

APPENDIX

Appendix A

Media preparation

Sabouraud Dextrose Agar (SDA)

Sabouraud dextrose agar powder	65.0g
dH ₂ O	1000ml

Yeast Peptone Glucose agar (YPG)

Yeast Extract	10.0g
Peptone	20.0g
Glucose	20.0g
Bacto Agar	18.0g
pH	6.8
dH ₂ O	1000ml

Glucose Yeast Malt Extract agar (GYMP)

NH ₄ Cl	1.0g
MgSO ₄ . 7H ₂ O	1.0g
KH ₂ PO ₄	1.0g
K ₂ HPO ₄	1.0g
Yeast Extract	8.0g
Peptone	8.0g
Glucose	15.0g
Malt extract	8.0g
Bacto Agar	20.0g
pH	7.2*
dH ₂ O	1000ml

* pH adjusted before putting agar

Mueller Hinton agar (MHA)

Mueller Hinton agar powder 38.0g

dH₂O 1000ml

Preparation : All material above was dissolved in 1000ml of distilled water. The media was stirred and then autoclaved at 121°C at 15 psi for 15 minutes. Once autoclaved the media was cooled before pouring into Petri dishes.

Appendix B

Buffer preparation for chromatography

10mMTris Hcl in 0.15M Nacl

Tris base 1.2114g

Nacl 8.766g

pH 7.4

Preparation : Tris base was dissolved in approximately 300ml of dH₂O. The pH of the solution was adjusted to pH 7.4 by titration with HCl and the volume was made up to 1000ml. Sodium chloride was added. Once prepared, the buffer was stored at 4°C.

Appendix C

Buffer and reagent preparation for Tricine SDS-PAGE

Tricine SDS Running buffer

Preparation of the buffer was made by adding 100 ml of 10X Novex Tricine SDS running buffer to 900 ml of deionized water.

Colloidal Coomassie Blue staining (Maldi ToF compatible)

85% phosphoric acid solution	2.0g
MiliQ water	80.0 ml
Ammonium persulfate	10.0g
5% (w/v) Coomassie Brilliant Blue G-250	2ml

Preparation : All materials above were dissolved in 1000 ml of MiliQ water and used as stock. The solution was shock vigorously before use for even distribution of the colloidal particles. For staining solution preparation before use, 20 ml Methanol was mixed with 80 ml stock stain solution.

Destaining solution

25% methanol in water was used for destaining. 250ml methanol was diluted to final volume of 1000ml with MiliQ water.

Fixing and Storing solution

25% (w/v) ammonium persulfate in MiliQ water was used for fixing and storage of the gel. Preparation of the solution was made by dissolving 250g ammonium persulfate in 1000 ml miliQ water.

Silver Staining

Fixing solution

Preparation of the solution was made by mixing 400 ml absolute Ethanol with 100 ml Acetic acid and top up to 1000 ml with distilled water.

Sensitizing solution

Ethanol	300 ml
Na-thiosulphate	2.0 g
Na-acetate	68.0 g
Gluteraldehyde	5 ml

Preparation of the solution was made by dissolving all materials in 1000 ml of distilled water except Gluteraldehyde which was added prior to use.

Silver Reaction solution

The solution was prepared by dissolving 2.5 g silver nitrate into 1000 ml of distilled water. 400µl formaldehyde was added prior to use.

Developing solution

Preparation of the solution was made by dissolving 25 g sodium carbonate in 1000 ml distilled water. 400µl formaldehyde was added prior to use.

Stopping solution

For one liter preparation of stopping solution, 14.6 g EDTA was dissolved in 1000 ml miliQ water.

Preserving solution

Preserving solution was prepared by mixing 100 ml of glycerol with 900 ml of distilled water.

Silver staining (Maldi compatible)

Fixing solution

For one liter preparation of fixing solution, 500 ml methanol, 120 ml acetic acid and 475 µl formaldehyde were mixed and the volume was made up to 1000 ml with miliQ water.

Sensitizing solution

Preparation of 1000 ml sensitizing solution involved dissolving 0.25 g of sodium thiosulfate in miliQ water.

Silver reaction solution

The solution was prepared by dissolving 2.0 g silver nitrate with 720µl formaldehyde in 1000 ml miliQ water.

Developing solution

Preparation of the solution was made by adding 0.47 ml formaldehyde, 60 g sodium carbonate and 20 ml sensitizing solution. The solution was made 1000 ml with miliQ water.

Stopping solution

Stopping solution was prepared by mixing 500 ml methanol and 120 ml acetic acid. The solution was made 1000 ml with miliQ water.

Preserving solution

The solution was made by 1% acetic acid in miliQ water. For preparation of one liter solution, 10 ml was added to 990 ml of miliQ water.

Appendix D

Table 1 : Ammonium Sulfate precipitation

This table specified the quantity of solid ammonium sulfate (in grams) to be added to one litre solution to produce a desired change in the presence saturation of ammonium sulphate.

Initial concentration (%)	Final concentration of ammonium sulfate (% saturation)									
	10	20	30	40	50	60	70	80	90	100
	Quantity of ammonium sulfate (g) added into 1 liter of solution									
0	56	114	176	243	313	390	472	561	662	767
10		57	118	183	251	326	406	494	592	694
20			59	123	189	262	340	424	520	619
30				62	127	198	273	356	449	546
40					63	132	205	285	375	469
50						66	137	214	302	392
60							69	143	227	314
70								72	153	237
80									77	157
90										77

* 662g/1L is needed for 90% saturation

Source : Green and Hughes in Methods Enzymology, 1, 67 – 90 (1995).

