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Promising antimicrobial protein from *Klebsiella*

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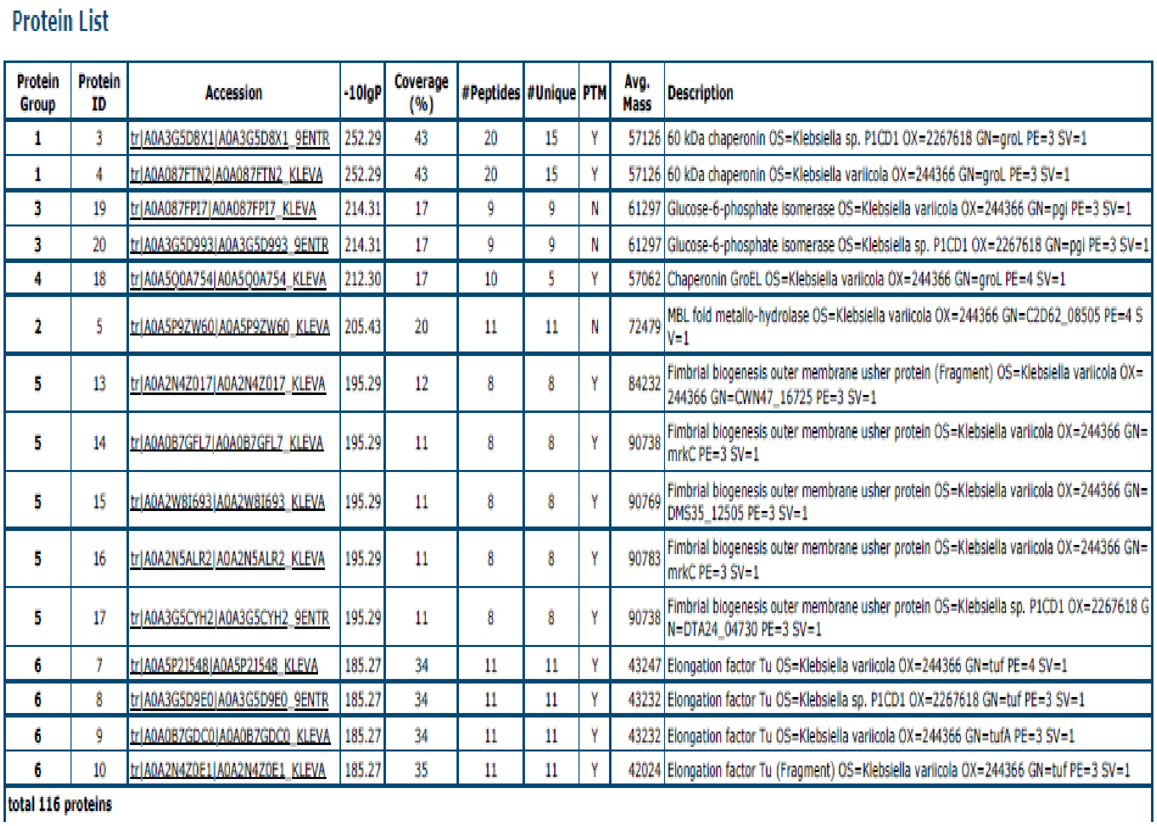
Supplementary Data

Suppl. Table S1 – Percentage purity of the protein obtained after Ammonium sulphate precipitation and HPLC

Procedure/ step	Fraction volume	Total proteins in µg/mL	Micro gram of Proteins in per mL of fraction	% Purity
Culture supernatant	125 mL	73.75 mg	0.59	59%
Ammonium sulphate precipitated, dialyzed &Lyophilized sample of 70% induced (ES)	1.0 mL	0.990mg	0.99	99%
Lyophilized reconstituted peak 5 from HPLC of 70% induced (ES)	500 µL	39.7µg	0.1588	15.88%

