5 RESULTS

5.1 CULTURING OF ISOLATE NO 6 AND *HALOMONAS SALINA*

Isolation of the Isolate No 6 was done by soil dilution method. The type strain *Halomonas salina* (DSMZ 5928) and Isolate No 6 were stocked in sterile 30 % glycerol solution and stored at -80ºC deep freeze. Brown medium containing 5 % and 20 % NaCl were used in culturing. As indicated in 4.2.1., each moderately halophilic microorganism studied was differentiated in four groups. At the time of culturing, the microorganisms were first pre-cultured in 5 ml of two different Brown medium at two different temperatures; at 37ºC and 45ºC under sterile conditions. The growths of microorganisms were controlled and the organisms reaching nearly 0.65 at OD_{600}, were inoculated at the same conditions. The growths of inoculated samples were again controlled from time to time at OD_{600}. Extra care was given not to lower the medium volume below 10 % by withdrawal of culture sample for OD_{600} control, in the incubation flask during the growth period. After the start of inoculations, microorganisms reaching OD_{600} 1.2, which was the exponential phase, were harvested. The growth information including the wet cell amount of the samples is given at table 5.1

Table 5.1 Growth information about moderately halophilic microorganisms

	No.6 Group1	**No.6 Group2**	**No.6 Group3**	**No.6 Group4**	***H.salina* Group1**	***H.salina* Group2**	***H.salina* Group3**	***H.salina* Group4**
Preculture	1 day	3 days	2 days	2 days	1 day	3 days	2 days	2 days
OD_{600}	0.65	0.68	0.8	0.67	0.72	0.7	0.72	0.74
Inoculation	1 day	3 days	2 days	3 days	1 day	3 days	2 days	3 days
OD_{600}	1.1	1.2	1.38	1.42	1.4	1.3	1.9	1.5
Wet cell (µg)	520	150	420	120	830	200	560	137

The harvested cells were washed 2 times with 4ºC cold 50 mM Tris buffer to eliminate high concentration of salt, which was present in the culturing medium. The cells were extracted by five cycles of freezing and thawing in liquid nitrogen, using "Sigma Total Prot" extracton kit with an external addition of protease inhibitor coctail. Although sonication was proposed for the cell disturption in the technical bulletin of the extraction kit used, after several trials, it was found that freezing and thawing in liquid nitrogen was more convenient because of its capability to inhibit foam formation. Whole cell protein concentrations of the extracted eight samples were determined by Bradford and the concentraions are given in table 5.2

Table 5.2 Whole cell protein concentrations determined by Bradford Method

Concentration	**Isolate No 6**	***Halomonas salina* (DSMZ 5928)**
Group 1	254,87 µg/100µl	291,12 µg/100µl
Group 2	277,35 µg/100µl	255.12 µg/100µl
Group 3	342,5 µg/100µl	301,25 µg/100µl
Group 4	287,5 µg/100µl	281,25 µg/100µl

5.2 2-DE PATTERNS OF ISOLATE NO 6 AND *HALOMONAS SALINA*

Isoelectric focusing gel composition was first prepared with ampholyte mixture ranged between pH 3 to pH 10. 80 µg of samples were applied to the anodic side of the first dimensional gels and focused vertically. Molecular mass separation was performed in the second dimension using SDS-PAGE with 15 % acrylamide gels. The analytical gels were silver stained [112] and it was clearly seen that the whole cell protein composition patterns' of the two microorganisms were mostly located at the acidic side of the gels. Therefore, it was decided to run the first dimension gels with an ampholyte mixture ranging between pH 3 to pH 7.8. Molecular mass separation was performed in the second dimension using SDS-PAGE with 15 % acrylamide gels. For the analytical gels, 80 µg of sample was loaded on 23 x 30 x 0.1 cm (W x L x D) gels and silver stain was used to visualize the whole cell protein pattern. The analytical gel images of 8 different samples are given between figure 5.1 and 5.8

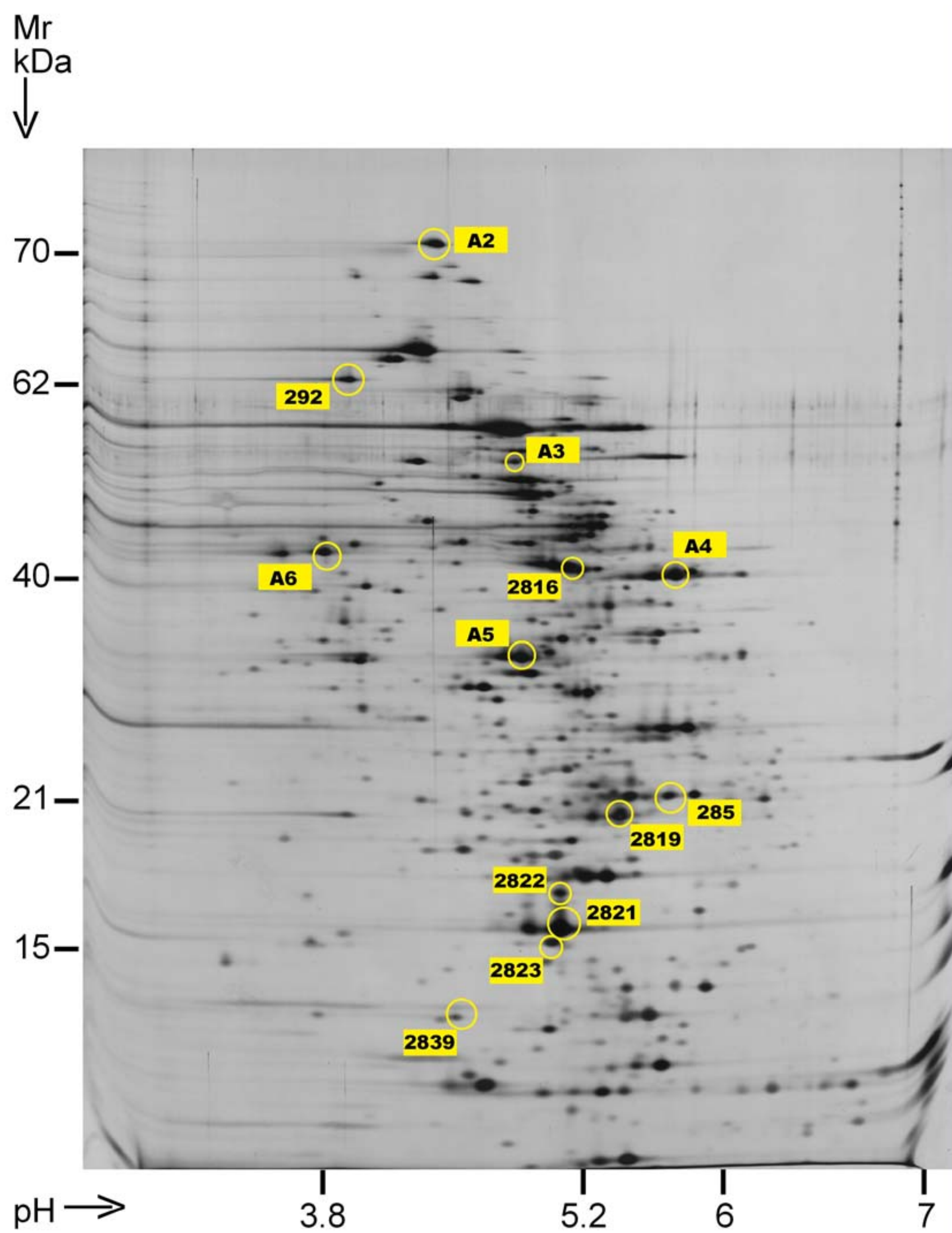

Figure 5.1 Silver stained 2-DE gel of *Halomonas salina* GRP 1. Identified spots are numbered.