Table 2 : Triplicate optical density reading at 280 nm for bovine serum albumin standard curve (BSA) (0.02 to 0.16 mg/ml)

Concentration BSA (mg/ml)	Optical density (280 nm)			
	Replicate 1	Replicate 2	Replicate 3	Mean ± SD
0.02	0.0009	0.0009	0.0007	0.00083
0.04	0.004	0.0042	0.0047	0.0043
0.06	0.0073	0.0078	0.0007	0.00737
0.08	0.0122	0.0120	0.0118	0.0121
0.10	0.014	0.014	0.0143	0.0141
0.12	0.018	0.0178	0.0182	0.018
0.14	0.02144	0.021	0.0216	0.02135
0.16	0.02488	0.0248	0.0248	0.0248

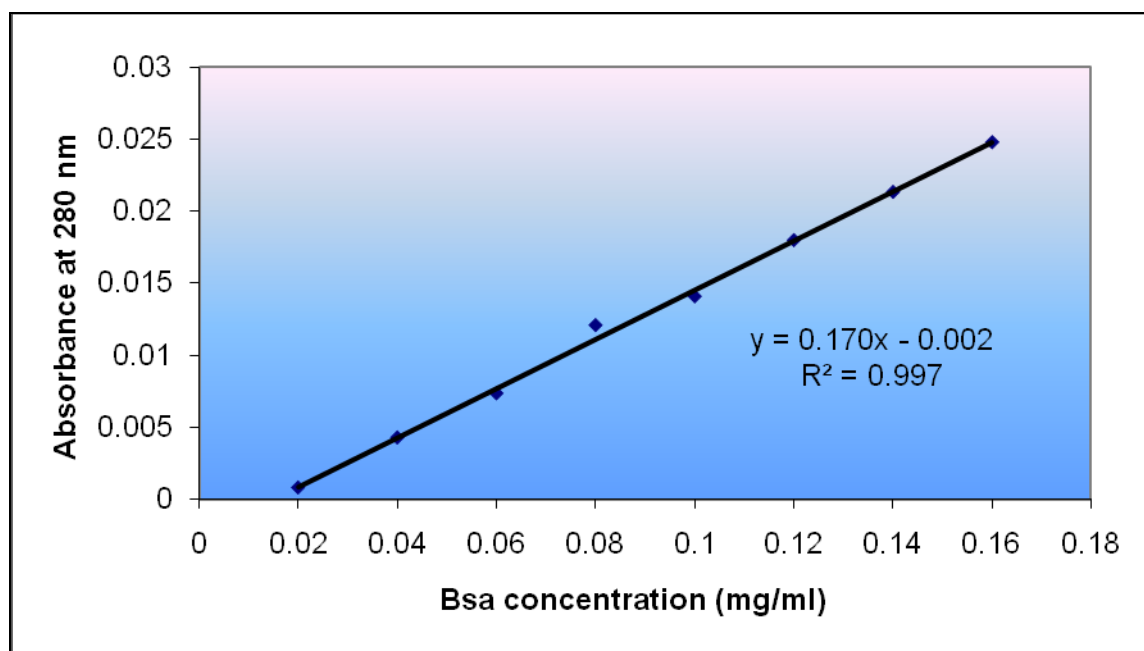


Figure 1 : Calibration standard curve of BSA (mg/ml)

Table 3 : Triplicate optical density reading at 280 nm for freeze dried fractions from sephacryl-S100, Superdex 75 and respective protein contents in 1mg crude and freeze dried fractions.

Protein fractions	Optical density (280 nm)				Protein contents in 1mg crude / fractions (ug)
	Replicate 1	Replicate 2	Replicate 3	Mean \pm SD	
Sephacryl s100					
Fraction 1	0.013	0.016	0.011	0.013 \pm 0.003	93 \pm 15
Fraction 2	0.033	0.030	0.037	0.033 \pm 0.004	210 \pm 21
Fraction 3	0.055	0.060	0.058	0.058 \pm 0.003	352 \pm 15
Fraction 4	0.044	0.042	0.045	0.044 \pm 0.002	270 \pm 9
Fraction 5	0.065	0.061	0.067	0.064 \pm 0.003	391 \pm 18
Fraction 6	0.055	0.052	0.050	0.052 \pm 0.003	321 \pm 15
Fraction 7	0.059	0.055	0.060	0.058 \pm 0.003	354 \pm 15
Fraction 8	0.019	0.021	0.019	0.020 \pm 0.001	130 \pm 7
Superdex 75 HR					
Fraction 1	0.002	0.003	0.003	0.003 \pm 0.0006	30 \pm 3
Fraction 2	0.009	0.008	0.009	0.009 \pm 0.0006	65 \pm 3

Table 4 : Observed monoisotopic mass used in Profound for protein identification.