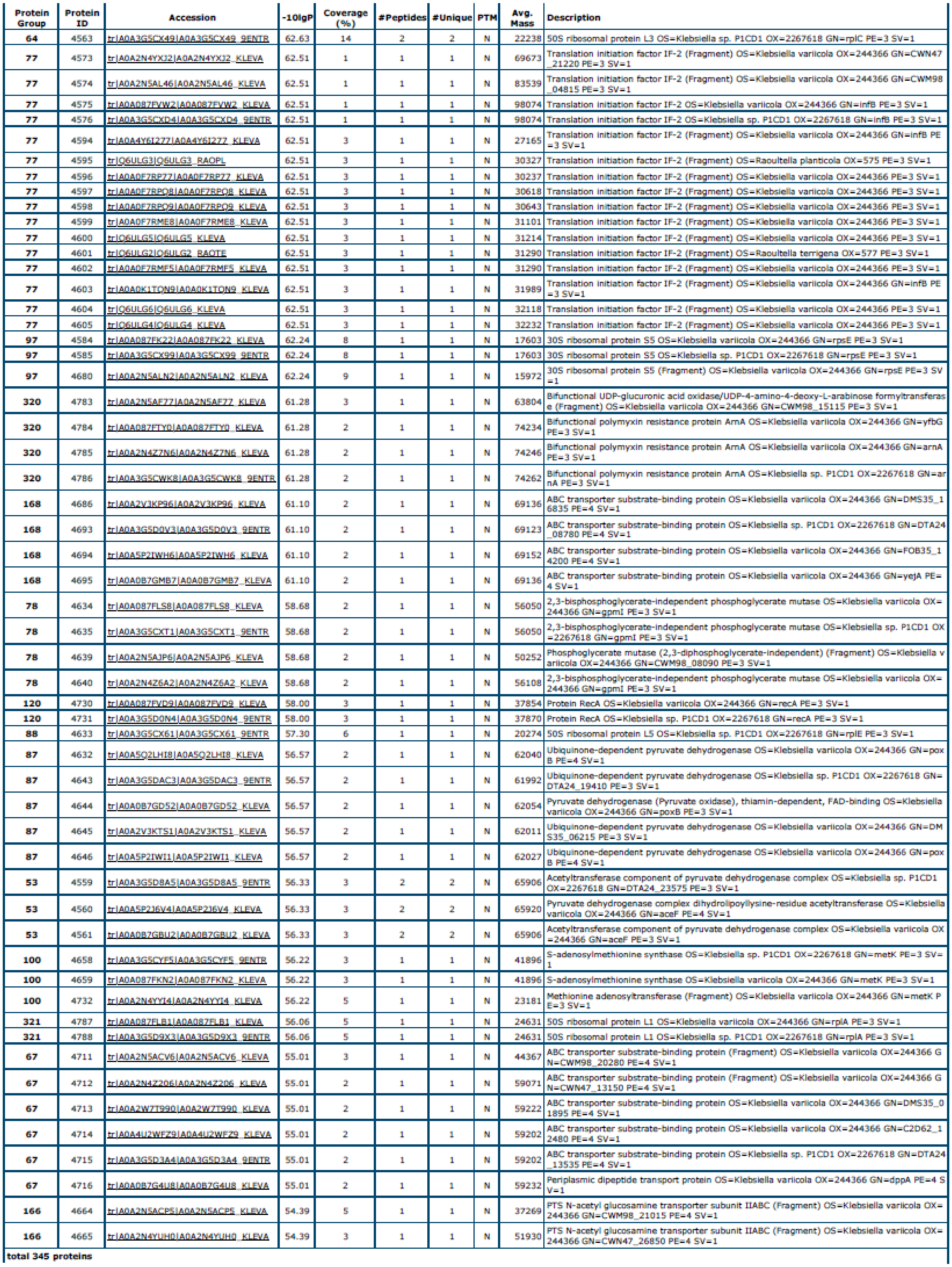


Protein List

Protein Group	Protein ID	Accession	-10lgP	Coverage (%)	#Peptides	#Unique	PTM	Avg. Mass	Description
1	3	tr|A0A3G5D8X1|A0A3G5D8X1_9ENTR	252.29	43	20	15	Y	57126	60 kDa chaperonin OS=Klebsiella sp. P1CD1 OX=2267618 GN=groL PE=3 SV=1
1	4	tr|A0A087FTN2|A0A087FTN2_KLEVA	252.29	43	20	15	Y	57126	60 kDa chaperonin OS=Klebsiella variicola OX=244366 GN=groL PE=3 SV=1
3	19	tr|A0A087FPI7|A0A087FPI7_KLEVA	214.31	17	9	9	N	61297	Glucose-6-phosphate isomerase OS=Klebsiella variicola OX=244366 GN=pgi PE=3 SV=1
3	20	tr|A0A3G5D993|A0A3G5D993_9ENTR	214.31	17	9	9	N	61297	Glucose-6-phosphate isomerase OS=Klebsiella sp. P1CD1 OX=2267618 GN=pgi PE=3 SV=1
4	18	tr|A0A5Q0A754|A0A5Q0A754_KLEVA	212.30	17	10	5	Y	57062	Chaperonin GroEL OS=Klebsiella variicola OX=244366 GN=groL PE=4 SV=1
2	5	tr|A0A5P9ZW60|A0A5P9ZW60_KLEVA	205.43	20	11	11	N	72479	MBL fold metallo-hydrolase OS=Klebsiella variicola OX=244366 GN=C2D62_08505 PE=4 SV=1
5	13	tr|A0A2N4Z017|A0A2N4Z017_KLEVA	195.29	12	8	8	Y	84232	Fimbrial biogenesis outer membrane usher protein (Fragment) OS=Klebsiella variicola OX=244366 GN=CWN47_16725 PE=3 SV=1
5	14	tr|A0A0B7GFL7|A0A0B7GFL7_KLEVA	195.29	11	8	8	Y	90738	Fimbrial biogenesis outer membrane usher protein OS=Klebsiella variicola OX=244366 GN=mrkC PE=3 SV=1
