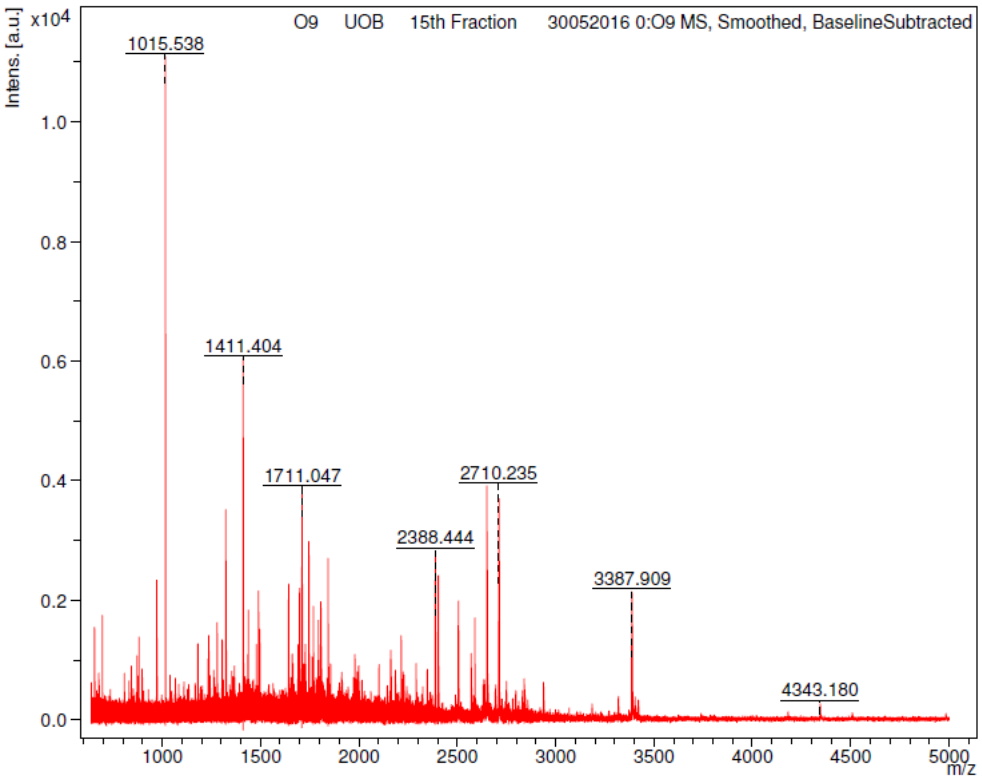
Appendices

Appendix 1

Figure A1: Mass spectrum of column purified fraction 15th



m/z	S/N	Quality Fac.	Res.	Intens.	Area
656.549	8.0	422	2481	1128.94	475
695.707	10.8	1508	3904	1524.63	438
872.237	6.4	1387	4625	925.26	311
883.003	6.2	354	2635	897.33	539
973.489	13.9	2779	4581	2033.58	822
1015.538	71.9	27863	5129	10630.44	4152
1181.904	7.7	994	7780	1236.25	421
1237.064	7.1	619	4542	1158.43	697
1280.190	8.1	2528	5832	1364.36	682
1304.259	8.2	745	6731	1377.65	614
1323.161	18.4	7158	5818	3132.40	1669
1411.404	31.6	18407	6520	5605.16	3003
1437.526	8.2	1083	5422	1458.79	954
1490.617	9.6	2045	5981	1732.95	1102
1641.990	11.2	3919	7510	2013.38	1248
1698.123	9.3	726	4712	1672.75	1749
1711.047	18.8	6086	5997	3384.51	2825
1746.197	13.4	4250	6151	2406.68	2031
1769.154	7.1	785	4077	1278.60	1681
1807.346	7.1	326	4878	1266.64	1441
1842.405	12.0	2654	6790	2121.06	1804
2215.233	8.1	1366	7314	987.85	995
2388.444	17.4	3596	6394	1727.81	2208
2403.515	16.2	6686	7093	1588.68	1845
2505.906	14.3	2109	6231	1236.31	1737
2571.062	8.2	1633	7191	647.41	815
2589.031	12.2	4034	7624	945.85	1138
2649.229	27.5	859	5966	1989.26	3135
2652.150	7.8	229	4767	565.11	1110
2710.235	33.5	2246	7251	2265.85	3092
2839.577	6.1	334	6688	353.06	561
3387.909	33.3	2806	7779	1037.88	1963
4343.180	6.2	631	7651	86.47	235