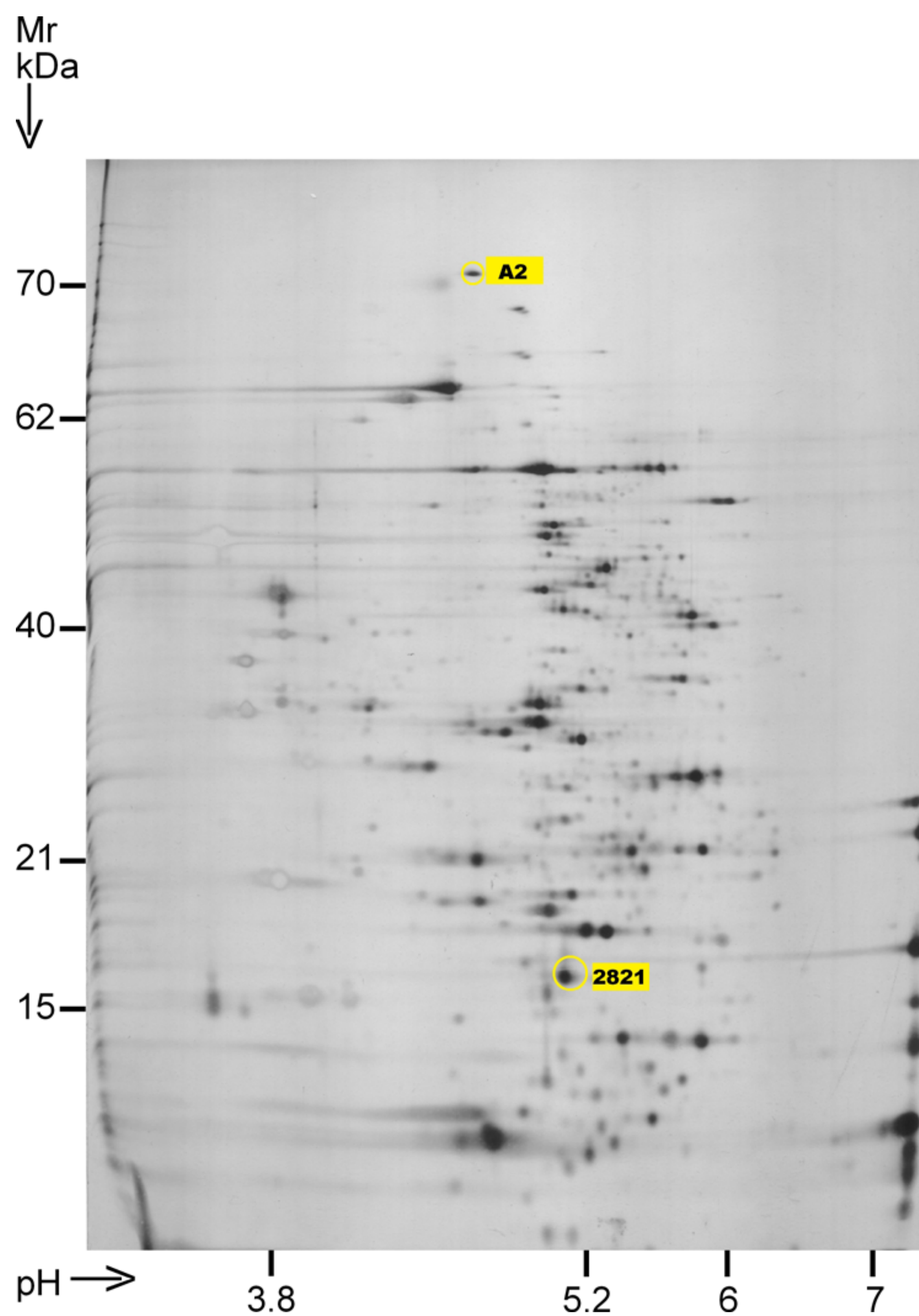

Figure 5.2. Silver stained 2-DE gel of *Halomonas salina* GRP 2. Identified spots are numbered.

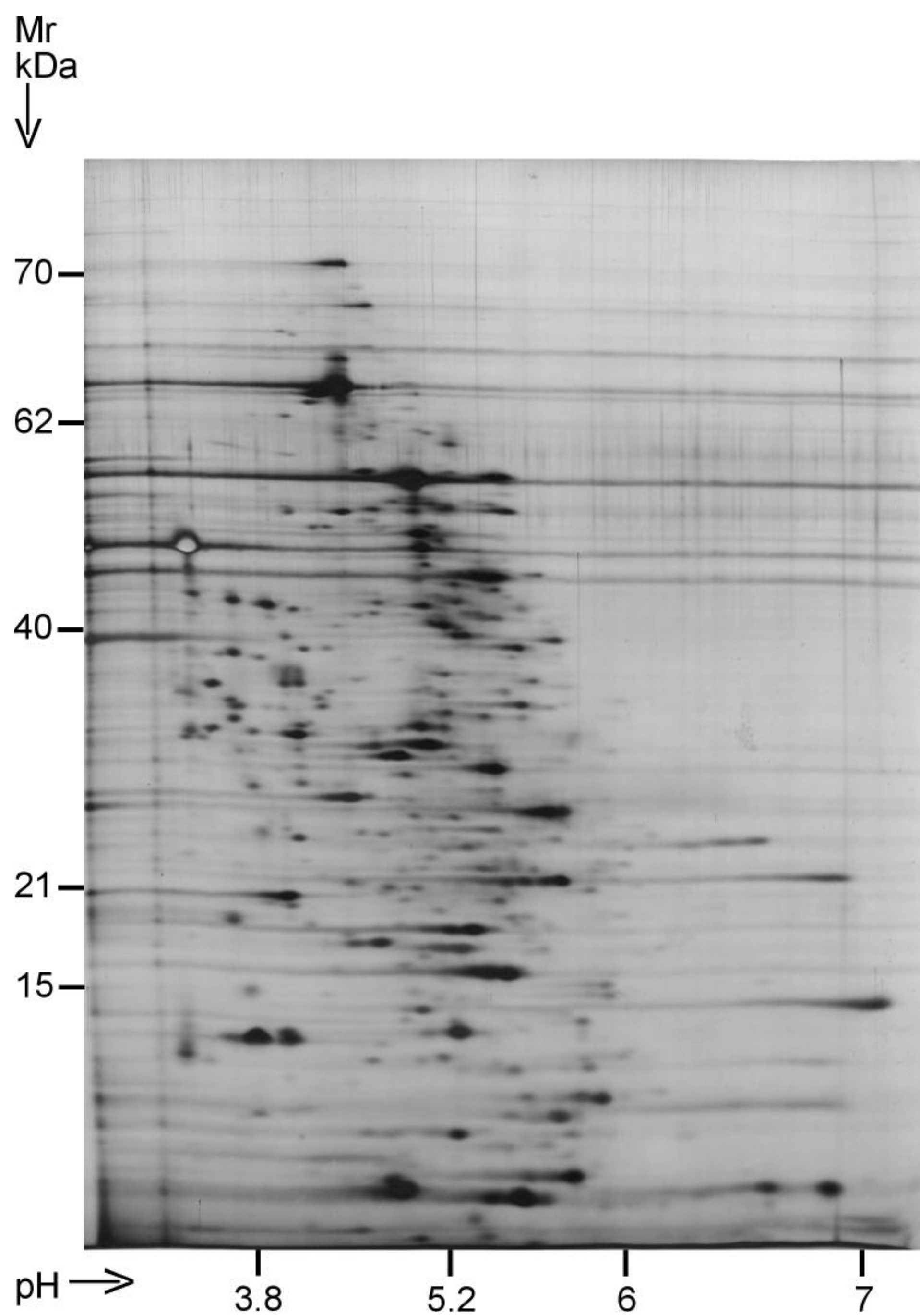

Figure 5.3. Silver stained 2-DE gel of *Halomonas salina* GRP 3.