5	15	tr|A0A2W8I693|A0A2W8I693_KLEVA	195.29	11	8	8	Y	90769	Fimbrial biogenesis outer membrane usher protein OS=Klebsiella variicola OX=244366 GN=DMS35_12505 PE=3 SV=1
5	16	tr|A0A2N5ALR2|A0A2N5ALR2_KLEVA	195.29	11	8	8	Y	90783	Fimbrial biogenesis outer membrane usher protein OS=Klebsiella variicola OX=244366 GN=mrkC PE=3 SV=1
5	17	tr|A0A3G5CYH2|A0A3G5CYH2_9ENTR	195.29	11	8	8	Y	90738	Fimbrial biogenesis outer membrane usher protein OS=Klebsiella sp. P1CD1 OX=2267618 GN=DTA24_04730 PE=3 SV=1
6	7	tr|A0A5P2J548|A0A5P2J548_KLEVA	185.27	34	11	11	Y	43247	Elongation factor Tu OS=Klebsiella variicola OX=244366 GN=tuf PE=4 SV=1
6	8	tr|A0A3G5D9E0|A0A3G5D9E0_9ENTR	185.27	34	11	11	Y	43232	Elongation factor Tu OS=Klebsiella sp. P1CD1 OX=2267618 GN=tuf PE=3 SV=1
6	9	tr|A0A0B7GDC0|A0A0B7GDC0_KLEVA	185.27	34	11	11	Y	43232	Elongation factor Tu OS=Klebsiella variicola OX=244366 GN=tufA PE=3 SV=1
6	10	tr|A0A2N4Z0E1|A0A2N4Z0E1_KLEVA	185.27	35	11	11	Y	42024	Elongation factor Tu (Fragment) OS=Klebsiella variicola OX=244366 GN=tuf PE=3 SV=1

total 116 proteins

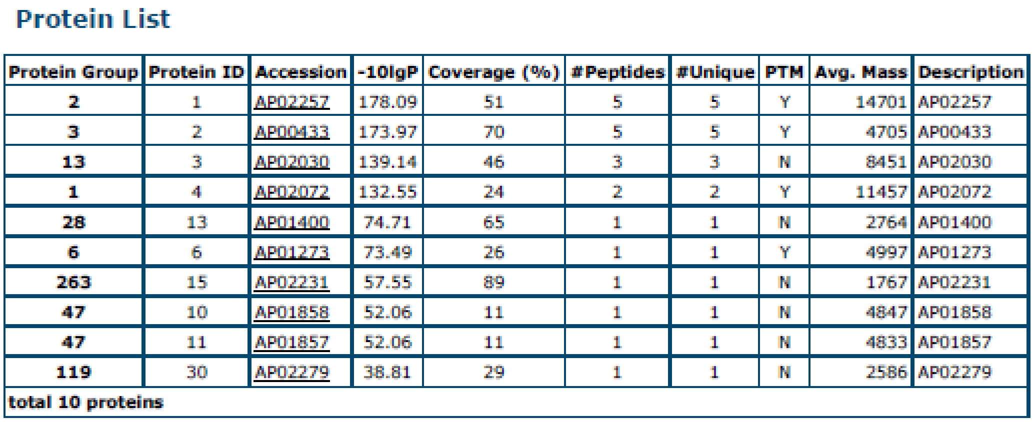
Suppl. Fig S1 – Sample list of 15 proteins/ peptide out of 116 proteins obtained after MS analysis