Query	Observed	Mr (expt)	Mr (calc)	Delta	Miss	Score	Expect	Rank	Protein
<u>137</u>	523.8812	1045.7478	1045.5404	0.2074	0	45	0.13	1	QLSAAVNTDK
<u>156</u>	577.3802	1152.7458	1152.5636	0.1822	1	34	1.5	1	QNR ^T AYSTGR
<u>169</u>	595.4033	1188.7920	1189.5575	-0.7655	0	33	2	1	SGGGDIESEIAR
<u>136</u>	521.3630	1040.7115	1040.6594	0.0521	1	32	2.5	1	KGSSVPLLLK
<u>116</u>	467.3643	932.7140	932.5443	0.1697	1	31	3.5	1	KGTLVPYR
<u>124</u>	481.8478	961.6810	961.5379	0.1432	1	31	4.1	1	IMKSQLR + Oxidation (M)
<u>275</u>	694.4298	2080.2676	2081.0501	-0.7825	1	29	2.5	1	ELNELQSKHTASQAE ^L QR
<u>148</u>	560.3509	1118.6873	1117.5186	1.1687	0	29	6	1	QMDAASVQPR + Oxidation (M)
<u>113</u>	460.3791	918.7437	919.4763	-0.7326	0	29	5	1	APSFNGSLK
<u>92</u>	720.3319	719.3246	720.3402	-1.0156	0	29	8.5	1	ATSNSNK
<u>123</u>	481.8460	961.6774	961.5127	0.1647	1	29	6.7	1	LMQSVGRR + Oxidation (M)
<u>37</u>	547.3854	546.3781	547.2788	-0.9007	1	28	12	1	MVGKN
<u>208</u>	725.9895	1449.9645	1450.6796	-0.7150	1	28	4.4	1	EMLEEIEKMGAR + Oxidation (M)
<u>143</u>	548.1086	1094.2026	1093.5074	0.6952	0	28	4.7	1	MATVTVDEGR + Oxidation (M)
<u>175</u>	627.4424	1252.8703	1253.5921	-0.7219	1	27	5.5	1	RTTESADSIMK + Oxidation (M)
<u>187</u>	463.9044	1388.6913	1389.6421	-0.9509	0	27	8.2	1	GGQGLGYIFGMMK + 2 Oxidation (M)
<u>172</u>	613.4046	1224.7946	1224.5405	0.2542	0	27	7	1	DSTLASASSACR
<u>130</u>	496.8432	991.6718	990.5345	1.1372	1	26	12	1	SSDKSNLLK
<u>134</u>	515.3954	1028.7763	1028.5614	0.2149	1	26	9.8	1	QLEVAAKDR
<u>310</u>	722.4858	2164.4356	2163.9273	0.5082	1	26	4.4	1	QQMGNHKMLADGTGNSGDGSK + 2 Oxidation (M)
<u>276</u>	695.4160	2083.2262	2084.0035	-0.7773	0	26	6.2	1	SFERPSERPVGDA ^S SGGPPR
<u>192</u>	469.3673	1405.0800	1405.7275	-0.6475	0	26	6.9	1	LVDLPATFDMLR + Oxidation (M)
<u>206</u>	725.5005	1448.9865	1449.7616	-0.7751	0	25	8.1	1	AGYVDALVPY ^N IR
<u>274</u>	694.0969	2079.2688	2079.1324	0.1364	1	25	6	1	LASTAQSERLLEAVLPEPR
<u>249</u>	601.4424	1801.3054	1800.9444	0.3609	0	25	6.2	1	GGMQIWSPADSLITVVK
<u>253</u>	613.4046	1837.1919	1837.9026	-0.7107	1	25	6.6	1	CLDLACGTGKSTSLLSR
<u>268</u>	691.4698	2071.3876	2071.0599	0.3277	1	25	5.3	1	AISHSKFHPTPADNPISPR
<u>282</u>	702.4434	2104.3085	2105.0324	-0.7239	1	25	6	1	GKGADVTSGLMVNLGQGSWGR + Oxidation (M)
<u>226</u>	548.1086	1641.3040	1641.8151	-0.5111	1	25	7.2	1	RYDSPVLTEYFPR

292	716.7785	2147.3137	2148.1110	-0.7972	1	25	6.8	1	GEMIDLPLDRGQLLNHVR + Oxidation (M)
232	561.4317	1681.2734	1680.8513	0.4220	0	24	7.7	1	AFALAVAMSMCALGIR
331	764.1583	2289.4531	2289.1053	0.3479	0	24	5.7	1	DPFEAYSTSTTIQTDIYIPK
254	627.4424	1879.3054	1878.9245	0.3810	0	24	7	1	VSVDSGGCEEITSIVIK
340	784.4668	2350.3784	2351.1036	-0.7252	1	24	6.7	1	MQSSAAEAANTPGHANSSHSIRK
261	674.1629	2019.4670	2019.9643	-0.4974	0	24	7	1	SQAASAATASLHSSSSMASLR
120	471.3662	940.7178	940.4688	0.2490	0	24	13	1	QPVMEPPK + Oxidation (M)
193	471.3662	1411.0766	1411.6952	-0.6185	1	24	9.7	1	RMYSMPVSAINK + Oxidation (M)
289	715.4807	2143.4203	2143.0527	0.3676	0	24	7	1	NVGLPHGSRPMHQNTMPVR + Oxidation (M)
319	732.4646	2194.3719	2195.3545	-0.9826	0	24	7.6	1	LTILLSVLLQLFSLAEVPK
163	589.3711	1176.7276	1177.6013	-0.8737	1	24	19	1	DGVTVGVKEMK + Oxidation (M)
115	463.9044	925.7942	925.5233	0.2709	0	24	12	1	GPSIGLASPK
244	591.3951	1771.1634	1769.9709	1.1925	0	23	10	1	TSLTGGLLHLITMEIR + Oxidation (M)
279	697.0577	2088.1514	2089.0125	-0.8611	1	23	11	1	LRQLYGMDAINFMPYGGK + Oxidation (M)
220	802.0344	1602.0542	1600.8937	1.1605	1	23	13	1	LPLVNFKTANLTDTR
181	659.4595	1316.9045	1317.7544	-0.8499	1	23	17	1	KDVLDIIFDLK
260	658.4410	1972.3013	1971.9063	0.3949	1	23	9.9	1	GSADMKSGMAAAMIATASAAK + 2 Oxidation (M)
285	703.8421	2108.5045	2108.1782	0.3263	0	23	9.2	1	EWGLSIGLGAISLPWGALIR
118	469.3440	936.6734	936.4916	0.1818	1	22	21	1	KLEAYDAK
219	770.4672	1538.9199	1539.8694	-0.9495	1	22	18	1	IDIATKELHLMK + Oxidation (M)
128	491.8780	981.7414	982.5560	-0.8146	1	22	17	1	AEKPPGTRK
264	679.1032	2034.2879	2034.0496	0.2383	1	22	11	1	IFNTVFDIKSYQSTLMK
252	611.4326	1831.2760	1830.8569	0.4192	1	22	13	1	TQDNDA TRTRPNDA TR
297	717.1046	2148.2919	2149.0448	-0.7529	0	22	13	1	DYINIAPNGMMYTKPHIR + Oxidation (M)
326	749.0088	2244.0045	2243.1896	0.8149	1	22	16	1	IESDPKTISQASETAEILAIK
204	483.4600	1447.3582	1446.6674	0.6908	1	22	18	1	RGSWHASTDGMVK + Oxidation (M)
262	674.4005	2020.1797	2020.9425	-0.7628	1	21	17	1	HPDPEWTHMKTLQER + Oxidation (M)
160	578.6700	1155.3254	1154.5179	0.8076	1	21	20	1	QEWKYDMR
78	658.4410	657.4338	656.3857	1.0480	0	21	48	1	ATPVAAK
298	718.4446	2152.3120	2151.2052	1.1069	1	21	14	1	AQANLPPTILDASPLKLSFR
337	777.4476	2329.3210	2329.1988	0.1222	0	21	16	1	QSYTTLGGDITLYVMLRPPSK
278	696.7965	2087.3678	2088.0820	-0.7141	1	21	14	1	TNMVTLNAATGAMIKNLGPR + Oxidation (M)
339	780.2349	2337.6828	2338.1144	-0.4316	1	21	12	1	DMQAMTTVQGNLNSLAKEVEK + 2 Oxidation (M)
166	590.4171	1178.8196	1178.6295	0.1901	1	21	26	1	IRLETYQEK