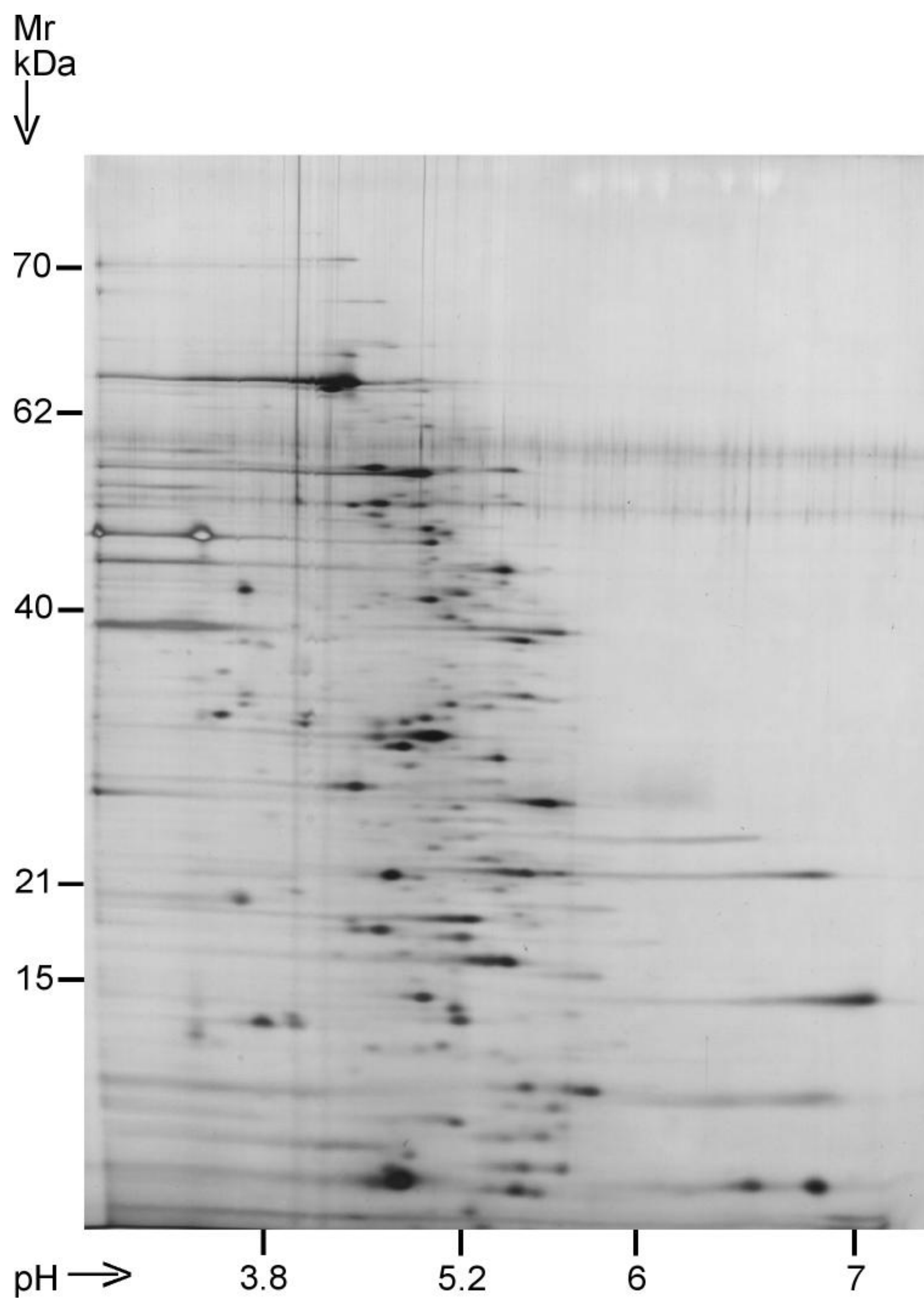

Figure 5.4. Silver stained 2-DE gel of *Halomonas salina* GRP 4.

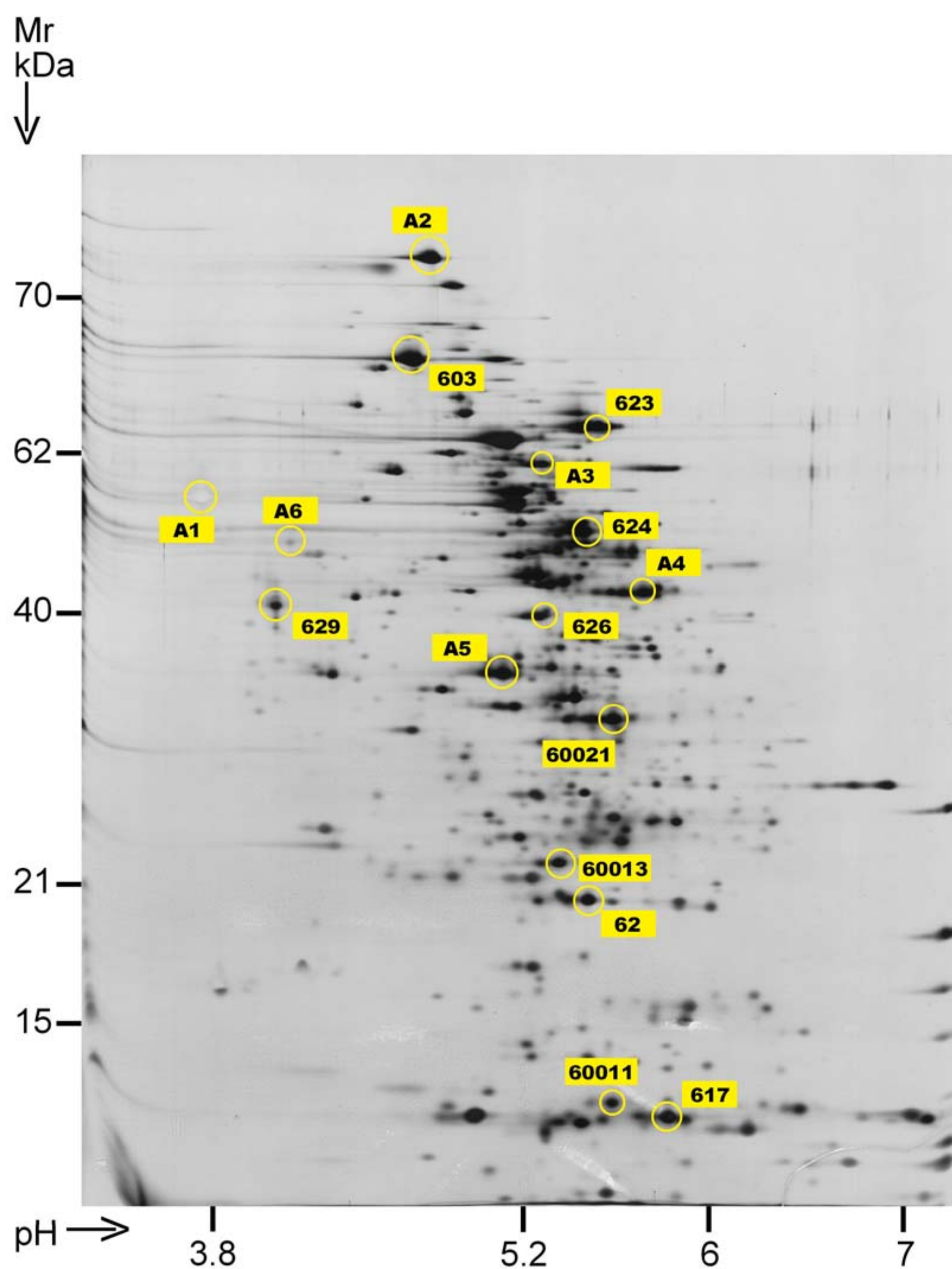

Figure 5.5 Silver stained 2-DE gel of Isolate No 6 GRP 1. Identified spots are numbered.