Protein Group	Protein ID	Accession	-10lgP	Coverage (%)	#Peptides	#Unique	PTM	Avg. Mass	Description
64	4563	tr|A0A3G5CX49|A0A3G5CX49_9ENTR	62.63	14	2	2	N	22238	50S ribosomal protein L3 OS=Klebsiella sp. P1CD1 OX=2267618 GN=rplC PE=3 SV=1
77	4573	tr|A0A2N4YXJ2|A0A2N4YXJ2_KLEVA	62.51	1	1	1	N	69673	Translation initiation factor IF-2 (Fragment) OS=Klebsiella variicola OX=244366 GN=CWN47_21220 PE=3 SV=1
77	4574	tr|A0A2N5AL46|A0A2N5AL46_KLEVA	62.51	1	1	1	N	83539	Translation initiation factor IF-2 (Fragment) OS=Klebsiella variicola OX=244366 GN=CWM98_04815 PE=3 SV=1
77	4575	tr|A0A087FVW2|A0A087FVW2_KLEVA	62.51	1	1	1	N	98074	Translation initiation factor IF-2 OS=Klebsiella variicola OX=244366 GN=infB PE=3 SV=1
77	4576	tr|A0A3G5CXD4|A0A3G5CXD4_9ENTR	62.51	1	1	1	N	98074	Translation initiation factor IF-2 OS=Klebsiella sp. P1CD1 OX=2267618 GN=infB PE=3 SV=1
77	4594	tr|A0A4Y6I277|A0A4Y6I277_KLEVA	62.51	3	1	1	N	27165	Translation initiation factor IF-2 (Fragment) OS=Klebsiella variicola OX=244366 GN=infB PE=3 SV=1
77	4595	tr|Q6ULG3|Q6ULG3_RAOPL	62.51	3	1	1	N	30327	Translation initiation factor IF-2 (Fragment) OS=Raoultella planticola OX=575 PE=3 SV=1
77	4596	tr|A0A0F7RP77|A0A0F7RP77_KLEVA	62.51	3	1	1	N	30237	Translation initiation factor IF-2 (Fragment) OS=Klebsiella variicola OX=244366 PE=3 SV=1
77	4597	tr|A0A0F7RPQ8|A0A0F7RPQ8_KLEVA	62.51	3	1	1	N	30618	Translation initiation factor IF-2 (Fragment) OS=Klebsiella variicola OX=244366 PE=3 SV=1
77	4598	tr|A0A0F7RPQ9|A0A0F7RPQ9_KLEVA	62.51	3	1	1	N	30643	Translation initiation factor IF-2 (Fragment) OS=Klebsiella variicola OX=244366 PE=3 SV=1
77	4599	tr|A0A0F7RME8|A0A0F7RME8_KLEVA	62.51	3	1	1	N	31101	Translation initiation factor IF-2 (Fragment) OS=Klebsiella variicola OX=244366 PE=3 SV=1
77	4600	tr|Q6ULG5|Q6ULG5_KLEVA	62.51	3	1	1	N	31214	Translation initiation factor IF-2 (Fragment) OS=Klebsiella variicola OX=244366 PE=3 SV=1
77	4601	tr|Q6ULG2|Q6ULG2_RAOTE	62.51	3	1	1	N	31290	Translation initiation factor IF-2 (Fragment) OS=Raoultella terrigena OX=577 PE=3 SV=1
77	4602	tr|A0A0F7RMF5|A0A0F7RMF5_KLEVA	62.51	3	1	1	N	31290	Translation initiation factor IF-2 (Fragment) OS=Klebsiella variicola OX=244366 PE=3 SV=1
77	4603	tr|A0A0K1TQN9|A0A0K1TQN9_KLEVA	62.51	3	1	1	N	31989	Translation initiation factor IF-2 (Fragment) OS=Klebsiella variicola OX=244366 GN=infB PE=3 SV=1
77	4604	tr|Q6ULG6|Q6ULG6_KLEVA	62.51	3	1	1	N	32118	Translation initiation factor IF-2 (Fragment) OS=Klebsiella variicola OX=244366 PE=3 SV=1
77	4605	tr|Q6ULG4|Q6ULG4_KLEVA	62.51	3	1	1	N	32232	Translation initiation factor IF-2 (Fragment) OS=Klebsiella variicola OX=244366 PE=3 SV=1
97	4584	tr|A0A087FK22|A0A087FK22_KLEVA	62.24	8	1	1	N	17603	30S ribosomal protein S5 OS=Klebsiella variicola OX=244366 GN=rpsE PE=3 SV=1
97	4585	tr|A0A3G5CX99|A0A3G5CX99_9ENTR	62.24	8	1	1	N	17603	30S ribosomal protein S5 OS=Klebsiella sp. P1CD1 OX=2267618 GN=rpsE PE=3 SV=1
