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Molecular evolution of adenylating domain of aminoadipate reductase

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Abstract

Background: Aminoadipate reductase (Lys2) is a fungal-specific protein. This enzyme contains an adenylating domain. A similar primary structure can be found in some bacterial antibiotic/peptide synthetases. In this study, we aimed to determine which bacterial adenylating domain is most closely related to Lys2. In addition, we analyzed the substitution rate of the adenylating domain-encoding region.

Results: Some bacterial proteins contain more than two similar sequences to that of the adenylating domain of Lys2. We compared 67 amino acid sequences from 37 bacterial and 10 fungal proteins. Phylogenetic trees revealed that the *lys2* genes are monophyletic; on the other hand, bacterial antibiotic/peptide synthase genes were not found to be monophyletic. Comparative phylogenetic studies among closely related fungal *lys2* genes showed that the rate of insertion/deletion in these genes was lower and the nucleotide substitution rate was higher than that in the internal transcribed spacer (ITS) regions.

Conclusions: The *lys2* gene is one of the most useful tools for revealing the phylogenetic relationships among fungi, due to its low insertion/deletion rate and its high substitution rate. Lys2 is most closely related to certain bacterial antibiotic/peptide synthetases, but a common ancestor of Lys2 and these synthetases evolutionarily branched off in the distant past.

Background

Not only fungi, but also certain prokaryotes synthesize lysine through the 2-aminoadipate pathway [1–3]. However, the prokaryotic pathway is not identical to that of fungi. The fungal process required to synthesize lysine from 2-aminoadipate differs from that of prokaryotes [4]. The first step of this fungal-specific pathway is the reduction of 2-aminoadipate.

Aminoadipate reductase converts 2-aminoadipate to 2-aminoadipate 6-semialdehyde via an adenosylated derivative. In *Saccharomyces cerevisiae*, this reaction requires

Mg²⁺ and the participation of the products of two genes, *lys2* and *lys5* [5]. Recently, it has been shown that aminoadipate reductase is encoded by only *lys2*, and that the Lys5 protein appears to be a specific phosphopantetheinyl transferase for Lys2, converting the inactive apo-Lys2 to the active holo-Lys2 [6,7].

The *lys2* gene is a fungal-specific gene and generally appears to be present in a single copy in the genome. The Lys2 protein has no extensive homologous protein in eukaryotes, with the exception of fungi, but it does possess similarity to some bacterial antibiotic/peptide synthetases

[4,8–10]. Recently, *Drosophila* and mouse were found to have the analogue of Lys2, which function under degradation of lysine [11]. However, Lys2 is more similar bacterial antibiotic/peptide synthetases than the animal proteins. Lys2 has an adenylating, a peptidyl carrier, and a reductive domain. This protein has twelve conserved motifs. The adenylating domain contains nine conserved motifs [12]. In this study, we aimed to reveal which bacterial adenylating domain is the most closely related to Lys2.

In addition, in order to determine the substitution rate of *lys2*, we compared the *lys2* sequences from closely related fungi. In this study, we sequenced *lys2* fragments [13] and compared them among black-koji molds of the *Aspergillus niger* group.

Results and Discussion

The deduced amino acid sequences (each 343 amino-acids long) from *Aspergillus awamori* IAM 2112, *A. awamori* IAM 2299, *A. awamori* IAM 2300, *A. saitoi* IAM 2210, *A. saitoi* IAM 2215, *A. saitoi* IAM 14608, *A. saitoi* var. *kagoshimaensis* IAM 2190, and *A. saitoi* var. *kagoshimaensis* IAM 2191 were identical. Those from *A. usamii* IAM 2185 and IAM 2186 differed from the other black-koji molds by one amino acid. The nucleotide sequences from *A. awamori* IAM 2112, IAM 2299, and IAM 2300 were identical. Those from *A. saitoi* IAM 2210 and IAM 2215 were identical. Those from *A. saitoi* var. *kagoshimaensis* IAM 2190 and IAM 2191 were identical. Those from *A. usamii* IAM 2185 and IAM 2186 were identical. *Aspergillus awamori*'s sequence was 10 nucleotides different from that of *A. saitoi*

IAM 2210 and IAM 2215, and 40 nucleotides different from that of *A. usamii*.

We deposited the sequences in the DNA Data Bank of Japan under accession numbers AB079758, AB085587, AB079759, AB085588, AB085589, AB079760, AB085590, AB079761, and AB085591 for *A. awamori* IAM 2299, *A. awamori* IAM 2300, *A. saitoi* IAM 2210, *A. saitoi* IAM 2215, *A. saitoi* IAM 14608, *A. saitoi* var. *kagoshimaensis* IAM 2190, *A. saitoi* var. *kagoshimaensis* IAM 2191, *A. usamii* IAM 2185, and *A. usamii* IAM 2186, respectively.