<u>266</u>	679.4814	2035.4222	2036.0248	-0.6026	1	21	14	1	VVAEGEYNLREMVEGVVK + Oxidation (M)
<u>102</u>	764.1706	763.1634	763.3534	-0.1901	0	21	35	1	TASNMPK + Oxidation (M)
<u>294</u>	716.8076	2147.4009	2147.2466	0.1543	1	21	15	1	YNIAKVLASYLLTAAGVKPR
<u>155</u>	577.3772	1152.7399	1152.5636	0.1764	1	21	32	1	QNRTAYSTGR
<u>157</u>	577.3953	1152.7761	1152.5809	0.1952	1	21	29	1	STVMDKLTSR + Oxidation (M)
<u>35</u>	531.4071	530.3998	530.2700	0.1298	0	20	91	1	ATDPK
<u>291</u>	716.7664	2147.2774	2146.9866	0.2909	0	20	19	1	ALSLITDNLNEDDLDDTR
<u>301</u>	720.4142	2158.2207	2157.1596	1.0611	1	20	21	1	ALAHLEFFGAPPKPGKHAGGR
<u>171</u>	611.4326	1220.8507	1220.5641	0.2865	1	20	29	1	EMHSM ^u SAKLR + 2 Oxidation (M)
<u>180</u>	658.4410	1314.8675	1315.7500	-0.8825	0	20	33	1	NILLDFNLNIK
<u>341</u>	808.0715	2421.1926	2421.1781	0.0145	1	20	20	1	NRENDLDDLCVGAMHIIPVPK + Oxidation (M)
<u>177</u>	636.1096	1270.2047	1270.5864	-0.3817	0	20	26	1	MTSDTTPSFLR + Oxidation (M)
<u>117</u>	937.6034	936.5962	937.5093	-0.9132	1	20	41	1	SPPRAPSAR
<u>125</u>	483.4600	964.9055	964.5454	0.3601	1	20	30	1	ATPRGAPAPK
<u>168</u>	595.3678	1188.7211	1187.5530	1.1680	1	20	44	1	EREADENVAR
<u>302</u>	720.5019	2158.4840	2159.0746	-0.5907	1	20	17	1	VSLDFIETNPAARLDDDLVG
<u>213</u>	489.3716	1465.0931	1464.7725	0.3206	1	20	26	1	AKAELSDKPPFFGR
<u>189</u>	696.4625	1390.9104	1391.7045	-0.7941	1	20	35	1	AETKFQIDGLDR
<u>311</u>	725.5005	2173.4797	2173.0156	0.4641	0	20	18	1	YEPWSTYSEPYLQHVFK
<u>121</u>	480.3285	958.6424	958.4832	0.1592	0	20	58	1	AVSQTSPNR
<u>159</u>	577.4043	1152.7941	1152.5887	0.2054	0	20	34	1	QLDVVSEHAR
<u>265</u>	679.4685	2035.3837	2034.9971	0.3866	0	20	20	1	SGSGGAQITEAQASFITTPR
<u>240</u>	587.4284	1759.2635	1759.8952	-0.6317	1	19	24	1	ALKQALSLGEDDGTSQK
<u>141</u>	1093.8886	1092.8813	1092.5815	0.2998	0	19	35	1	ISNYEIINK
<u>145</u>	559.3851	1116.7556	1117.5914	-0.8358	1	19	52	1	QQSAMVQAKK
<u>188</u>	696.4426	1390.8707	1391.7871	-0.9165	1	19	45	1	SISSSLKTSIEK
<u>127</u>	488.3852	974.7559	975.4985	-0.7426	1	19	50	1	EATANSKQK
<u>295</u>	716.8125	2147.4157	2146.9412	0.4745	1	19	22	1	LSGHTCDEGKEMWDPLTR + Oxidation (M)
<u>119</u>	469.3673	936.7200	937.4287	-0.7087	1	19	40	1	NDSAM ^u RTK + Oxidation (M)
<u>131</u>	497.3765	992.7384	993.5356	-0.7972	0	19	45	1	HTQPLLSVR
<u>250</u>	902.5164	1803.0182	1802.0203	0.9980	1	19	38	1	TQFLLPPIEPHLKNR
<u>263</u>	679.0109	2034.0108	2035.1174	-1.1066	1	19	35	1	HPGGGIGKITSSTNLTLQVLR
<u>71</u>	635.4072	634.3999	633.3486	1.0513	0	19	84	1	VFGSPK
<u>324</u>	740.4883	2218.4429	2218.0100	0.4330	0	19	22	1	TIMDPYEAEDLTPTSTGSTR