97	4680	tr|A0A2N5ALN2|A0A2N5ALN2_KLEVA	62.24	9	1	1	N	15972	30S ribosomal protein S5 (Fragment) OS=Klebsiella variicola OX=244366 GN=rpsE PE=3 SV=1
320	4783	tr|A0A2N5AF77|A0A2N5AF77_KLEVA	61.28	3	1	1	N	63804	Bifunctional UDP-glucuronic acid oxidase/UDP-4-amino-4-deoxy-L-arabinose formyltransferase (Fragment) OS=Klebsiella variicola OX=244366 GN=CWM98_15115 PE=3 SV=1
320	4784	tr|A0A087FTY0|A0A087FTY0_KLEVA	61.28	2	1	1	N	74234	Bifunctional polymyxin resistance protein ArnA OS=Klebsiella variicola OX=244366 GN=yfbG PE=3 SV=1
320	4785	tr|A0A2N4Z7N6|A0A2N4Z7N6_KLEVA	61.28	2	1	1	N	74246	Bifunctional polymyxin resistance protein ArnA OS=Klebsiella variicola OX=244366 GN=arnA PE=3 SV=1
320	4786	tr|A0A3G5CWK8|A0A3G5CWK8_9ENTR	61.28	2	1	1	N	74262	Bifunctional polymyxin resistance protein ArnA OS=Klebsiella sp. P1CD1 OX=2267618 GN=arnA PE=3 SV=1
168	4686	tr|A0A2V3KP96|A0A2V3KP96_KLEVA	61.10	2	1	1	N	69136	ABC transporter substrate-binding protein OS=Klebsiella variicola OX=244366 GN=DMS35_16835 PE=4 SV=1
168	4693	tr|A0A3G5D0V3|A0A3G5D0V3_9ENTR	61.10	2	1	1	N	69123	ABC transporter substrate-binding protein OS=Klebsiella sp. P1CD1 OX=2267618 GN=DTA24_08780 PE=4 SV=1
168	4694	tr|A0A5P2IWH6|A0A5P2IWH6_KLEVA	61.10	2	1	1	N	69152	ABC transporter substrate-binding protein OS=Klebsiella variicola OX=244366 GN=FOB35_14200 PE=4 SV=1
168	4695	tr|A0A0B7GMB7|A0A0B7GMB7_KLEVA	61.10	2	1	1	N	69136	ABC transporter substrate-binding protein OS=Klebsiella variicola OX=244366 GN=yejA PE=4 SV=1
78	4634	tr|A0A087FLS8|A0A087FLS8_KLEVA	58.68	2	1	1	N	56050	2,3-bisphosphoglycerate-independent phosphoglycerate mutase OS=Klebsiella variicola OX=244366 GN=gpmI PE=3 SV=1
78	4635	tr|A0A3G5CXT1|A0A3G5CXT1_9ENTR	58.68	2	1	1	N	56050	2,3-bisphosphoglycerate-independent phosphoglycerate mutase OS=Klebsiella sp. P1CD1 OX=2267618 GN=gpmI PE=3 SV=1
78	4639	tr|A0A2N5AJP6|A0A2N5AJP6_KLEVA	58.68	2	1	1	N	50252	Phosphoglycerate mutase (2,3-diphosphoglycerate-independent) (Fragment) OS=Klebsiella variicola OX=244366 GN=CWM98_08090 PE=3 SV=1
78	4640	tr|A0A2N4Z6A2|A0A2N4Z6A2_KLEVA	58.68	2	1	1	N	56108	2,3-bisphosphoglycerate-independent phosphoglycerate mutase OS=Klebsiella variicola OX=244366 GN=gpmI PE=3 SV=1
120	4730	tr|A0A087FVD9|A0A087FVD9_KLEVA	58.00	3	1	1	N	37854	Protein RecA OS=Klebsiella variicola OX=244366 GN=recA PE=3 SV=1
120	4731	tr|A0A3G5D0N4|A0A3G5D0N4_9ENTR	58.00	3	1	1	N	37870	Protein RecA OS=Klebsiella sp. P1CD1 OX=2267618 GN=recA PE=3 SV=1
88	4633	tr|A0A3G5CX61|A0A3G5CX61_9ENTR	57.30	6	1	1	N	20274	50S ribosomal protein L5 OS=Klebsiella sp. P1CD1 OX=2267618 GN=rplE PE=3 SV=1
87	4632	tr|A0A5Q2LHI8|A0A5Q2LHI8_KLEVA	56.57	2	1	1	N	62040	Ubiquinone-dependent pyruvate dehydrogenase OS=Klebsiella variicola OX=244366 GN=poxB PE=4 SV=1
87	4643	tr|A0A3G5DAC3|A0A3G5DAC3_9ENTR	56.57	2	1	1	N	61992	Ubiquinone-dependent pyruvate dehydrogenase OS=Klebsiella sp. P1CD1 OX=2267618 GN=DTA24_19410 PE=3 SV=1
87	4644	tr|A0A0B7GD52|A0A0B7GD52_KLEVA	56.57	2	1	1	N	62054	Pyruvate dehydrogenase (Pyruvate oxidase), thiamin-dependent, FAD-binding OS=Klebsiella variicola OX=244366 GN=poxB PE=3 SV=1