Comparisons between *A. awamori* and *Penicillium chrysogenum* (Table 1) and between *A. awamori* and *A. fumigatus* (Table 2) showed that the rate of insertion/deletion in *lys2* was lower and the nucleotide substitution rate was higher than that in ITS regions. We therefore believe that *lys2* is a more powerful tool to reveal phylogenetic relationships among fungi than are the ITS regions.

The result of the homology search using BLAST showed that Lys2 had a more similar sequence to that of certain bacterial antibiotic/peptide synthetases than did any other existing proteins. In addition, some bacterial antibiotic/peptide synthetases were shown to contain more than two homologous regions. For example, RS05859 in *Ralstonia solanacearum* GMI1000 has five homologous regions. Therefore, we obtained 57 amino acid sequences, with a value of $E < 10^{-25}$, from 39 proteins (see Materials and Methods).

Table 1: Comparison between *Aspergillus awamori* and *Penicillium chrysogenum*

Region	Alignment length (A)	Insertions/Deletions (B)	Substitutions (C)	B/A	C/(A-B)
18S rDNA	1734	3	25	1.7×10^{-3}	1.4×10^{-2}
ITS1	185	10	34	5.4×10^{-2}	1.9×10^{-1}
ITS2	169	5	22	3.0×10^{-2}	1.3×10^{-1}
<i>lys2</i>	1032	9	242	8.7×10^{-3}	2.4×10^{-1}

Table 2: Comparison between *Aspergillus awamori* and *A. fumigatus*

Region	Alignment length (A)	Insertions/Deletions (B)	Substitutions (C)	B/A	C/(A-B)
18S rDNA	1733	0	11	0	6.3×10^{-3}
ITS1	188	6	23	3.2×10^{-2}	1.3×10^{-1}
ITS2	170	4	22	2.4×10^{-2}	1.3×10^{-1}
<i>lys2</i>	1032	0	202	0	2.0×10^{-1}

The phylogenetic tree (Fig. 1ab) shows that the adenylating domains from some bacterial antibiotic/peptide synthetases are distributed quite widely, and that duplications and/or horizontal transfers occurred many times. For example, *Anabaena* sp. PCC 7120 has 12 similar sequences within itself. In this tree, these 12 sequences were distributed among at least 6 groups. The present findings indicate that duplication and/or horizontal transfer occurred in the genome of *Anabaena* sp. PCC 7120. On the other hand, the adenylating domains from Lys2 formed a monophyletic cluster. However, the neighbor-joining tree presented here did not clarify which bacterial domain was most closely related to that of Lys2.

In order to determine which bacterial domain was most closely related to that of Lys2, a maximum likelihood analysis using PHYLIP version 3.6 [14] was carried out. We selected 27 amino acid sequences from the 67 sequences used in the neighbor-joining analysis. The alignment used in maximum likelihood analysis is shown in Fig. 2. The phylogenetic tree (Fig. 3) indicates that a protein (AGR L 2311) from *Agrobacterium tumefaciens* is most closely related to a common ancestor of Lys2, but this result had only weak bootstrap support (17%). In the bootstrap consensus tree (Fig. 3), the branch points at the early stage of evolution are very weak support. Animals and plants have no Lys2. If the common ancestor of eukaryotes had a similar protein, the other eukaryotes except for fungi had lost it.

Conclusions

This study indicated that Lys2 is more closely related to certain bacterial antibiotic/peptide synthetases than it is to any other known proteins. However, in the distant past, a common ancestor of Lys2 branched off from the bacterial antibiotic/peptide synthetase. This study did not find evidence for a direct horizontal transfer (i.e., at least not a recent horizontal transfer) between bacteria and a common ancestor of fungi. The *lys2* gene has been inherited during fungal evolution. On the other hand, in the course of bacterial evolution, the duplication and/or horizontal transfer have occurred.

Materials and Methods

In this study, we used *Aspergillus awamori* IAM 2299, *A. awamori* IAM 2300, *A. saitoi* IAM 2210, *A. saitoi* IAM 2215, *A. saitoi* IAM 14608, *A. saitoi* var. *kagoshimaensis* IAM 2190, *A. saitoi* var. *kagoshimaensis* IAM 2191, *A. usamii* IAM 2185, and *A. usamii* IAM 2186. Potato dextrose agar was used for the cultivation. Genomic DNA isolation, DNA amplification, and the sequencing of *lys2* fragments were performed according to the method of An *et al.* [13].