<u>238</u>	869.5028	1736.9911	1737.7887	-0.7976	1	19	40	1	NAVSSSRGNGVGSMAK
<u>258</u>	654.9072	1961.6998	1960.9953	0.7044	0	19	27	1	AGNTTTTTPTATTEVPVTA
<u>174</u>	414.3291	1239.9656	1240.5836	-0.6180	0	19	37	1	TYNVAGFGNGNK
<u>150</u>	568.3431	1134.6717	1134.6549	0.0167	1	19	66	1	AAIPAKYFVR
<u>241</u>	589.4628	1765.3665	1765.0032	0.3632	1	18	29	1	LRHGLSVIMASGLGNK
<u>335</u>	772.4627	2314.3662	2315.1402	-0.7741	1	18	27	1	MGFDEAIISLALCYRSGTVR
<u>146</u>	559.3863	1116.7581	1117.5992	-0.8412	1	18	62	1	AFAGDRVGLGR
<u>248</u>	900.9971	1799.9797	1798.9061	1.0736	1	18	43	1	NSLSKSSDDPIVPDGR
<u>147</u>	559.3890	1116.7634	1117.6091	-0.8457	0	18	62	1	AISSQSLLGR
<u>222</u>	808.0715	1614.1284	1613.8202	0.3082	0	18	34	1	HLFGVGEVFLEDPR
<u>308</u>	721.7917	2162.3531	2162.1041	0.2490	1	18	27	1	KMDYDTPNLDVIARPLASK + Oxidation (M)
<u>330</u>	759.5389	2275.5948	2276.2376	-0.6427	1	18	23	1	LSLNKSSSTTALQNTLNFLPK
<u>170</u>	601.4174	1200.8203	1201.7547	-0.9343	1	18	55	1	VGGLLSKFILR
<u>154</u>	1153.6720	1152.6647	1151.5645	1.1002	0	18	60	1	MEVFIDGGIR + Oxidation (M)
<u>162</u>	587.4284	1172.8423	1173.5455	-0.7031	0	18	53	1	YQYGNAPSFK
<u>334</u>	1157.7129	2313.4112	2313.0801	0.3311	1	18	26	1	ITDTEYPHIKLFETSSGFDN
<u>22</u>	474.3596	473.3524	473.2486	0.1038	0	18	1.7e+002	1	SATPV
<u>273</u>	693.0935	2076.2587	2077.1320	-0.8733	1	18	34	1	RLINVPPLGEALES DIWR
<u>142</u>	547.8877	1093.7609	1094.6196	-0.8587	1	18	57	1	APTPVTPRR
<u>36</u>	536.3808	535.3735	534.2914	1.0821	0	18	1.1e+002	1	GHPPK
<u>144</u>	559.0213	1116.0280	1116.6516	-0.6236	1	18	55	1	HRHLSQVIK
<u>158</u>	577.3995	1152.7844	1152.6979	0.0865	1	18	55	1	ALATPIGQKVR
<u>231</u>	559.3890	1675.1451	1674.9781	0.1671	1	18	39	1	AIIERLQDLHQVLK

**Table 5 : Sequences producing significant alignments with antifungal protein
[*Aspergillus giganteus*]:**

Accession	Description	Max score	Total score	Query coverage	E value
<u>P17737.2</u>	RecName: Full=Antifungal protein; Flags: Precursor >emb CAA43181.1 antifungal protein precursor [<i>Aspergillus giganteus</i>]	<u>151</u>	151	100%	2e-35
<u>CAA37523.1</u>	antifungal protein [<i>Aspergillus giganteus</i>]	<u>149</u>	149	100%	1e-34
<u>XP_001267787.1</u>	antifungal protein Afp [<i>Aspergillus clavatus</i> NRRL 1] >gb EAW06361.1 antifungal protein Afp [<i>Aspergillus clavatus</i> NRRL 1]	<u>140</u>	140	100%	6e-32
<u>ABR10398.1</u>	antifungal protein precursor [<i>Aspergillus clavatus</i>]	<u>140</u>	140	100%	7e-32
<u>ADC55278.1</u>	antimicrobial peptide [<i>Aspergillus clavatus</i>]	<u>135</u>	135	97%	1e-30
<u>1AFP_A</u>	Chain A, Solution Structure Of The Antifungal Protein From <i>Aspergillus giganteus</i> . Evidence For Disulphide Configurational Isomerism	<u>100</u>	100	66%	8e-20
<u>XP_002566698.1</u>	Pc24g00380 [<i>Penicillium chrysogenum</i> Wisconsin 54-1255] >gb AAA92718.1 antifungal protein [<i>Penicillium chrysogenum</i>] >gb ABE96639.1	<u>59.7</u>	59.7	100%	1e-07

	antifungal protein [<i>Penicillium chrysogenum</i>] >gb ABE96640.1 antifungal protein [<i>Penicillium chrysogenum</i>] >emb CAP86946.1 Pc24g00380 [<i>Penicillium chrysogenum</i> Wisconsin 54-1255] >prf 2204241A paf gene				
<u>XP_001262586.1</u>	hypothetical protein NFIA_112130 [<i>Neosartorya fischeri</i> NRRL 181] >gb EAW20689.1 conserved hypothetical protein [<i>Neosartorya fischeri</i> NRRL 181] >emb CAQ42994.1 <i>Neosartorya fischeri</i> antifungal protein	<u>57.0</u>	57.0	100%	9e-07
<u>CAR79017.1</u>	antifungal protein [<i>Fusarium poae</i>]	<u>45.8</u>	45.8	90%	0.002
<u>CAR79014.1</u>	antifungal protein [<i>Fusarium cerealis</i>] >emb CAR79015.1 antifungal protein [<i>Fusarium polyphialidicum</i>] >emb CAR79016.1 antifungal protein [<i>Fusarium sporotrichioides</i>]	<u>45.8</u>	45.8	90%	0.002
<u>XP_384921.1</u>	hypothetical protein FG04745.1 [<i>Gibberella zeae</i> PH-1] >emb CAR79009.1 antifungal protein [<i>Fusarium boothii</i>]	<u>45.4</u>	45.4	90%	0.002
<u>CAR79010.1</u>	antifungal protein [<i>Fusarium boothii</i>] >emb CAR79011.1 antifungal protein [<i>Gibberella zeae</i>] >emb CAR79012.1 antifungal protein [<i>Gibberella zeae</i>] >emb CAR79013.1 antifungal protein [<i>Fusarium pseudograminearum</i>]	<u>45.4</u>	45.4	90%	0.002