87	4645	tr|A0A2V3KTS1|A0A2V3KTS1_KLEVA	56.57	2	1	1	N	62011	Ubiquinone-dependent pyruvate dehydrogenase OS=Klebsiella variicola OX=244366 GN=DMS35_06215 PE=3 SV=1
87	4646	tr|A0A5P2IWI1|A0A5P2IWI1_KLEVA	56.57	2	1	1	N	62027	Ubiquinone-dependent pyruvate dehydrogenase OS=Klebsiella variicola OX=244366 GN=poxB PE=4 SV=1
53	4559	tr|A0A3G5D8A5|A0A3G5D8A5_9ENTR	56.33	3	2	2	N	65906	Acetyltransferase component of pyruvate dehydrogenase complex OS=Klebsiella sp. P1CD1 OX=2267618 GN=DTA24_23575 PE=3 SV=1
53	4560	tr|A0A5P2J6V4|A0A5P2J6V4_KLEVA	56.33	3	2	2	N	65920	Pyruvate dehydrogenase complex dihydrolipoyllysine-residue acetyltransferase OS=Klebsiella variicola OX=244366 GN=aceF PE=4 SV=1
53	4561	tr|A0A0B7GBU2|A0A0B7GBU2_KLEVA	56.33	3	2	2	N	65906	Acetyltransferase component of pyruvate dehydrogenase complex OS=Klebsiella variicola OX=244366 GN=aceF PE=3 SV=1
100	4658	tr|A0A3G5CYF5|A0A3G5CYF5_9ENTR	56.22	3	1	1	N	41896	S-adenosylmethionine synthase OS=Klebsiella sp. P1CD1 OX=2267618 GN=metK PE=3 SV=1
100	4659	tr|A0A087FKN2|A0A087FKN2_KLEVA	56.22	3	1	1	N	41896	S-adenosylmethionine synthase OS=Klebsiella variicola OX=244366 GN=metK PE=3 SV=1
100	4732	tr|A0A2N4YYI4|A0A2N4YYI4_KLEVA	56.22	5	1	1	N	23181	Methionine adenosyltransferase (Fragment) OS=Klebsiella variicola OX=244366 GN=metK PE=3 SV=1
321	4787	tr|A0A087FLB1|A0A087FLB1_KLEVA	56.06	5	1	1	N	24631	50S ribosomal protein L1 OS=Klebsiella variicola OX=244366 GN=rplA PE=3 SV=1
321	4788	tr|A0A3G5D9X3|A0A3G5D9X3_9ENTR	56.06	5	1	1	N	24631	50S ribosomal protein L1 OS=Klebsiella sp. P1CD1 OX=2267618 GN=rplA PE=3 SV=1
67	4711	tr|A0A2N5ACV6|A0A2N5ACV6_KLEVA	55.01	3	1	1	N	44367	ABC transporter substrate-binding protein (Fragment) OS=Klebsiella variicola OX=244366 GN=CWM98_20280 PE=4 SV=1
67	4712	tr|A0A2N4Z206|A0A2N4Z206_KLEVA	55.01	2	1	1	N	59071	ABC transporter substrate-binding protein (Fragment) OS=Klebsiella variicola OX=244366 GN=CWN47_13150 PE=4 SV=1
67	4713	tr|A0A2W7T990|A0A2W7T990_KLEVA	55.01	2	1	1	N	59222	ABC transporter substrate-binding protein OS=Klebsiella variicola OX=244366 GN=DMS35_01895 PE=4 SV=1
67	4714	tr|A0A4U2WFZ9|A0A4U2WFZ9_KLEVA	55.01	2	1	1	N	59202	ABC transporter substrate-binding protein OS=Klebsiella variicola OX=244366 GN=C2D62_12480 PE=4 SV=1
67	4715	tr|A0A3G5D3A4|A0A3G5D3A4_9ENTR	55.01	2	1	1	N	59202	ABC transporter substrate-binding protein OS=Klebsiella sp. P1CD1 OX=2267618 GN=DTA24_13535 PE=4 SV=1
67	4716	tr|A0A0B7G4U8|A0A0B7G4U8_KLEVA	55.01	2	1	1	N	59232	Periplasmic dipeptide transport protein OS=Klebsiella variicola OX=244366 GN=dppA PE=4 SV=1
166	4664	tr|A0A2N5ACP5|A0A2N5ACP5_KLEVA	54.39	5	1	1	N	37269	PTS N-acetyl glucosamine transporter subunit IIABC (Fragment) OS=Klebsiella variicola OX=244366 GN=CWM98_21015 PE=4 SV=1
166	4665	tr|A0A2N4YUH0|A0A2N4YUH0_KLEVA	54.39	3	1	1	N	51930	PTS N-acetyl glucosamine transporter subunit IIABC (Fragment) OS=Klebsiella variicola OX=244366 GN=CWN47_26850 PE=4 SV=1