We compared the nuclear small subunit rRNA genes (18S rDNAs), ITS1 regions, ITS2 regions, and *lys2* genes be-

tween *A. awamori* and *Penicillium chrysogenum* and between *A. awamori* and *A. fumigatus*. The following nucleotide-sequence accession numbers were used: D63695, *A. awamori* 18S rDNA [15]; U03518, *A. awamori* ITS1 [16]; U03519, *A. awamori* ITS2 [16]; AB076077, *A. awamori lys2* [13]; M55628, *P. chrysogenum* 18S rDNA; AJ270768, *P. chrysogenum* ITS1 and ITS2 [17]; Y13967, *P. chrysogenum lys2* [18]; AB008401, *A. fumigatus* 18S rDNA [19]; AF455542, *A. fumigatus* ITS1 and ITS2. The preliminary sequence of *lys2* was obtained from The Institute for Genomic Research website at <http://www.tigr.org>.

We performed a homology search using BLAST [20] with the parameter values given in the Kyoto Encyclopedia of Genes and Genomes [21]. The query amino acid sequence was a fragment of *Saitoella complicata* Lys2 [13]. In this study, we phylogenetically analyzed 57 amino acid sequences (all sequences had a value of $E < 10^{-25}$, according to the BLAST search results) separately from those of fungi. Multiple alignment was created using CLUSTAL W [22] among the 57 high-scoring sequences and those of 10 fungal Lys2 proteins. A neighbor-joining phylogenetic tree was constructed using MEGA version 2.1 [23] with 1,000 bootstrap replicates. Based on this tree, we selected 27 amino acid sequences for a maximum likelihood analysis, which was performed using PHYLIP version 3.6 [14]. We used three programs (consense, proml, and seqboot) for constructing phylogenetic tree with 100 bootstrap replicates.

The protein names used in this study are AGR_L_2311, *Agrobacterium tumefaciens* C58 (Cereon) AGR_L_2311; all1647, *Anabaena* sp. PCC 7120 peptide synthetase; all2642, *Anabaena* sp. PCC 7120 multifunctional peptide synthetase; all2643, *Anabaena* sp. PCC 7120 microcystin synthetase B; all2644, *Anabaena* sp. PCC 7120 peptide synthetase; all2645, *Anabaena* sp. PCC 7120 peptide synthetase; all2647, *Anabaena* sp. PCC 7120 microcystin synthetase B; all2648, *Anabaena* sp. PCC 7120 peptide synthetase; all2649, *Anabaena* sp. PCC 7120 probable non-ribosomal peptide synthetase; all1695, *Anabaena* sp. PCC 7120 probable peptide synthetase; Atu3682, *Agrobacterium tumefaciens* C58 (U.Washington/Dupont) non-ribosomal peptide synthetase; b0586, *Escherichia coli* K-12 MG1655 enterobactin synthetase component F; BG10168, *Bacillus subtilis* 168 surfactin synthetase subunit 1; BG10169, *Bacillus subtilis* 168 surfactin synthetase subunit 2; BG10170, *Bacillus subtilis* 168 surfactin synthetase subunit 3; BG10970, *Bacillus subtilis* 168 peptide synthetase; BG10971, *Bacillus subtilis* 168 peptide synthetase; BG10972, *Bacillus subtilis* 168 peptide synthetase; BG11243, *Bacillus subtilis* 168 probable non-ribosomal peptide synthetase; BG11961, *Bacillus subtilis* 168 peptide synthetase; ECs0625, *Escherichia coli* O157:H7 Sakai enterobactin synthetase component EntF; JW0578, *Es-*