<u>CAR79018.1</u>	antifungal protein [<i>Fusarium avenaceum</i>] >emb CAR79019.1 antifungal protein [<i>Fusarium culmorum</i>] >emb CAR79020.1 antifungal protein [<i>Fusarium culmorum</i>] >emb CAR79021.1 antifungal protein [<i>Fusarium culmorum</i>] >emb CAR79022.1 antifungal protein [<i>Fusarium culmorum</i>]	<u>45.1</u>	45.1	90%	0.004
<u>CAR79023.1</u>	antifungal protein [<i>Fusarium asiaticum</i>]	<u>43.1</u>	43.1	90%	0.011
<u>XP_002557660.1</u>	Pc12g08290 [<i>Penicillium chrysogenum</i> Wisconsin 54-1255] >sp B6GXZ8.1 AFP_PENCW RecName: Full=Antifungal protein; Flags: Precursor >emb CAP80456.1 Pc12g08290 [<i>Penicillium chrysogenum</i> Wisconsin 54-1255]	<u>38.1</u>	38.1	100%	0.35
<u>2KCN_A</u>	Chain A, Solution Structure Of The Antifungal Protein Paf From <i>Penicillium Chrysogenum</i>	<u>37.7</u>	37.7	62%	0.56
<u>D0EXD3.1</u>	RecName: Full=Antifungal protein; Flags: Precursor >gb ACX54052.1 antifungal protein preproprotein [<i>Penicillium chrysogenum</i>]	<u>37.4</u>	37.4	100%	0.70
<u>ZP_03542506.1</u>	Rieske (2Fe-2S) domain protein [<i>Comamonas testosteroni</i> KF-1] >gb EED66792.1 Rieske (2Fe-2S) domain protein [<i>Comamonas testosteroni</i> KF-1]	<u>35.0</u>	35.0	85%	3.3

<u>YP_003278931.1</u>	Rieske iron-sulfur protein [Comamonas testosteroni CNB-2] >gb ACY33635.1 Rieske iron-sulfur protein [Comamonas testosteroni CNB-2]	<u>34.7</u>	34.7	85%	4.7
<u>ZP_07043439.1</u>	Rieske iron-sulfur protein [Comamonas testosteroni S44] >gb EFI62922.1 Rieske iron-sulfur protein [Comamonas testosteroni S44]	<u>33.5</u>	33.5	85%	8.4

Appendix E

Data and Statistical Tables

Table 1 : Multiple sample comparison : Antibacterial activity (between fractions from Sephacryl S 100 and standard proteins)

Bacteria	Source of variation	Sum of squares	d.f	Mean square	F-ratio	Significance level
<i>Staphylococcus aureus</i> (between S1, S5, S6, S7 and std. proteins)	Between groups	41.7778	5	8.35556	66.84	0.0000*
	Within groups	1.5	12	0.125		
	Total	43.2778	17			
<i>Bacillus cereus</i> (between S1, S5, S6, S7 and std. proteins)	Between groups	34.2917	5	6.85833	61.73	0.0000*
	Within groups	1.33333	12	0.11111		
	Total	35.625	17			
<i>Bacillus subtilis</i> (between S1, S3, S6, S7 and std. proteins)	Between groups	2.94444	5	0.588889	5.3	0.0084**
	Within groups	1.33333	12	0.111111		
	Total	4.27778	17			
<i>Escherichia coli</i> <i>O157:H7</i> (between S1, S6, and std. proteins)	Between groups	26.25	3	8.75	70.00	0.0000*
	Within groups	1.0	8	0.125		
	Total	27.25	11			
<i>Escherichia coli</i> (between S6, S7 and std. proteins)	Between groups	3.5625	3	1.1875	9.50	0.0052**
	Within groups	1.0	8	0.125		
	Total	4.5625	11			
<i>Salmonella sp.</i> (between S5, S6, S7 and std. proteins)	Between groups	25.6	4	6.4	76.80	0.0000*
	Within groups	0.833333	10	0.0833333		
	Total	26.4333	14			

*p = 0; **p < 0.05

Table 2 : Multiple range tests : Antibacterial activity (between fractions from Sephacryl S 100 and cecropin and dermaseptin as standard proteins)

Contrast (S1, S5, S6, S7 and std. proteins)	<i>Staphylococcus aureus</i>		<i>Bacillus cereus</i>	
	Difference	Limits + / -	Difference	Limits + / -
Cecropin – Dermaseptin	0.333333	0.62897	- 0.166667	0.592999
Cecropin – S1	*3.66667	0.62897	*3.0	0.592999
Cecropin – S5	*3.33333	0.62897	*1.66667	0.592999
Cecropin – S6	0.0	0.62897	0.0	0.592999
Cecropin – S7	*1.33333	0.62897	*3.0	0.592999
Dermaseptin – S1	*3.33333	0.62897	*3.16667	0.592999
Dermaseptin – S5	*3.0	0.62897	*1.83333	0.592999
Dermaseptin – S6	- 0.333333	0.62897	0.166667	0.592999
Dermaseptin – S7	*1.0	0.62897	*3.16667	0.592999
S1 – S5	- 0.333333	0.62897	*- 1.33333	0.592999
S1 – S6	*- 3.66667	0.62897	*- 3.0	0.592999
S1 – S7	*- 2.33333	0.62897	0.0	0.592999
S5 – S6	*- 3.33333	0.62897	*- 1.66667	0.592999
S5 – S7	*- 2.0	0.62897	*1.33333	0.592999
S6 – S7	*1.33333	0.62897	*3.0	0.592999