Sequence of 15th fraction:

MKSLLGPLLL GASRLATATN GADSLVARAE SSLKWEPCKL DLPDAAKELL
KAGDCATIEV PLDYTDKKSD KTVELQLIRY NATKEPFKGS VLWNPGGPGI
SGIETLAYLG QDFRDIMGGH HNIISFDPRG VGRTIPFACG KNASTELTRR
SLEPLPQADL WEYVKNEGWQ TMQATAESCY ETQQEHGR**FL STAFTAR**DMM
KIVDALGEDG KLRFWGISYG TILGQVAAAM FPDRIGRLLL DSNSLADAYL
TSTGVGGPHD AEKSLVHLFA ECVELGTKYC KVANYSGSST TVEDLRDATV
ETFQKLKDLK TLPDGLSSKD YPYAGNSILK QLKYGIMNLL SSPFNYPSVV
EILSYAFEGD YKKALSVYKE DTSEWNLGTN AFQGIACSES SFRVSNPEDL
YSMYQAHLAG SSFGDAIAAD YVACAAWK**FD AAEGVDTNTL R**NVNTSFPVL

Note: The Red colour represents peptide match.

Appendix 2: Media, buffer and reagent preparation

10X TEB buffer (pH 8.0):	**100 ml**
Tris base	10.8 g
EDTA	0.92 g
Boric acid	5.5 g

Dissolve in 80 ml of sterile filtered DW; adjust the pH to 8.0 with 10N NaOH or Conc. HCl. Make up to 100 ml with sterile filtered DW. Sterilize by autoclaving and store at RT.

2X Loading solution	**10 ml**
Glycerol	5 ml
10X TEB buffer (pH 8.0)	1 ml
10% Bromophenol blue	0.1 ml
Sterile filtered DW	3.8 ml

First take 3.8 ml of DW and add other reagents with constant mixing. Sterilize by autoclaving, store at RT.

1X Phosphate buffered saline (PBS):	**100 ml**
NaCl	800 mg
KCl	20 mg
Na_2HPO_4	144 mg
K_2HPO_4	24 mg

Dissolve in 80 ml of sterile filtered D/W; adjust the pH to 8.0. Make up to 100 ml with sterile filtered DW. Sterilize by autoclaving and store at RT.

1X Electrophoresis buffer for SDS-PAGE:	**1000 ml**
SDS	1gm
Tris base	3gm
Glycine	14.4gm

Dissolve in 800 ml of sterile filtered DW. Make up to 100 ml with sterile filtered DW.

Gel stock for SDS-PAGE (30%-0.8%):	**100 ml**
Acrylamide	30 gm
Bisacrylamide	0.8 gm

Dissolve in 80 ml of D/W, make up to 100 ml and store at 4°C.

YMB media	**100 ml**
Yeast extract	0.3 gm
Malt extract	0.3 gm
Glucose	1 gm
Peptone	0.5 gm

Reagent for silver staining:

I) Fixing solution

a) Solution A	**100 ml**
Methanol	50 ml
Acetic acid	16 ml
D/W	34 ml
Formaldehyde	20 µl

b) Solution B	**100 ml**
Ethanol	50 ml
D/W	50 ml

II) Staining Solution

a) 0.2 % Silver nitrate solution	**100 ml**
Silver nitrate	0.2 g
Formaldehyde	10 µl
Sterile Filtered DW	100 ml

b) 1% Nitric acid	
Nitric acid	1ml
D/W	99 ml

III) Developing Solution	**100 ml**
Sodium carbonate	6 g
Formaldehyde	150 µl
Sodium thio sulphate	0.2 g
D/W	100 ml