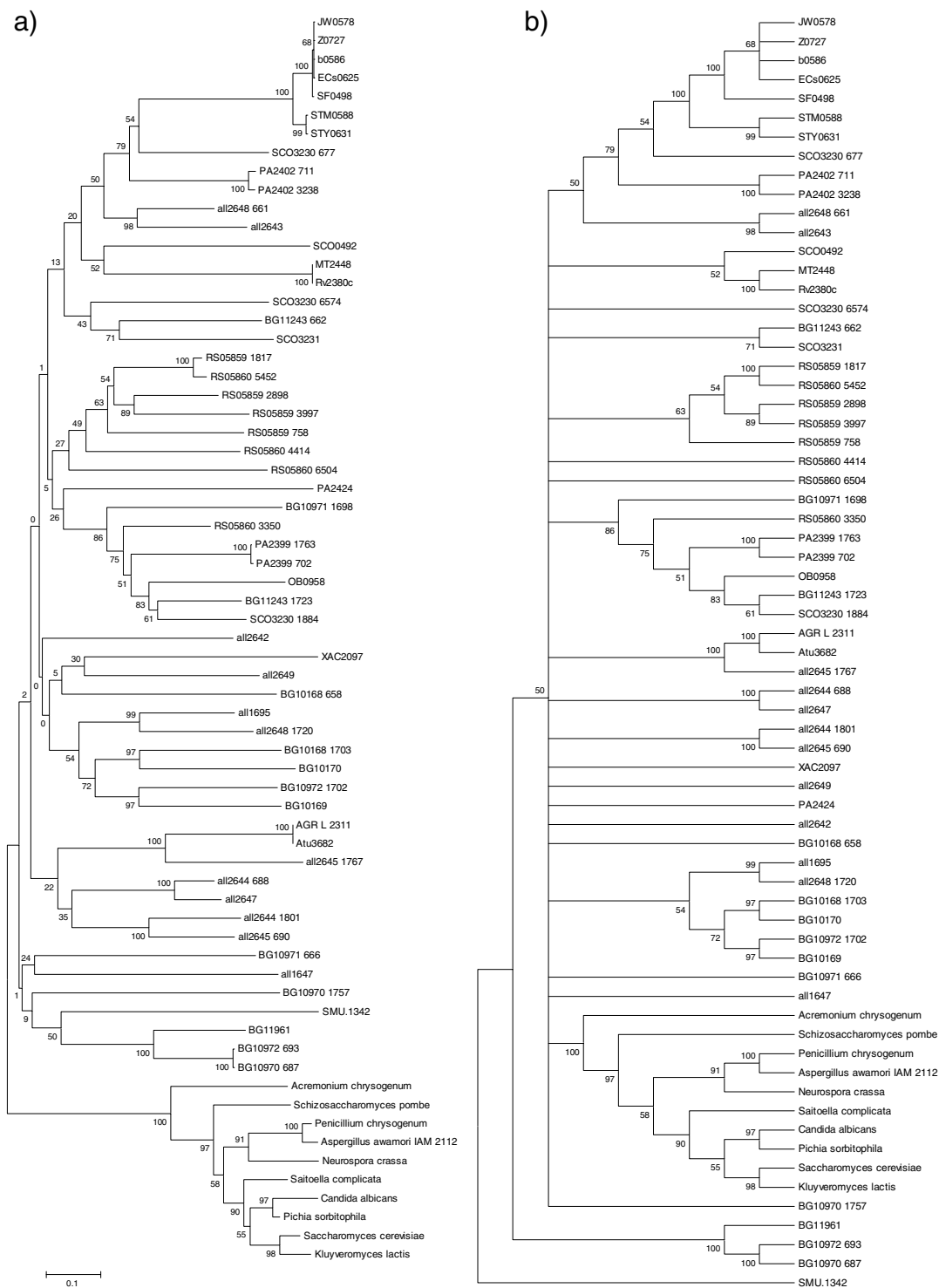


Figure 1

a) Phylogenetic relationships among 67 amino acid sequences from the adenylating domain of Lys2 and bacterial antibiotic/peptide synthetase. A total of 176 amino acid sites were considered without gap regions in alignment. b) The bootstrap consensus tree. The cut-off value for consensus was 50%. Protein names were shown in Materials and Methods.