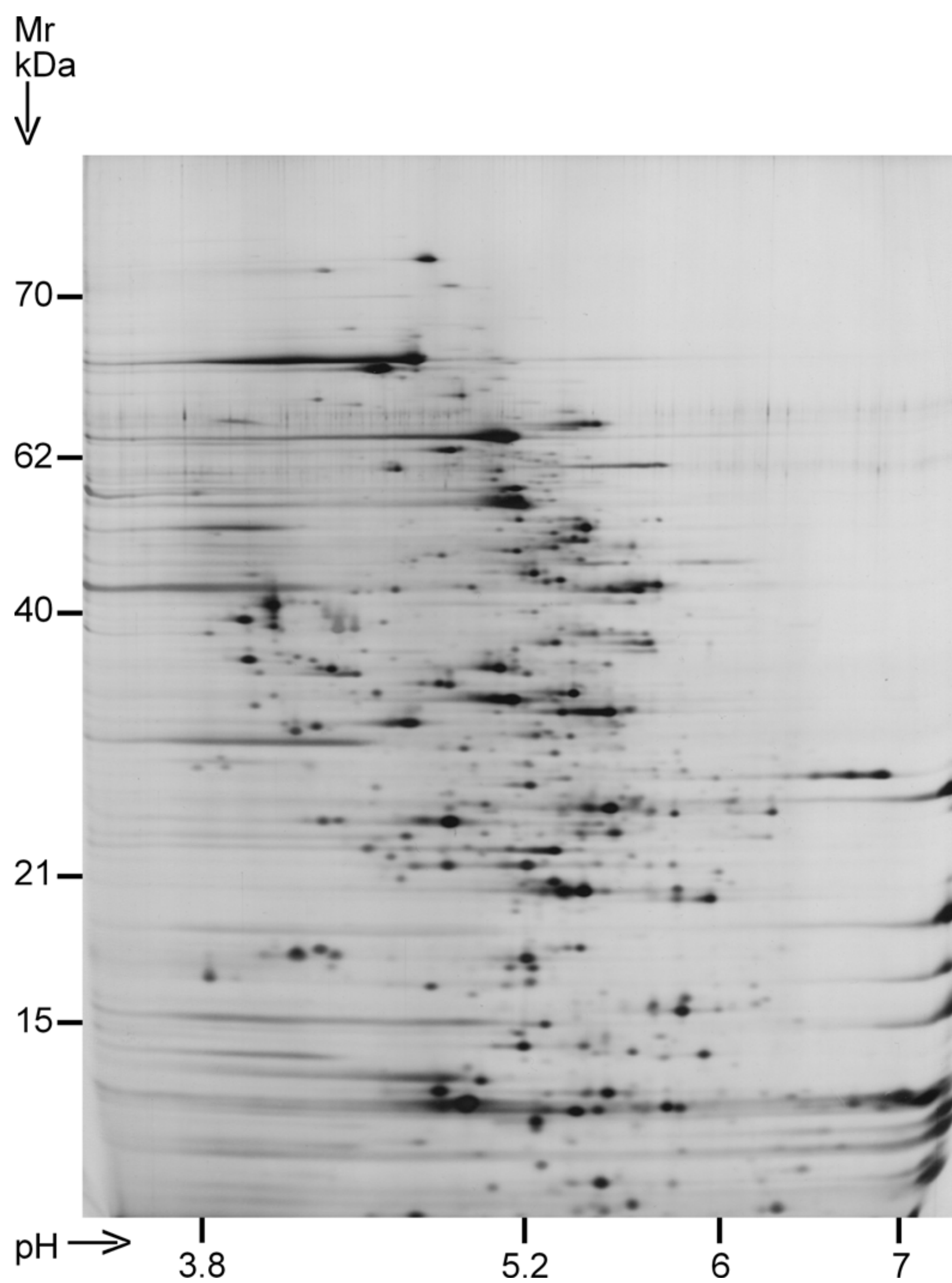

Figure 5.6 Silver stained 2-DE gel of Isolate No 6 GRP 2.

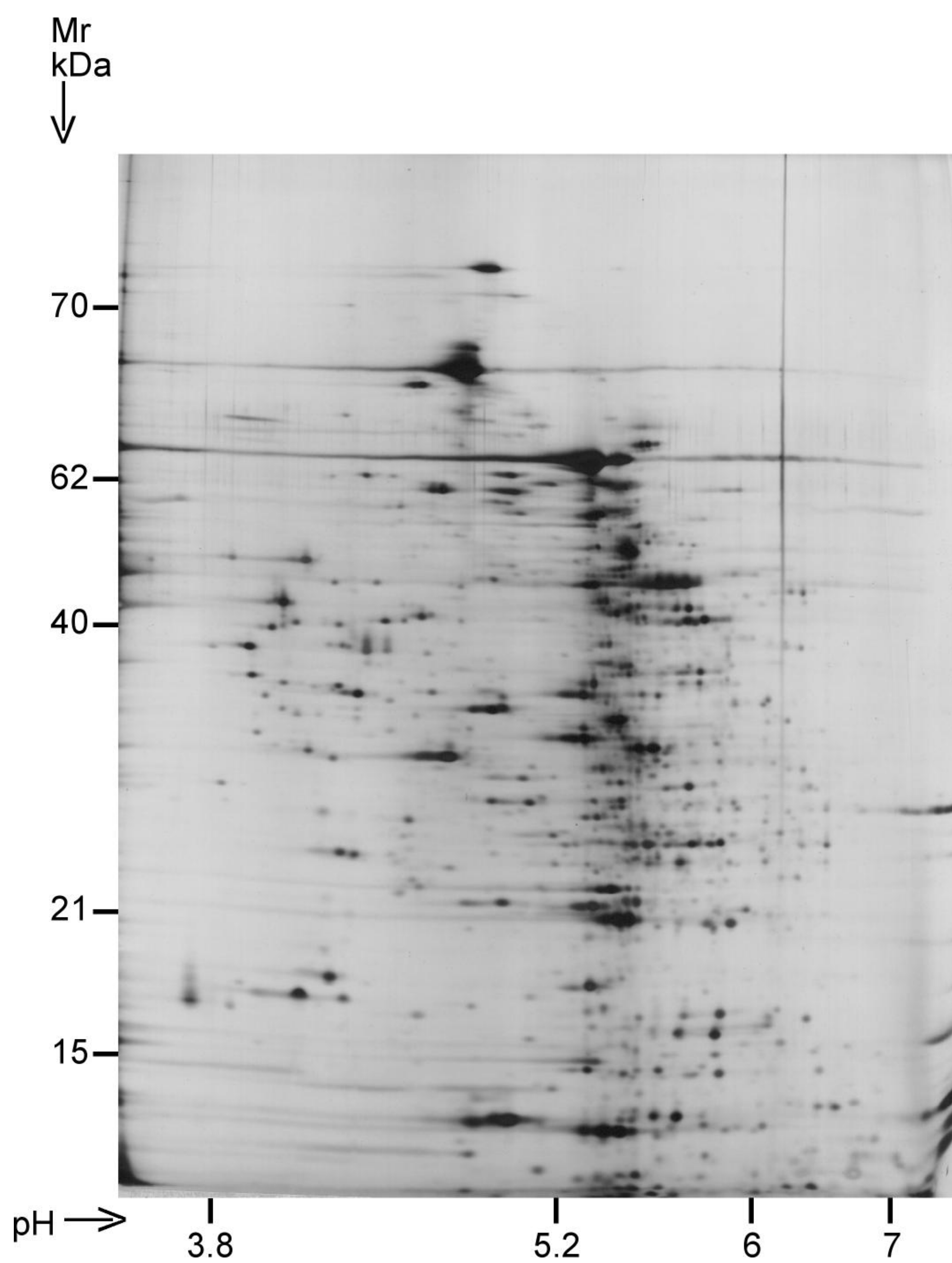

Figure 5.7 Silver stained 2-DE gel of Isolate No 6 GRP 3.

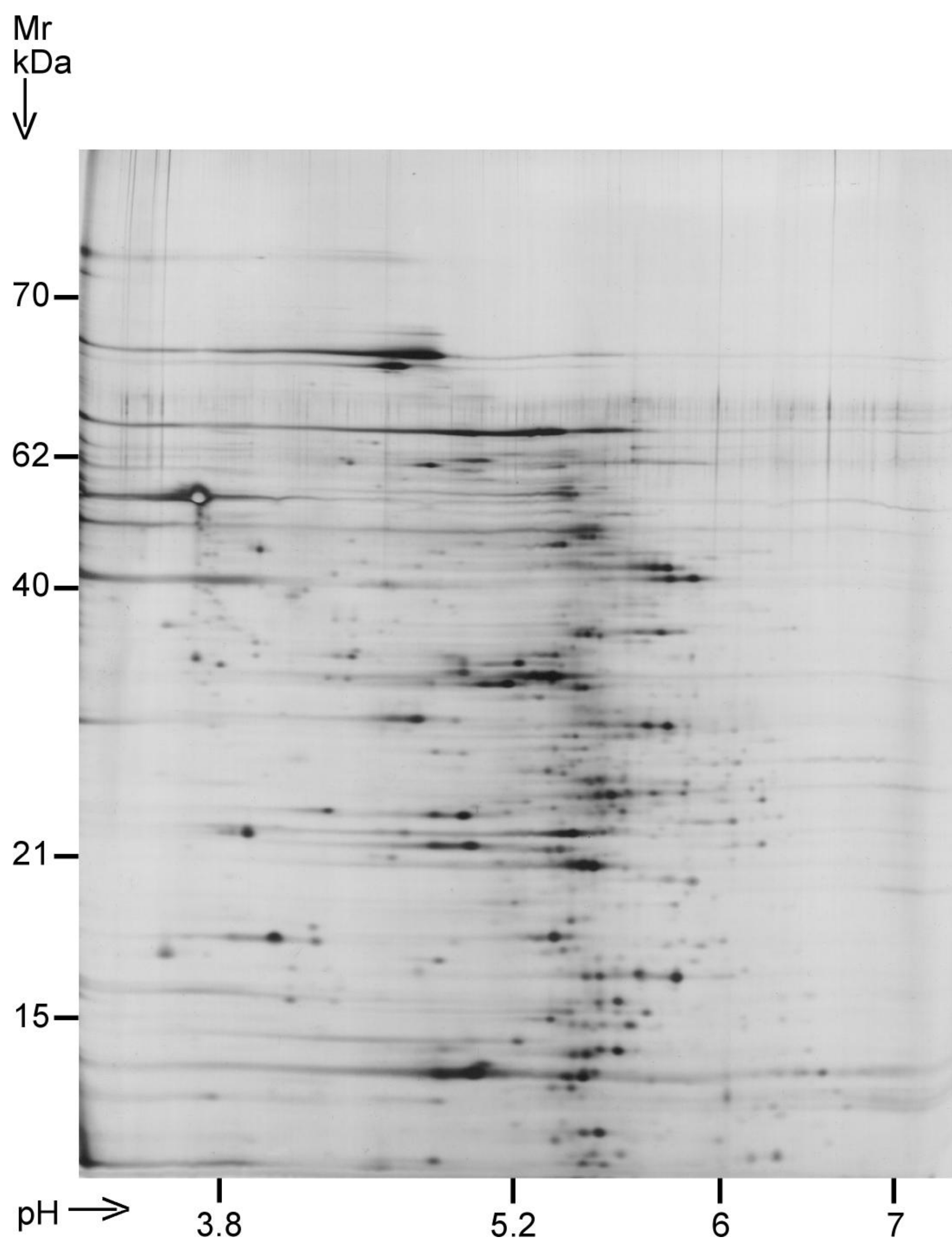

Figure 5.8 Silver stained 2-DE gel of Isolate No 6 GRP 4.