Contrast (S1, S3, S6, S7 and std. proteins)	<i>Bacillus subtilis</i>		<i>Escherichia coli O157:H7</i>	
	Difference	Limits + / -	Difference	Limits + / -
Cecropin - Dermaseptin	- 0.5	0.592999	*- 1.0	0.665688
Cecropin – S1	0.333333	0.592999	*2.0	0.665688
Cecropin – S3	0.333333	0.592999	-	-
Cecropin – S6	0.333333	0.592999	*- 2.0	0.665688
Cecropin – S7	*0.833333	0.592999	-	-
Dermaseptin – S1	*0.833333	0.592999	*3.0	0.665688
Dermaseptin – S3	*0.833333	0.592999	-	-
Dermaseptin – S6	*0.833333	0.592999	*- 1.0	0.665688
Dermaseptin – S7	*1.333333	0.592999	-	-
S1 – S3	0.0	0.592999	-	-
S1 – S6	0.0	0.592999	*- 4.0	0.665688
S1 – S7	0.5	0.592999	-	-
S3 – S6	0.0	0.592999	-	-
S3 – S7	0.5	0.592999	-	-
S6 – S7	0.5	0.592999	-	-

Contrast (S5, S6, S7 and std. proteins)	<i>Escherichia coli</i>		<i>Salmonella sp.</i>	
	Difference	Limits + / -	Difference	Limits + / -
Cecropin - Dermaseptin	*1.0	0.665688	0.333333	0.525179
Cecropin – S5	-	-	*3.333333	0.525179
Cecropin – S6	*1.0	0.665688	0.0	0.525179
Cecropin – S7	*1.5	0.665688	0.0	0.525179
Dermaseptin – S5	-	-	*3.0	0.525179
Dermaseptin – S6	0.0	0.665688	- 0.333333	0.525179
Dermaseptin – S7	0.5	0.665688	- 0.333333	0.525179
S5 – S6	-	-	*- 3.333333	0.525179
S5 – S7	-	-	*- 3.333333	0.525179
S6 – S7	0.5	0.665688	0.0	0.525179

* denotes a statistically significant difference

Table 3 : Sample comparison : Antibacterial activity (between purified protein from Superdex 75 HR and cecropin as standard protein).

Bacteria	Source of variation	Sum of squares	d.f	Mean square	F-ratio	Significance level
<i>Escherichia coli O157:H7</i>	Between groups	6.0	1	6.0	-	0.0000*
	Within groups	0.0	4	0		
	Total	6.0	5			
<i>Salmonella typhi</i>	Between groups	6.0	1	6.0	16.0	0.0161**
	Within groups	1.5	4	0.375		
	Total	7.5	5			

*p = 0; **p < 0.05

Table 4 : Multiple range tests : Antibacterial activity (between purified protein and cecropin as standard protein).

Contrast	<i>Escherichia coli</i>		<i>Salmonella sp.</i>	
	Difference	Limits + / -	Difference	Limits + / -
Cecropin – purified protein	*- 2.0	0.0	*2.0	1.38823

* denotes a statistically significant difference

Table 5 : Sample comparison : Antibacterial activity using broth micro dilution method (between different concentrations of purified protein)

Bacteria	Source of variation	Sum of squares	d.f	Mean square	F-ratio	Significance level
<i>Escherichia coli</i> <i>O157:H7</i>	Between groups	7244.53	4	1811.13	880.60	0.0000*
	Within groups	20.5671	10	2.05671		
	Total	7265.1	14			
<i>Salmonella typhi</i>	Between groups	2736.1	4	684.026	105.57	0.0000*
	Within groups	64.7934	10	6.47934		
	Total	2800.9	14			

*p = 0

Table 6 : Multiple range tests : Antibacterial activity using broth micro dilution method (between different concentrations of purified protein)

Contrast (purified protein in mg/ml)	<i>Escherichia coli O157:H7</i>		<i>Salmonella typhi</i>	
	Difference	Limits + / -	Difference	Limits + / -
0.0005 – 0.005	*- 3.92	2.60906	*-5.40667	4.63087
0.0005 – 0.05	*- 9.8	2.60906	*-11.2633	4.63087
0.0005 – 0.5	*- 13.07	2.60906	*-13.9667	4.63087
0.0005 – 5.0	*- 60.4567	2.60906	*- 39.1933	4.63087
0.005 – 0.05	*- 5.88	2.60906	*- 5.85667	4.63087
0.005 – 0.5	*- 9.15	2.60906	*- 8.56	4.63087
0.005 – 5.0	*- 56.5367	2.60906	*-33.7867	4.63087
0.05 – 0.5	*- 3.27	2.60906	-2.70333	4.63087
0.05 – 5.0	*- 50.6567	2.60906	*- 27.93	4.63087
0.5 – 5.0	*- 47.3867	2.60906	*- 25.2267	4.63087

* denotes a statistically significant difference

Table 7 : Antibacterial activity against *Escherichia coli* O157:H7 and *Salmonella typhi* (% inhibition) using different concentration of purified protein (5mg/ml – 0.0005 mg/ml)

Protein concentration (mg / ml)	Source of variation	Sum of squares	d.f	Mean square	F-ratio	Significance level
5	Between groups	225.339	1	225.339	38.39	0.0034*
	Within groups	23.4767	4	5.86917		
	Total	248.815	5			
0.5	Between groups	147.114	1	147.114	21.56	0.0097*
	Within groups	27.2959	4	6.82397		
	Total	174.41	5			
0.05	Between groups	164.431	1	164.431	64.72	0.0013*
	Within groups	10.1626	4	2.54065		
	Total	174.594	5			
0.005	Between groups	165.165	1	165.165	112.64	0.0004*
	Within groups	5.86507	4	1.46627		
	Total	171.03	5			
0.0005	Between groups	121.68	1	121.68	26.22	0.0069*
	Within groups	18.5603	4	4.64007		
	Total	140.24	5			