BG11961 ---ELFWPY AGASVYLLPQ GGEKEP--EV IAKAIEEQKI TAMHFVPSML HAFLEQIKYR SVPIKTNRLK RVFSGG---- EQLGTHLVSR
Rv2380c ---EIFGTLA CGARMVIRPR GGLTDI--GY LTALLRDEGI TAMHFVPSLL GLFLSLPG-- --VSQWRTLQ RVPIGG---- EPLPEGEVADK
SCO0492 ---EIFLPLV SGRRLVLRP GGERDP--HH LLSVIDEQRV TFTYLVSSML DVLLMAGD-- --SGRLDSL RHMVCGG---- EVLTPELYER
a112643 ---EFFWTL NGRLVMSKP GGHQDP--NY LLEITAQHKI TTLHFVPTML GVFLAEPNL-- --NERCHSLK RVICSG-- EALSIEIQNR
RS05859_18 ---ELFWPLL AGARLVNARP EGHKAP--AY LAATIEQAGI TTLHFVPSML QLFLDQVEAG ---RCQGLR RMLCSG---- EALPHALQQR
RS05859_75 ---DVMVPLL NGRRVVIDQ PTVLAP--ER FAQALRRGQV SVLWMTAGQF HQYAPSLIG-- ---VFPQLR YLMVGG---- DVLDPATIAM
RS05860_44 ---L---LL CGACLVLAPA QALMPG--AA LTHLLDRERI THVTLPPAVL ALMPEQALP- ---ADC HLIVAG---- EACPPSLVR-
RS05860_65 ---KNLGLPLL AGGRLHLAPG --FVP--DA LVAQIRREGI THNLSPSAF HALIDAAGAE ---GLGGLR RVVLGG---- EPQTARLQG
PA2424 ---LLAPLL CGARRVLRQA -GQWGA--EE ICELIRAEV SVLGFTPSYG SQAQWLESQ G---RQLPVR MCITGG---- EALTGHLQR
XAC2097 ---LYLPLI CGGTTELLPE -RDEIE--AL LKRVCAQDFI CLVKITPAHL DVL TQQLAAC G---GTPSVS LRVVGG---- EALHASTVKR
BG11243_66 ---EYLFPI SGAQIVIAKK ETIREP--QA LAQMIENFDI NIMQATPTLW HALVTSEPEK ---LRGLR VLVGG-- ALPSGLQEL
SCO3230_65 ---EMWVPLV SGGTVVAPP G-HLDP--AA ITDLITAHDI TAIHLTAGFF RVVAEEAPEK ---FAGVR EVLTGG---- DVVSPAAR
a112644_68 ---EIFLPI VGACLVLVER EVTLDGER-- LAQATAHQI TFHQATPATW RLLLASWEG ---K-QDL KILCGG---- EALDNTLAQQ
a112649 ---EIFVPLS WGGCVILADN ALQLP----- -ELPAAQV TINTVPSAA RELLRLNGIA ---A-TVQ TVNLAG---- EPLPKSLVDE
a112642 ---EYTPLL VGKAVILLPE AEIEEALKNA LSSARNFSLV KLTPAHSIL SPLLPSADLP- ---G-YPQ AFIIQG---- EALTEQHLEF
a112642 ---LIYMALG SGAKLCLAKS ESLLPG--ET LKLLLRDNVA THITITPSAL SLLPSADLP- ---HLR MVLVGG---- EAPSPETIAK
a111695 ---EWAALL NGKLVLMPI NIPSLQEIG- --MAIKQYHV TLLWLTAGLF NLMVEQIEH ---LKSLR QLLAGG---- DVSVYHVSK
BG10168_65 ---KQIFASLL LGQTLVYVK KTVTNG--AA LTAYYRKNSI EADTGPAPL Q--MLAAGD FEG--LKLK HMLIGEGELS SVLSDKLLKL
BG10169 ---EVFGALL NGAALVYVK RHVLD--KQ FAALFREQSI TTMHLTSPLF N--QLAAKDA GHF--GTLR HLIIGG---- DALVPHIVSK
BG10971_16 ---EMFGALL NGSTLVVSK ETARDP--QA FRLLKKERV TVLNQPTAF YGLMLEDQNH TDH---LNIR YVIFGG---- EALQPLGLS
BG10971_66 ---LFTPLL SGACVLLTD DEAKD--VLA LKRKIARYKV SHMIIVPSL RVLLEVMAD ---DAKSLR IVTFAG---- EAVTDLHLQ
a111647 ---EIFWTLM SGATICPVQR EVVLPN--WE FARWJQETQI NVMHFVPSLF GEFISALENE TWS--FPQLR WLMFSG---- EALPMSFIQR
JW0578 ---EFFWPI AGAKLVMAEP EHRDP--LA MQQFAEYGV TTFHFVPSML AAFVASLTPQ TARQSCATLK QVFCSG---- EALPADLCRE
BG10970_17 FSGDLARTL NGGTLVCPD ETRLEP--AE IYKIKSQRI TVMESTPALI IPMVEVYRN ---QFKLPDL ILLGS---- EMLKQDFKT
SMU_1342 ---ELFGWTF EGAVLVLEN GEEKDP--QR IIEIINSQNI SKLHFVPSML NVFLEFCERE N-KDSLKSLS IVFSSG---- DALTQVQIK
AGR_L_2311 ---DIFGPLA VGGAIVIPVR EESVDN--AR WMKLLQHRV TVWNSVPALA QLLLAELPAL R---EKPLLR MIMMSG---- DWIPVSLPPA
Acremonium ---RDIFTPFL LGAKIIPPA DVIAIY--EL LAQWKNDRV TVTHLTPAMG QILVGGATAQ ---IPSLR NAFFVG---- DLLSKKDTTR