2-DE gels were correlated to each other by overlaying the original 2-DE gels on each other and compared on the basis of migration on 2-DE gels. Two different comparisons have been made.

The first comparison was between two different microorganisms namely a new isolate (Isolate No 6) from soil, and a type strain *Halomonas salina*. This comparison was made between group 1 gels (organisms were cultured at 37ºC in Brown medium containing 5 % NaCl). The protein spots numbered with letter A (Figure 5.1, 5.5) were the analyzed spots present in both organisms.

The second was the comparison of each microorganism within its four different groups. The numbered spots on group 1 of both microorganisms' gels are the protein spots which show expression differences between 4 groups of the same organism. Spot A2 and 2821 were analyzed from both group1 and group2 gels of *Halomonas salina*.

5.3 IDENTIFICATION METHODS AND PARAMETERS OF SELECTED PROTEIN SPOTS

In this study, different identification methods were applied in order to obtain satisfying results.

One of the identification method used was N-terminal sequencing. After the separation of whole cell proteins of the halophilic microorganisms by 2-DE large gels (23 x 30 x 2.5 cm) the spots were directly blotted on PVDF membranes. The blotted membranes were stained with Coomassie Brilliant Blue R-250 and dried. To perform N-terminal Edman degradation, the spots were cut carefully matching the dimensions of the sequencers cartridge. After applying the membrane to the cartridge, Applied Biosystems Model 492A Procise seqencer (WITA GmbH, Teltow, Germany) was run automatically. 10 protein spots, which were subjected to Edman degradation, were successfully sequenced. The amino acid sequences obtained from the analysis were subjected to similarity search (Blast tool) at ExPASy Proreomics Server. Amino acid sequences were edited in raw format at the "Blast" searches. The searches were restricted by limiting the taxonomy to archaea and bacteria. Through the 10 sequenced proteins, 8 of them were identified by database searches and the rest 2 spots could not be matched because of probable absence in the sequence databases.

Another method used for protein identification was Nano LC ESI-QTOF MS/MS. For this analysis, the protein spots from 150 µg of extracts loaded onto the first

dimensional gel and staining of the second dimension 2-DE gels by colloidal CBB-G250 were used. The interesting spots were cut carefully by the help of an automatic micropipette tip carefully, avoiding any kind of contamination from the environment. These were enzymatically digested with trypsin in centrifuge compatible protein purification plate to avoid loss of sample. Tryptic peptides were separated on a Waters BEH130C18 nanoAQUITY UPLC column (75µm x 100mm 1.7µm) connected to a nano ACQUITY UPLC capillary liquid chromatography system delivering a gradient of 5 to 50 % acetonitrile. Eluting peptides were ionised by electrospray ionisation (ESI) on a Q-Tof Premier mass spectrometer (Waters Corporation, Milford, MA, USA). MS/MS analyses were conducted by using collision energy profiles chosen on the basis of the m/z value and the charge state of the parent ion. Fragment ion masses and intensities were correlated to the protein databases NCBInr and Swiss-Prot using the Mascot software.

The partial amino acid sequences obtained were used for similarity searching of amino acid sequences against the NCBI BLAST non-redundant protein sequence database using mascot search at “matrixscience” on the Internet.

Totally 30 protein spots were analysed by Nano LC ESI-QTOF MS/MS and among them 20 spots were identified as homologous proteins from bacterial and achaeal species. Unidentified protein spots had either very low match scores or had poor resolution in their spectra. During the evaluation of the spectra, the Mascot search engine was used. In the search parameters, carbamidomethyl at cysteine and oxidation at methionine sites were set as variable modifications, monoisotopic mass values were selected and fragment and peptide mass tolerances were accepted within ± 0.4 Da.

MALDI-TOF was applied as third possibility to identify proteins by peptide mass fingerprinting (PMF). For this analysis, the protein spots from 150 µg of extract loaded onto the first dimension gels and staining by colloidal CBB-G 250 of the second dimension gels were used. The interesting spots were cut carefully by the help of an automatic micropipette tip carefully, avoiding any kind of contamination from the environment and then enzymatically digested with trypsin. After digestion, the elution derived from the weak spots were further concentrated. Elution of the weak spots were frozen on dry ice and then centrifuged in a speedvac centrifuge to dryness. Then, samples were prepared with only 2 µl of elution buffer. Totally 5 out of 20 spots were analyzed by MALDI-TOF applying several trials and variations of the method, but satisfying and unambiguous results could not be obtained. The main problems were low scores of the data analysis and poor sequence coverage. For the

PMF search, monoisotopic masses were used. The peptide mass tolerance was ±1.2 Da, maximum missed cleavages was set as 1.

For *Halomonas salina* and isolate No 6 the identification methods used and the results are given in Table 5.3 and Table 5.4 respectively.

Table 5.3 List of identified proteins of moderately halophilic bacteria, *Halomonas salina* (DSMZ 5928)

Spot No	Database Identification	Accession No.	Score	pI		Mr (kDa)		Identification Methods
				2-DE	Pred.	2-DE	Pred.	
A2	Aconitate hydratase 1	gi 92114180	695	5.1	4.96	90	94.6	NanoLC ESI QTOF
A3	Succinic semialdehyde dehydrogenase	gi 92114412	96	4.8	4.86	55	51.8	NanoLC ESI QTOF
A4	Acetyl-CoA C-acetyltransferase	gi 92115183	79	5.8	5.64	41.8	40.2	NanoLC ESI QTOF
A5	Malate dehydrogenase	gi 84701538	109	4.8	4.92	36	34.4	NanoLC ESI QTOF
A6	Periplasmic phosphate binding protein	gi 92113600	94	3.8	4.16	42	37.2	NanoLC ESI QTOF
285	Electron transfer flavoprotein beta subunit	gi 92113737	91	5.8	5.62	21	26.8	NanoLC ESI QTOF
292	Solute-binding protein	gi 92113996	58	4.0	4.60	62	58.9	NanoLC ESI QTOF
2816	Putative cobalt-precorrin-6A synthase	gi 48428102	59	5.0	6.20	41.8	36.1	Maldi-TOF
2819	Single-strand binding protein	gi 92112583	128	5.4	5.15	21	20.6	NanoLC ESI QTOF
2821	Hypothetical protein ECA3428	gi 50122352	194	5.1	5.13	16.8	19.1	NanoLC ESI QTOF
2822	Hypothetical protein SSO0826	gi 15897726	69	5.1	10.0	14.8	12.1	Maldi-TOF
2823	Nucleoside diphosphate kinase	gi 27597163	92	5.1	4.72	15	15.2	NanoLC ESI QTOF
2839	30S ribosomal protein S20	gi 59798831	45	4.5	11.8	12	9.5	Maldi-TOF