* p < 0.05

Table 8 : Multiple range tests : Antibacterial activity against *Escherichia coli* O157:H7 and *Salmonella typhi* (% inhibition) using different concentration of purified protein (5mg/ml – 0.0005 mg/ml)

Contrast	5 mg/ml		0.5 mg/ml	
	Difference	Limits + / -	Difference	Limits + / -
<i>E. coli</i> O157:H7 - <i>S. typhi</i>	*12.2567	5.49203	*-9.90333	5.92193

Contrast	0.05 mg/ml		0.005 mg/ml		0.0005 mg/ml	
	Difference	Limits + / -	Difference	Limits + / -	Difference	Limits + / -
<i>E. coli</i> O157:H7 - <i>S. typhi</i>	*- 10.47	3.61341	*- 10.4933	2.74506	*- 9.00667	4.88323

* denotes a statistically significant difference

Table 9 : Different concentration comparison of Anti HIV-1 Reverse transcriptase activity (between different concentrations of purified protein)

Activity	Source of variation	Sum of squares	d.f	Mean square	F-ratio	Significance level
Anti HIV-1 RT	Between groups	28245.3	8	3530.66	2328.09	0.0000*
	Within groups	27.2979	18	1.51655		
	Total	28272.6	26			

Table 10: Multiple range tests : Anti HIV-1 Reverse transcriptase (between different concentration of purified protein)

Contrast (purified protein in mg/ml)	Difference	Limits + / -
0.039 – 0.078	*-2.59567	2.11249
0.039 – 0.156	*-5.204	2.11249
0.039 – 0.313	*-6.46933	2.11249
0.039 – 0.625	*-14.735	2.11249
0.039 – 1.25	*-37.247	2.11249
0.039 – 2.5	*-76.2773	2.11249
0.039 - 5	*-77.5723	2.11249
0.078 – 0.156	*- 2.59667	2.11249
0.078 – 0.313	*- 5.2	2.11249
0.078 – 0.625	*- 6.47	2.11249
0.078 – 1.25	*- 14.7333	2.11249
0.078 – 2.5	*- 37.2467	2.11249
0.078 - 5	*- 77.5733	2.11249
0.156 - 0.313	*- 2.60333	2.11249
0.156 - 0.625	*- 3.87333	2.11249
0.156 - 1.25	*- 12.1367	2.11249
0.156 - 2.5	*- 34.65	2.11249
0.156 - 5	*- 74.9767	2.11249
0.313 - 0.625	- 1.27	2.11249
0.313 - 1.25	*- 9.53333	2.11249
0.313 - 2.5	*- 32.0467	2.11249
0.313 - 5	*- 72.3733	2.11249
0.625 – 1.25	*- 8.263333	2.11249
0.625 – 2.5	*- 30.7767	2.11249
0.625 - 5	*- 71.1033	2.11249
1.25 – 2.5	*- 22.5133	2.11249
1.25 - 5	*- 62.84	2.11249
2.5 – 5	*- 40.3267	2.11249

* denotes a statistically significant difference

Table 11 : Hemolytic activity (between different concentrations of melittin as standard protein)

Activity	Source of variation	Sum of squares	d.f	Mean square	F-ratio	Significance level
Hemolytic activity	Between groups	59150.1	9	6572.23	976.08	0.0000
	Within groups	134.666	20	6.73331		
	Total	59284.8	29			

Table 12 : Multiple range tests : Hemolytic activity of melittin (between different concentration)

Contrast (melittin in ug/ml)	Difference	Limits + / -
0.27 - 0.55	*-7.29667	4.41953
0.27 - 1.09	*-9.49333	4.41953
0.27 - 2.19	*-12.3933	4.41953
0.27 - 4.38	*-59.9267	4.41953
0.27 - 8.75	*-100.0	4.41953
0.27 - 17.5	*-100.07	4.41953
0.27 - 35	*-101.883	4.41953
0.27 - 70	*-101.667	4.41953
0.27 - 140	*-102.753	4.41953
0.55 - 1.09	-2.19667	4.41953
0.55 - 2.19	*-5.09667	4.41953
0.55 - 4.38	*-52.63	4.41953
0.55 - 8.75	*-92.7033	4.41953
0.55 - 17.5	*-92.7733	4.41953
0.55 - 35	*-94.5867	4.41953
0.55 - 70	*94.37	4.41953
0.55 - 140	*-95.4567	4.41953
1.09 - 2.19	-2.9	4.41953
1.09 - 4.38	*-50.4333	4.41953
1.09 - 8.75	*-90.5067	4.41953
1.09 - 17.5	*-90.5767	4.41953
1.09- 35	*-92.39	4.41953
1.09 - 70	*-92.1733	4.41953
1.09- 140	*-93.26	4.41953
2.19 - 4.38	*-47.5333	4.41953
2.19 - 8.75	*-87.6067	4.41953
2.19 - 17.5	*-87.6767	4.41953
2.19 -35	*-89.49	4.41953
2.19 - 70	*-89.2733	4.41953
2.19 - 140	*-90.36	4.41953
4.38 - 8.75	*-40.0733	4.41953
4.38 - 17.5	*-40.1433	4.41953
4.38 - 35	*-41.9567	4.41953
4.38- 70	*-41.74	4.41953
4.38 - 140	*-42.8267	4.41953
8.75 - 17.5	-0.07	4.41953
8.75 - 35	-1.88333	4.41953
8.75 - 70	-1.66667	4.41953
8.75 - 140	-2.75333	4.41953
17.5 - 35	-1.81333	4.41953
17.5- 70	-1.59667	4.41953
17.5 - 140	-2.68333	4.41953
35 - 70	0.216667	4.41953
35 - 140	-0.87	4.41953
70 - 140	-1.08667	4.41953

* denotes a statistically significant difference