FYELLPNVS- -ITNSYGPT EATVEAAFFDC P-----P HEKLERIPIG KPVHVRVLYL LNQNQ--RML PVGCIGELYI AGAGVARGYL NRPA----LT
FHATFD-AL- -LHNFGYPT TVINASRFKV V-----G PQGTRNFIQV RPKINTMHL LDDSL--QPV PTGIGIYI GTHVAYGYH RRAG----LI
FRTRLD-IP- -LYHGYGPAE TTIGVSHVYV R-----G AERLSTSIG RANPNTRYLV LDDSL--RPV PVGVGGELYA GGLLGRGVY NAPG----LT
FRLDLD-AE- -LHNYGPT EADIVTYMWC Q-----P TDNLHTVPIG RPIANTQIYL LDNDL--QPV PLGIGIYI GGVGARGYL KRPD----LT
SLARFPHE- -LHNYGPT EADIVTAMWC -----NA EIHPGVPIG RPIANTQIYV LDAYR--QPV PLGVTGEIYI GGAGVARGYL NRPE----LT
VLREGAPQH- -LLNGYGPT TTTFATTHLI -----QA VAAGRGPIG RPIANTQIYV LDAYQ--QPV PLGVTGEIYV GGAGVGLYL NRPE----LT
--LWSEGR- -MNAVYPT ATVCATMS-- -----RA LTAQDAPSIG GPGINVRVYV LDAYL--QPV PVGVTGEIYI AGSGLARGYL QRAG----LT
--LPEPRR- -FNSYGPTE CSDVVAWAL DA---E-LD RYRAASVPLG RSVNRVLYV LDAH--QPV PTGVAGEIYV GGAGVGRYV NRPE----LT
IRQAFAPAS- -FNAYGPT TVMPLACLA PE---R-LE EG--AASVPIG SVVGARVAYI LDADL--ALV POGATGEIYV GGAGLARGYL ERPA----LS
LRQLAPHAR- -VNYEGYPT TVGCVAYEI PL---D-WD AGTLATPIG RPDNMRVLY L DANR--QTV PAGVAGELCI AGSQVTRGYL NRPE----LT
QDLHCS- -LHNYGPT TTIWASAAFL EE---G--L KG--VPPIG KPIWNTQYV LDNGL--QPV PPGVGEIYI AGTLARGYL HRPD----LT
VLAHHPRIV- -LRHLYGPT TILCVTQHEV TA---PYEA RG---SLPVG RATGNTRAVY LDYRL--QPV PAGVPELFI SGSGLARGYL DRPD----LT
LLSCT-QE- -VWNYGPT TTIWS----- -AAQK LSIDEPVTIG HPIANTQFYV DDEHL--QPV PIGVPELYI GGAGVAKGYL QRPD----LT
LYQSTIER- -VWNYGPT DTYTSTH- -----ALIP RNSQAPPTIG QPIANTQYI LDQNL--QPV PVGIGIYI SGAGLARGYL KRPK----LT
WRSYFPQTK- -LNEYGPT TVGCCIYD- -----ASQG KSSKNVPIG RPIANTQYI LDYRL--QPV PIGVPELYI GGAGVARGYL NRPE----LT
WSQGR--R- -FINAYGPT VTNVNS----- -MVLG GNGHPLVPTI RPSANKLYI LDNYL--QPV PIGVIGELYI GGTLARGYL NRPD----LT
VIEELPNCQ- -LNGYGPTE NTFCTCHK- -----ITVN DLIKDSPIG RPIANTQYI LDDVL--QLV PIGIAGELYI GGDGLARGYL NKPD----LT
FKEAGTAPR- -LNVYGPTE TCVDASVHPV IP---ENAV QSAY--VPIG KALGNRLYI LDQKG--RLQ PEGVAGELYI AGDVGRGYL HLPD----LT
VKQASPSL- -LWNGYGPTE NTFSTSLI DR---EYGG S-----IPIG KPIGNSTAYI MDEQQ--CLQ PIGAPGELCV GGVGARGYL NLPE----LT
WNEQYHTD- -LHMYGITE TTVHVTFKKL SA---ADIA KNK---SNIG RPLSTLQAHV MDAH--NLQ PIGVPELYI GGVGARGYL NRPE----LT
NQIICPSAE- -LANEYGPTE NSVATTILR- -----H LNKRIITIG HPIRNTKVYV LHGN--QMQ PTGAAGELCI SGAGLARGYL QRQE----LT
WIDRHLKGT- -LANLYGPT ASIDVTCHLI TE---R-PD ERLTQPIG KADINVYKV LDGGM--QPV PQGNMELW GGVQALQYL KDPE----KT
WQQLTG--AP -LHNYGPT AAVDVSWYPA FG---EELA QVRGSSVPIG YPWNVTGLRI LDAMM--HPV PPGVAGDLYL TGQLAQLYV GRPD----LT
LDRFGQSMR- -IINSYGPT ATIDSSFYET SM---G--G ECTGDNVPIG SPLPNVHYV LSQTD--QIQ PIGVAGELCI GGAGVAKGYL HKPD----LT
FYSIFNDKP QLNLGPT EATIEVYFDC SN-----L DYKSDVPIG EPLDNVAYV LNDKK--QKC PIGVPELYI GGIQVAGYL NKED----AT
LKAQLPAD- -LISLGGATE ASIWSIFPHI G-----EA LRDWTSIPY QPLANQRVYV LDDQG--RRC PPWVTGRLFI GGVGARGYL GRPQ----LT
LRSIAPNV- -VNLNGYPT SGRVSVFKV PSRAKDPHFL DSLPDIPIVG GGMQNVQLLV VDPNDKMLC DLGEGELYV RAAGLAEGYL GDDEKTAELN

