae</i>	0.720	0.070		

Mean divergence time, Mya:  $58.8 \pm 2.5$

**Supplementary Table 5:** Estimation of the divergence time between *E. gansuensis* and *E. glyceriae*.

<b>Gene</b>	<b>Species</b>	<b><math>K_s</math></b>	<b><math>K_a</math></b>	<b>Average <math>K_s</math></b>	<b>Divergence time, Mya</b>
Glutamate dehydrogenase	<i>E. gansuensis</i>	0.108	0.017	0.108	6.99
	<i>E. gansuensis</i> var <i>inebrians</i>	0.108	0.011		
Isoleucine tRNA synthase	<i>E. gansuensis</i>	0.080	0.009	0.080	5.22
	<i>E. gansuensis</i> var <i>inebrians</i>	0.080	0.011		
MCM7	<i>E. gansuensis</i>	0.117	0.010	0.117	9.87
	<i>E. gansuensis</i> var <i>inebrians</i>	0.117	0.010		
TSR1	<i>E. gansuensis</i>	0.094	0.017	0.087	6.71
	<i>E. gansuensis</i> var <i>inebrians</i>	0.080	0.016		

Mean divergence time, Mya:  $7.2 \pm 0.97$