total 345 proteins

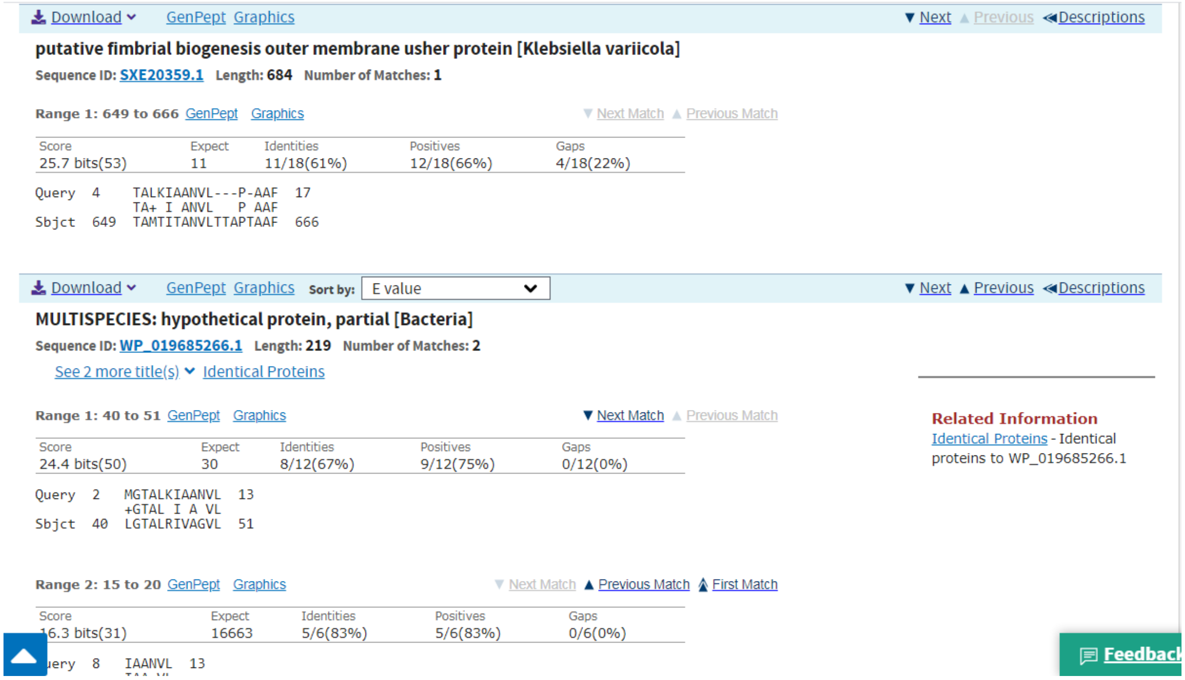
Suppl. Fig S2 – Sample list of Proteins obtained in MS analysis for *Klebisella* v*ariicola*