EERFLEDPFY ERMKYTGQVA RMLPDGNVEF LGRTDDQVKI RGYRIEPEGI EAALRSIEGV REAAVTRTD --EVAQLER LLPGYMVPAY MIEMEQUWPV
AERFVADPN SRMYRSGDLA RRNADGIEF VGRADEQVKI RGFRIELGIV AAAIADVPTV GQAVVVSDL --RIRARVAA ALPEYMLPAA YVLDIPI
ASRFVANPFA SRLYRTGDLA RFPADGSLDF LGRADNQVKI RGMRIEEDV EVGLAEHPRV RHTCVVARKN --EVKAWAGE HMVEYMLPST VVMTFEPFL
AERFVGGT STLYKTGDLA RYLPDGNLEY LGRLDNQVKI RGLRIELGEI EAVINQHPDV QQAVVILDSQ --ELEKLLAS QLPEYMLPST FVMSLPL
AERFVNPFH ERYMRTDGLG RMLPDGSLEY QGRADAQVKL RGFRIELGEI EARLSQCAGV SEAVVAMRED --TLREQLQA SLPEYMLPAA YVLEHLPL
AERFVNPFH ERYMRTDGLG RMLPDGSLEY QGRADAQVKL RGFRIELGEI EARLSQCAGV SEAVVAMRED --TLREQLQA SLPEYMLPAA YVLEHLPL
AERFMANPFA ERYMRTDGLG RMLPDGSLEY QGRADAQVKL RGFRIELGEI EARLSQCAGV SEAVVAMRED --TLREQLQA SLPEYMLPAA YVLEHLPL
GACFLADPFA ARMYKTGDG RMLANGSLEY LGRSDDQVKL RGFRIELGEI GASLARCDGV REAAVLAREDS --ALRRHMGA QLPEHMPAA YVLEHLPL
AERFVPPFA -RLYRTGDLY RLCNNGQVEY VGRIDHGVKI RGFRIELGEI EARLLEHPQV REALVLALDS --ALKTHLQK QLPDYMPAH LLLASLPL
EQRFVDPFG QRMVCSGDLA RMPDGTLEY LGRNDQIKL RGFRIEPAEV SSRILDNPLV ADAAVVHTA --RLREQLQA RLPDYMPAV YVLEHLPL
AERFVADPY TRMYRTGDQA RWRADGSLDY IGRADHQIKI RGFRIELGEI DAVLANHPHI EQAAVVRED --ELRRYMG A SLPDYMPVA FVMEDELPL
CERFVADPY ERYMRTDGLV RYNAAGELEY LARADDQVKI RGFRIELGEI EAVLATRPEL AQAAVVRED --ALRAFSRQ ALPDYMPVA FVLTGLPL
AERFLNQQS- -TLYKTGDRV RYLPDQKLEY LGRLDQVKI RGFRIELGEI EAVLAHQPHI SQAVVSVQED --DLQQLAN KLPKYMIPGV FVLTALPL
DERFIKQS- -TLYKTGDRA RYLPDGNIEY LGRFDHQVKI RGFRIELGEI EALLHQHPEL TQAVAVRND --ELRQFLAA KLPYMLPPTA FVLTLEPL
AERFISQSS- -TLYKPGDRA RYLSGDTIEY LGRLDQVKI RGFRIELGEI EAILKAHPSV QEAVVILQKV --DFRQYLAT KLPYMLPSPA FVLEQLPL
AERFIRDWGL SRLYKTGDLA YYPDGRIRL LGRVDNQVKI RGFRIEPEV ETLQCQHPGV RAGVIVRED --SLRAFMR E KLPEYVPSA FVLLTDPL
AEKFIHPH- -LYKTGDRV RMLPDGTIEF LGRIDFQVKI RGFRIELGEI EAILAQHPSV RASAVLAQED --ELRHFLQK KLPYMLPSPA FISLEKPL
EEKFLQDPF DRMYRTGDV RMLPDGTIEY LGREDDQVKV RGYRIELGEI EAVIQAPDV AKAVVLARPD --GLREHAAR QLPDYMPVA FTEVTEIPL
EKQFLDPPF RMYRTGDVA RMLPDGNIEF LGRIDNQVKV RGFRIELGEI ETKLNMAEHS TEAAVIRKN --ELRKTLSQ SLPDYMPVA FVMDLPL
ADRFSVNPYL DRLYRTGDLA KRLSNGELEY LGRIDEQVKV RGHRIELGEI QAALLQYPMI KEAAVITRAD --DIRTYLKN ALPDMPLPAR MIQIDSPV
QKAFSDHPL ERYMRTGDG IGRFDQVKI RGFRIELREI ETVLRQDVKI KEAAVLARDV --DLYQHLAG TLPYMLPSPA IINISQML
QAQFCNPPT DYLYRTGDLY KELPDGTIEY HGRIDHGVKI RGFRIELGEI ESVLTHPDV REAAALAVDY --FLKEYLEQ KLPYMLPQV FLWQLPL
ASRFIADPFA ERYMRTGDVA RMLDNGAVEY LGRSDDQVKI RGFRIELGEI RGRQALPDV EQAVTHACVI --ALQAQRE LTPHMPVPR LLQLPQL
QMKFTENPV ERYMRTGDRA CHLNGTIRL LGRMDYQVKI RGYRIETEEI ESVLQTLGV REAAVAVQHD --ALRAALTK ELPYMLPSPA LIPLVMPPL
KKSFVRLPKI SRLYATGDV KWTSEKLI IGRSDDQVKI RGYRIELGEI EYLLKKSQV NCLVSLQNKL --KIKEELKT LLPDYMPST IYVPEPFI
AERFIPDFA LLLYETDGL LGRFDQVKI RGFRIELGEI EALLQENV AEAVVTMGQ -----PP ALIAYVPSH KGSIG----
RSKFVANV FV DRLYRTDGLG RRRADGSEV TGRIDSQVKI RGFRIELGEI DSHLSQHPYV RENITLVRRD SEDCKFLSA KVPKYAVPSL LIPLARMP