Table 5.4 List of identified proteins of moderately halophile, isolate No 6

Spot No	Database Identification	Accession No.	MS Score/ Blast Homology*	pI		Mr (kDa)		Identification Methods
				2-DE	Pred.	2-DE	Pred.	
A1	Major outer membrane protein precursor	gi 32363216	74*	3.8	-	60	-	N-Termin.seq./Blast Search
A2	Aconitate hydratase	gi 92115078	322	5.1	4.83	90	99.3	NanoLC ESI QTOF
A3	NAD^+ dependent aldehyde dehydrogenase	gi 15076881	591	5.2	4.98	62	54.8	NanoLC ESI QTOF
A4	Acetyl-CoA C-acetyltransferase	gi 86132046	80	5.9	6.86	43	42.8	Maldi-TOF
A5	Malate dehydrogenase	gi 114319648	270	5.0	5.00	38	35.1	NanoLC ESI QTOF
A6	Periplasmic phosphate binding protein	gi 92113600	76	4.0	4.16	50	37.2	NanoLC ESI QTOF
62	Alkyl hydroperoxide reductase/ thiol specific antioxidant	gi 123083929	86*	5.5	-	20	-	N-Termin.seq./Blast Search
603	Acetate—CoA ligase	gi 92112993	196	4.5	4.99	65	71.8	NanoLC ESI QTOF
617	UspA (Universal stress protein A)	gi 123387377	69*	5.9	-	13	-	N-Termin.seq./Blast Search
623	Dihydrolipoamide dehydrogenase	gi 92113345	127	5.6	5.11	64	50.4	NanoLC ESI QTOF
624	Translation elongation factor Tu	gi 92112552	817	5.4	4.89	50	43.3	NanoLC ESI QTOF
626	Aspartate semialdehyde dehydrogenase	gi 92114568	95	5.2	5.20	40	40.1	NanoLC ESI QTOF
629	Type1 restriction-modification system, M subunit	gi 126665709	72	4.0	5.10	40	56.5	Maldi-TOF
60011	L-ectoine synthase (fragment)	gi 75442224	71*	5.6	-	13	-	N-Termin.seq./Blast Search
60013	Superoxide dismutase	gi 81678313	77*	5.3	-	22	-	N-Termin.seq./Blast Search
60021	Succinyl-CoA ligase [ADP-forming] subunit alpha	gi 84027802	81*	5.6	-	30	-	N-Termin.seq./Blast Search

5.4 2-DE GEL COMPARISON AND IDENTIFICATION OF GROUP 1 PROTEINS OF ISOLATE NO 6 AND *HALOMONAS SALINA*

Group1 of both organisms were compared on the basis of their migration of protein spots on 2-DE gels. Both organisms have acidic proteins expressed and similar protein spots distribution through the whole gels. Between these two moderately halophilic bacteria, 4 protein spots; A2, A4, A5, A6 (Figure 5.1 and 5.5) were identified as the same protein, and protein spot no A3 identified from the same enzyme family using different identification techniques as Nano LC-ESI MS-MS, and MALDI-TOF as given in Table 5.3 and Table 5.4.

5.4.1 ACONITATE HYDRATASE (Spot A2)

As it is seen in (Figure 5.9), spot A2 from group1 of Isolate No 6 obviously expressed more than spot A2 from group1 of *Halomonas salina*. They are both located near 70 kDa Mr value and pH 5 on 2-DE gels. Both spots were identified using Nano LC-ESI MS-MS. For both spots no A2, the proteins identified were aconitate hydratase, from *Chromohalobacter salexigens DSM 3043* with score of 322 from Isolate No 6 and with score of 695 from *Halomonas salina* sequence. The same protein aconitate hydratase was also matched with high scores from other microorganisms like *Marinobacter aquaeolei* VT8, *Idiomarina loihiensis* L2TR, and *Pseudoalteromonas atlantica* T6c.12 peptides from spot A2 of *Halomonas salina* fragmented by MS/MS are listed below. Individual scores of peptides that were greater then 47 indicate identity or extensive homology:

NPPAGEEEAILDLLTNR at the positions 41-57 with an ion score 50

VPPGVDEAAYVK at the positions 58-69 with an ion score 67

GEAESPLIDR at the positions 79-88 with an ion score 68

AGGFCFGGK at the positions 274-282 with an ion score 48 and cysteine carbamidomethylation at Cys-5

IAPIFFNTMEDSGALPIEMDVEK at the positions 283-305 with an ion score 40 and oxidations at Met-9 and Met-19

HGTDEVLTTFELK at the positions 324-336 with an ion score 55

ESLGLAPSDVFR at the positions 364-375 with an ion score 57

MTTVGSQDTTGPMTR at the positions 413-427 with an ion score 53 and oxidations at Met-1 and Met-13

MLLPDTVGTGGDSHTR at the positions 491-506 with an ion score 67

WMIANGYGDAR at the positions 634-644 with an ion score 58 and oxidation at Met-2

LWLAPPTK at the positions 741-748 with an ion score 32

YMSEFDAMAGEIYR at the positions 835-848 with an ion score 66 and oxidation at Met-8

8 peptides from spot A2 of Isolate No 6 fragmented by MS/MS are listed. Individual scores greater then 46 indicate identity or extensive homology:

EIGYRPAR at the positions 77-84 with an ion score 26

VLMQDFTGVPGVVDLAAMR at the positions 85-103 with an ion score 21 and oxidation at Met-18

DNVAIEMER at the positions 143-151 with an ion score 34 and oxidation at Met-7

ERYEFLR at position 154-160 with an ion score 25

WGQQAFDNFR at the positions 161-170 with an ion score 53

VVPPGTGICHQVNLEYLGK at the positions 171-189 with an ion score 64 and cysteine carbamidomethylation at Cys-9

FVEFYGDGLK at the positions 283-292 with an ion score 54

AVIAESYER at the positions 813-821 with an ion score 45

Aconitate hydratase, also known as aconitase, is an iron-sulphur protein that contains a [4Fe-4S]-cluster which takes role in the tricarboxylic acid (TCA) cycle that catalyzes the reversible, stereo-specific izomerisation of citrate to isocitrate via cis-aconitate in the TCA cycle.

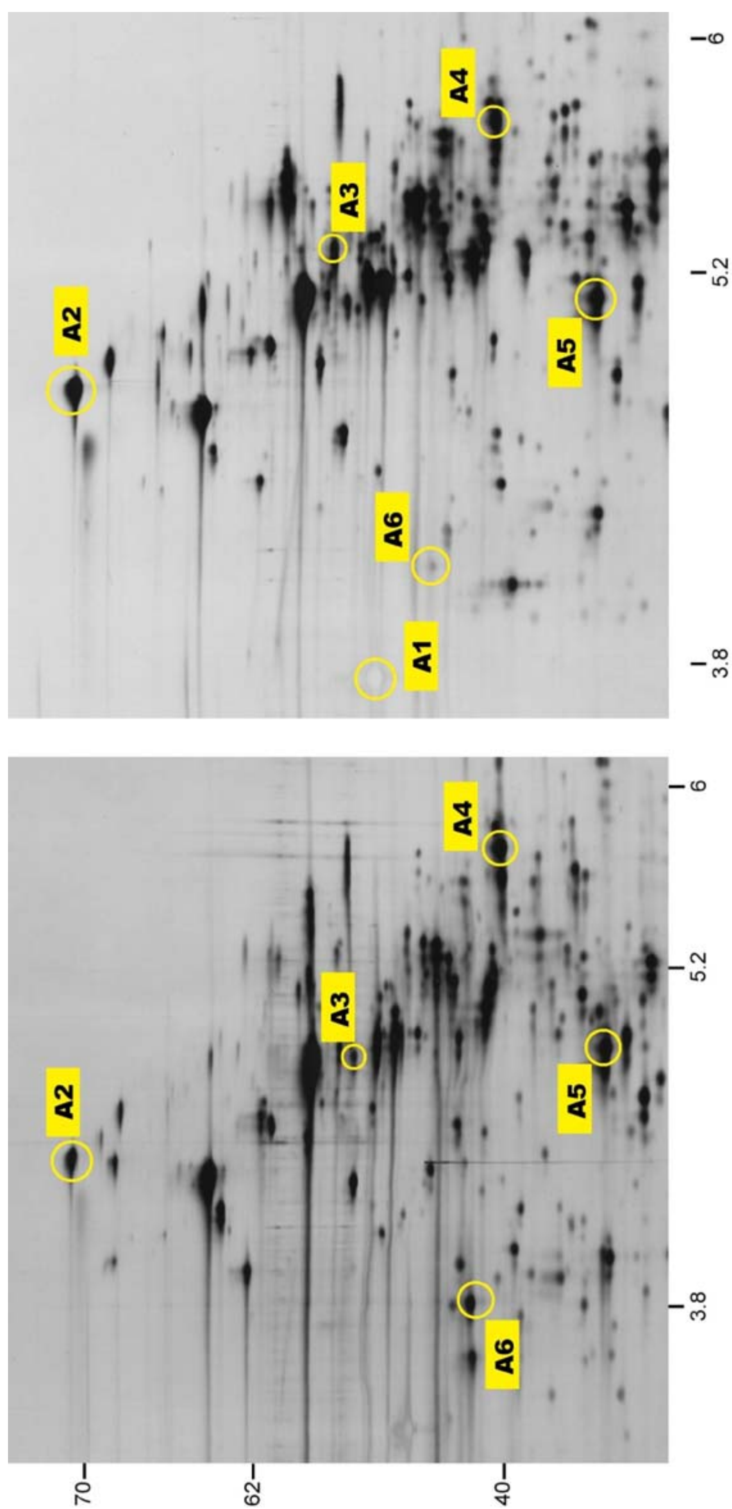

Figure 5.9 Zoomed silver stained 2-DE-gel representation of group 1 of *Halomonas salina* on the left and group 1 of Isolate No 6 on the right.