Protein List

Protein Group	Protein ID	Accession	-10lgP	Coverage (%)	#Peptides	#Unique	PTM	Avg. Mass	Description
2	1	AP02257	178.09	51	5	5	Y	14701	AP02257
3	2	AP00433	173.97	70	5	5	Y	4705	AP00433
13	3	AP02030	139.14	46	3	3	N	8451	AP02030
1	4	AP02072	132.55	24	2	2	Y	11457	AP02072
28	13	AP01400	74.71	65	1	1	N	2764	AP01400
6	6	AP01273	73.49	26	1	1	Y	4997	AP01273
263	15	AP02231	57.55	89	1	1	N	1767	AP02231
47	10	AP01858	52.06	11	1	1	N	4847	AP01858
47	11	AP01857	52.06	11	1	1	N	4833	AP01857
119	30	AP02279	38.81	29	1	1	N	2586	AP02279
total 10 proteins									

Suppl. Fig S3 – List of antimicrobial peptides obtained from APD database



putative fimbrial biogenesis outer membrane usher protein [Klebsiella variicola]

Sequence ID: SXE20359.1 **Length:** 684 **Number of Matches:** 1

Range 1: 649 to 666 GenPept Graphics

Score	Expect	Identities	Positives	Gaps
25.7 bits(53)	11	11/18(61%)	12/18(66%)	4/18(22%)

```
Query  4    TALKIAANVL---P-AAF  17
            TA+ I ANVL   P AAF
Sbjct  649  TAMTITANVLTTAPTAAF  666
```

MULTISPECIES: hypothetical protein, partial [Bacteria]

Sequence ID: WP_019685266.1 **Length:** 219 **Number of Matches:** 2

Range 1: 40 to 51 GenPept Graphics

Score	Expect	Identities	Positives	Gaps
24.4 bits(50)	30	8/12(67%)	9/12(75%)	0/12(0%)

```
Query  2   MGTALKIAANVL  13
           +GTAL I A VL
Sbjct  40  LGTALRIVAGVL  51
```

Related Information

Identical Proteins - Identical proteins to WP_019685266.1

Range 2: 15 to 20 GenPept Graphics

Score	Expect	Identities	Positives	Gaps
6.3 bits(31)	16663	5/6(83%)	5/6(83%)	0/6(0%)

```
uery  8   IAANVL  13
```

Suppl. Fig S4 – BLASTp of the peptide FMGTALKI obtained from APD database

APD ID: AP02279
Name/Class: Brevinin-1SN2 (XXU; 1S=S, UCSS1a; frog, amphibians, animals)
Source: skin, fine-spined frog, *Hylarana spinulosa*, Hainan, China, Asia
Sequence: FMGTALKIAANVLPAAFCKIFKKC
Length: 24
Net charge: 4
Hydrophobic residue%: 66%
Boman Index: -0.67 kcal/mol
3D Structure: Unknown
SwissProt ID: Reference ID: Ref
Activity: Anti-Gram+ & Gram-, Antifungal, Hemolytic,
Crucial residues:
Additional info: To be updated.

Title: Identification of multiple antimicrobial peptides from the skin of fine-spined frog, *Hylarana spinulosa* (*Ranidae*).
Author: Yang X, Hu Y, Xu S, Hu Y, Meng H, Guo C, Liu Y, Liu J, Yu Z, Wang H.2013

Suppl. Fig S5 – APD Predictions of Identified peptide of FMGTALKIAANVLPAAFCKIFKKC

Predict Antimicrobial Peptides

Results with Support Vector Machine (SVM) classifier

Seq. ID.	Class	AMP Probability
1	AMP	0.977

Results with Random Forest Classifier

Seq. ID.	Class	AMP Probability
1	AMP	0.9855

Results with Artificial Neural Network (ANN) classifier

Seq. ID.	Class
1	AMP

Results with Discriminant Analysis classifier

Seq. ID.	Class	AMP Probability
1	AMP	0.994

Suppl. Fig S6 – CAMPR3 Predictions of Identified peptide of FMGTALKIAANVLPAAFCKIFKKC