Figure 2
Alignment of the selected 27 amino acid sequences. This alignment was used in the maximum likelihood analysis. Protein names were shown in Materials and Methods.

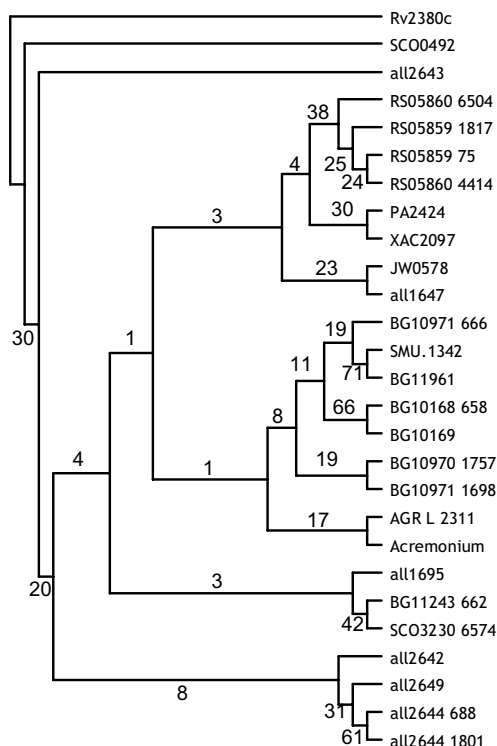


Figure 3

The bootstrap consensus tree among the selected 27 amino acid sequences based on the maximum likelihood analysis. The JTT model was used as the model of amino acid substitution. Number of times of bootstrap replicates was 100. Number of times to jumble in the proml program was 5. Protein names were shown in Materials and Methods.

Escherichia coli K-12 W3110 Enterochelin synthetase component F; MT2448, *Mycobacterium tuberculosis* CDC1551 peptide synthetase; OB0958, *Oceanobacillus iheyensis* HTE831 monomodular nonribosomal peptide synthetase; PA2399, *Pseudomonas aeruginosa* PA01 pyoverdine synthetase D; PA2402, *Pseudomonas aeruginosa* PA01 probable non-ribosomal peptide synthetase; PA2424, *Pseudomonas aeruginosa* PA01 probable non-ribosomal peptide synthetase; RS05859, *Ralstonia solanacearum* GMI1000 probable peptide synthetase protein; RS05860, *Ralstonia solanacearum* GMI1000 probable peptide synthetase protein; Rv2380c, *Mycobacterium tuberculosis* H37Rv mbtE; SCO0492, *Streptomyces coelicolor* A3(2) putative peptide synthetase; SCO3230, *Streptomyces coelicolor* A3(2) CDA peptide synthetase I; SCO3231, *Streptomyces coelicolor* A3(2) CDA peptide synthetase II; SF0498, *Shigella flexneri* 301 (serotype 2a) ATP-dependent serine activating enzyme; SMU.1342, *Streptococcus mutans* UA159

(serotype C) putative bacitracin synthetase 1; STM0588, *Salmonella typhimurium* LT2 enterobactin synthetase, component F (nonribosomal peptide synthetase); STY0631, *Salmonella typhi* enterobactin synthetase component F; XAC2097, *Xanthomonas axonopodis* pv. *citri* 306 ATP-dependent serine activating enzyme; Z0727, *Escherichia coli* O157:H7 EDL933 enterobactin synthetase component F.

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