5.4.2 PERIPLASMIC PHOSPHATE BINDING PROTEIN (Spot A6)

Spot A6 is located at the acidic side of group 1 2-DE gels of both organisms near pH 3.8 and 40 kDa area but expressed more in *Halomonas salina* as shown in figure 5.9. They were both identified by Nano LC-ESI MS-MS as periplasmic phosphate binding protein from *Chromohalobacter salexigens* DSM 3043 with scores of 76 for Isolate No 6 and 94 for *Halomonas salina*. 2 peptides were selected for MS/MS fragmentation from isolate No 6 and *Halomonas salina*.

2 peptides of spot A6 from Isolate No 6, which were fragmented by MS/MS, are listed below. Individual scores of peptides, which were greater then 47, indicate identity or extensive homology:

MKPSEFER at the positions 91-98 with an ion score 26

DAFEELVMEAASEEMDAYGGEGYTDIR at the positions 182-208 with an ion score 50 and oxidations at Met-8 and Met-15

2 peptides of spot A6 from *Halomonas salina* which were further fragmented in MS/MS is given below:

MKPSEFER at the positions 91-98 with an ion score 41

SLFFYVK at the positions 274-280 with an ion score 36

Periplasmic phosphate binding protein is a 341 amino acid long protein with molecular weight of 37.2-kDa and pI value of 4.16. These types of proteins found in the periplasmic space of gram-negative bacteria, and serve as initial high-affinity receptors in the active uptake of specific nutrient, phosphate. After binding their specific substrate molecule, they interact with a membrane bound complex, triggering a series of events that result in translocation of the phosphate. These proteins work with the ABC transport system. Transport works equally well in either direction and is driven by chemiosmotic source of energy where chemiosmotic source of energy include uniport symport or antiport.

5.4.3 DEHYDROGENASE (Spot A3)

Spot No A3 appears near pH value 5.1 but having a little shift to the more acidic site on group 1 gel of *Halomonas salina*. The protein expression levels of the spot A3 in each microorganism do not show very dramatic differences. Spot A3 from *Halomonas salina* was identified as NAD+-dependent aldehyde dehydrogenase from *Halomonas salina* with a very high score of 591. The same protein was also matched

with high scores from *Pseudomonas entomophila* L48, *Aeromonas hydrophila* subsp. Hydrophila and *Kineococcus radiotolerans* SRS30216. Spot No A3 from Isolate No 6 was identified as succinic semialdehyde dehydrogenase (SSADH) from *Chromohalobacter salexigens* DSM 3043 with a score of 96. The same protein was also matched from *Deinococcus radiodurans* R1 with significantly high score. For both A3, the identification method used was Nano LC-ESI MS-MS. Individual ion scores greater than 46 indicated identity or extensive homology.

9 peptides were further fragmented in MS/MS from spot A3 of Isolate No 6. These are:

YGNYIGGEFVPPVK at the positions 19-32 with an ion score 46

ETLNADLPLAVDHFR at the positions 112-126 with an ion score 86

LAPALAAGNCVVLKPAEQTPASILK at the positions 175-199 with an ion score 39 and cysteine carbamidomethylation at Cys-10

IAFTGSTPVGSHILK at the positions 236-250 with an ion score 77

CAAENIIPSTVELGGK at the positions 251-266 with an ion score 83 and cysteine carbamidomethylation at Cys-1

GNPLDTDVK at the positions 331-339 with an ion score 37

VGAQASQEQFDK at the positions 340-351 with an ion score 90

EEGAEFLTGGDK at the positions 361-372 with an ion score 57

VALEHYQQTK at the positions 483-492 with an ion score 64

2 peptides fragmented in MS/MS from spot A3 of *Halomonas salina* are given below:

GVFNVVPGR at position 205-213 with an ion score 35

NAGQTCVCTNR at position 285-295 with an ion score 62 and cysteine carbamidomethylations at Cys-6 and Cys-8.

Spot A3 of group 1 2-DE gel of Isolate No 6 was identified as NAD+-dependent aldehyde dehydrogenase. This type of dehydrogenase act on a wide variety of aliphatic and aromatic aldehyde substrates, using NADP as a cofactor. The typical members of the family are aldehyde dehydrogenase, succinic semialdehyde dehydrogenase, lactaldehyde dehydrogenase, acetaldehyde dehydrogenase, and benzaldehyde dehydrogenase.

Spot No A3 of group 1 2-DE gel of *Halomonas salina* was identified as SSADH, a family member of aldehyde dehydrogenase family. It shares a common evolutionary origin and enzymatic mechanism with lactaldehyde dehydrogenase [113]. Succinic semialdehyde dehydrogenase, catalyses the (NAD(P)+)-dependent catabolism reaction of succinic semialdehyde to succinate for metabolism by the TCA cycle.

5.4.4 ACETYL-CoA C-ACETYLTRANSFERASE (Spot A4)

As seen on figure 5.9, on group 1 2-DE gels of Isolate No 6 and *Halomonas salina,* spot A4 appears at the same localization, near pH value pH 5.9 and Mr value 40 with similar spot intensities. Both A4 spots were identified as acetyl-CoA C-acetyltransferase but from different origins of organism and with different identification methods. Spot A4 from Isolate No6 was identified with MALDI-TOF MS with a score of 80. Totally 10 peptides were matched of the identified acetyl-CoA C-acetyltransferase which was from *Cellulophaga sp.*MED134. The matched peptides are:

EAYIIDGVRTPIGNYK at the positions 2-17

TPIGNYKGTLSAVR at the positions 11-24

LCSSGLSAIIHANRAIK at the positions 88-104 and cysteine carbamidomethylation at Cys-2

AGDGDLFISGGVENMTR at the positions 105-121

GPYVMAKPSTAFGGDSKMYDSTFGWR at the positions 122-147 and oxidation at Met-5

MYDSTFGWR at the positions 139-147 and oxidation at Met-1

DPIIFKDDEFIKPNSSK at the positions 215-231

IMGIGPVQASNKALQK at the positions 294-309 and oxidation at Met-2

EWGLADDDAR at the positions 336-345

YALVTMCIGVGQGYAAVIENVAV at the positions 381-403 and cysteine carbamidomethylation at Cys-7

Spot A4 from *Halomonas salina* was identified with Nano LC-ESI MS-MS

with a score of 79. Totally 2 peptides were fragmented in identified acetyl-CoA acetyltransferase which was from *Chromohalobacter salexigens DSM 3043*. The peptides, which were fragmented in MS/MS, are:

ALHLATQAIR at the positions 94-103 with an ion score 46

AYANAGVDPSIMGIGPAPATR at the positions 278-298 with an ion score 33

Acetyl-CoA C-acetyltransferase belongs to the thiolase family, which catalyzes the thiolysis of a linear fatty acid CoA. Acetoacetyl-CoA thiolase (also called thiolase II) is specific for the thiolysis of acetoacetyl-CoA and involved in biosynthetic pathways such as poly β-hydroxybutyrate synthesis or steroid biogenesis. Its main function is the synthesis of acetoacyl-CoA from two molecules of acetyl-CoA, which shows its importance in several biosynthetic pathways.

5.4.5 MALATE DEHYDROGENASE (Spot A5)

Spot No A5 appears near pH 5.2 and Mr 38 on group1 2-DE gels of both microorganisms (Figure 5.9). Spot No A5 of Isolate No 6 on group 1 2-DE gel seems to be a bit more shifted to the acidic side. The spot A5 of each microorganism have similar spot intensities. Both A5 spots were identified as malate dehydrogenase with Nano LC-ESI MS-MS

Spot No5 of *Halomonas salina* was identified with a score of 109 from *Parvularcula bermudensis*. Also with a score of 100 the same protein was matched from *Alcanivorax borkumensis* SK2. List of peptides fragmented by MS/MS are:

IASGDMLGK at the positions 24-32 with an ion score 46 and oxidation at Met-6

GASSAASAASAAIDHMR at the positions 234-250 with an ion score 63

Spot No 5 of Isolate No 6 was identified with a score of 270 from *Alkalilimnicola ehrlichei* MLHE-1. Also with a score of 76 which is above the minimum score limit, the same protein was matched from *Desulfotalea psychrophila* LSv54. The peptides further fragmented by MS/MS are listed below:

KDLLEANAAIFSAQGK at the positions 99-114 with an ion score 83

DDLLEANAAIFSAQGK at the positions 100-114 with an ion score 100

DWYENDFIPTVQQR at the positions 212-225 with an ion score 87

The second important identification score for A5 of Isolate No 6 was reported from *Parvularcula bermudensis* with a score of 76, and the matched peptide was GASSAASAASAAIDHMR at position 234-250, same as in the result of Spot No5 of *Halomonas salina.*

Malate dehydrogenase, which participates in TCA cycle, belongs to MDH (malate dehydrogenase) type 2 family of LDH/MDH (Lactate dehydrogenase / Malate dehydrogenase) superfamily and catalyzes the reversible oxidation of malate to oxaloacetate utilizing the NAD/NADH cofactor system.

5.5 IDENTIFIED PROTEINS OF ISOLATE NO 6 FROM GROUP 1 GEL AND 2-DE GEL COMPARISON

4 groups of Isolate No.6 were compared within each other according to their 2-DE migration profiles. The spots, which showed migration or expression differences between the 4 groups of the same organism, were numbered on group 1 gels and the spots which correspond to those spots' orientation on the other groups gels were also numbered. The numbered spots on group 1 gels and (spot No A2 and 2821) both from group1 and group 2 gels of *H. salina*, were studied with different identification methods as Nano LC-ESI MS-MS, MALDI-TOF or N-Terminal sequencing. The identified spots are listed in table 5.4.

5.5.1 MAJOR OUTER MEMBRANE PROTEIN PRECURSOR (Spot No A1)

As seen in figure 5.10., the selected A1 spots are located near 3.8 pH value and 60 kDa Mr value on large 2-DE gels. Spot A1 is negatively stained with silver nitrate in group 1 and 4 where in group 4 gel it is darker. It is expressed weaker in group 2 and cannot be seen in group 3 gel images. Spot A1 from group 1 gel of Isolate No 6 was subjected to automated Edman sequence analysis. The sequence ATVYNQDGTKL was determined from this analysis. Applying a blast search at ExPASy server site, with a score of 34.6 bits (74) and the "E" threshold value 0.37, the spot showed similarity to major outer membrane protein precursor from various microorganisms.

Major outer membrane proteins of gram negative bacteria acts as a filter for hydrophilic compounds. Proteins known as porins are responsible for the molecular sieve properties of the outer membrane. Porins form large water-filled channels, which allow the diffusion of hydrophilic molecules into the periplasmic space. Some porins form general diffusion channels that allow any solutes up to a certain size to cross the membrane, while some are specific for a solute and contain a binding site for that solute inside the pores. As porins are the major outer membrane proteins, they also serve as receptor sites for the binding of phages and bacteriocins.

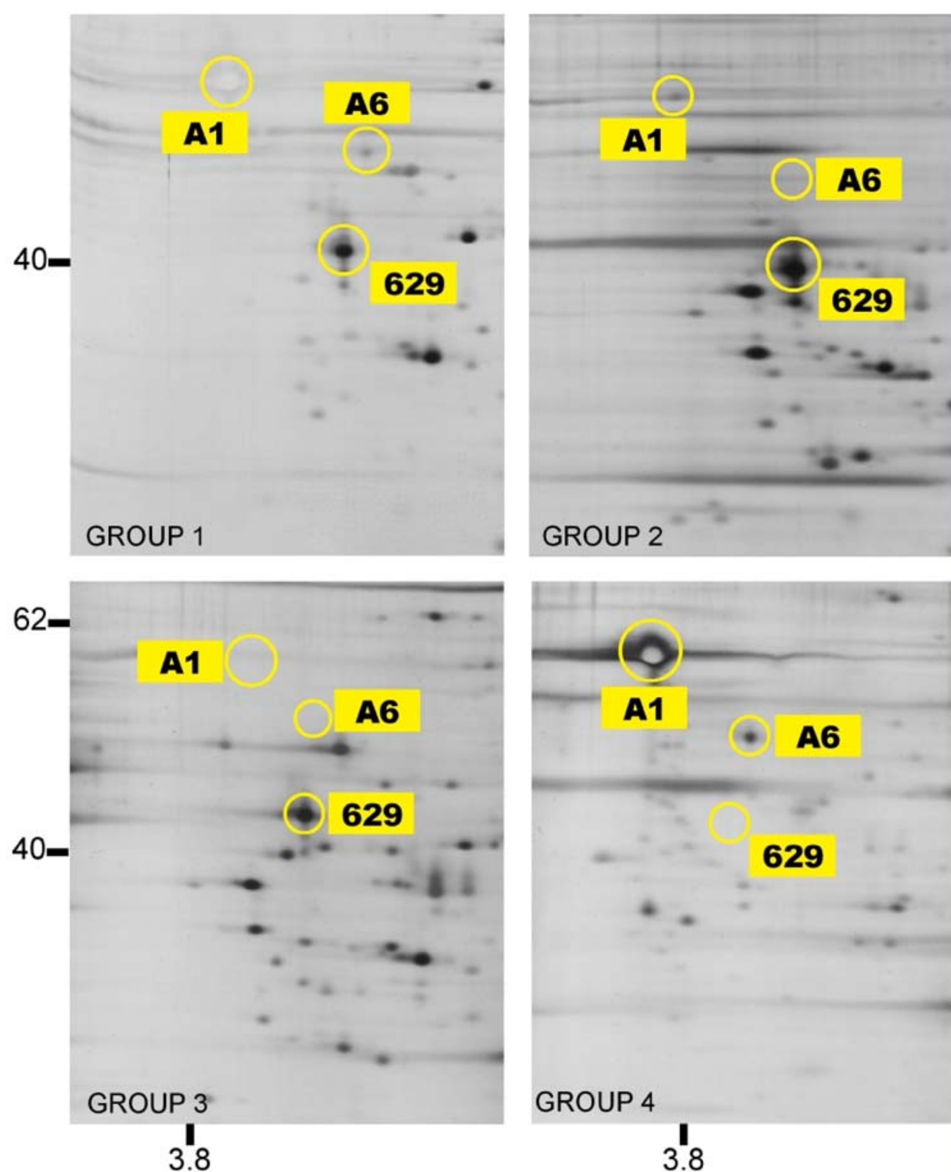

Figure 5.10 Zoomed silver stained 2-DE-gel representations of 4 groups of Isolate No 6 for spots A1, A6 and 629.

5.5.2 TYPE I RESTRICTION-MODIFICATION SYSTEM, M SUBUNIT (Spot No 629)

Spot No 629 is located near pH 4 and 40 kDa Mr value on groups 1, 2 and 3 but not detectable on group 4 2-DE gels of Isolate No 6, as seen in figure 5.10. In group 2, the expression level of the protein seems to be the highest of the four. Spot No 629 was analyzed by MALDI-TOF from the group 1 gel of Isolate No 6. The best match was Type I restriction-modification system, M subunit with a score of 72 from *Marinobacter sp.* ELB17. The matched 13 peptides are:

HELGDMYEQILK at the positions 142-153

AVTQFMVNR at the positions 169-177

AVTQFMVNRVDPK at the positions 169-181

VDPKLEEK at the positions 178-185

TRETADLFMTLFIHLLR at the positions 296-312

TNLLFFTK at the positions 364-371

SYNKTKPMR at the positions 391-399

EETEQAWK at the positions 421-428

EETEQAWKVSIDDIK at the positions 421-435

VSIDDIKAR at the positions 429-437

QAEVQEIR at the positions 467-474

DILAEALSDRSAGGDA at the positions 479-494

Restriction –modification (R-M) systems protect a bacterial cell against invasion of foreign DNA by endonucleolytic cleavage of DNA that lacks a site-specific modification. The R-M system is a complex containing three polyeptides as M, S and R [114].

The M and S subunits together form a methyltransferase that methylates two adenine residues in complementary strands of aDNA recognition sequence. When the target site is unmodified the DNA is cut, when hemimethylated the complex acts as a maintenance methyltransferase and both strands become methylated.

5.5.3 ACETATE-CoA LIGASE (Spot No 603)

As seen in figure 5.11, spot No 603 is detected near pH 4.5 and 65 kDa Mr value on 2-DE gels of 4 groups of Isolate No 6. The spot is detectable in all 4 groups but most dense in group 3 of Isolate No 6. This protein spot is analyzed by using Nano LC-ESI MS-MS and identified as acetate-CoA ligase from *Chromohalobacter salexigens* DSM 3043 with a score of 196. The list of peptides, which were fragmented in MS/MS, is:

VVITADESVR at the positions 183-192 with an ion score of 74

DNVDAALTR at the positions 201-209 with an ion score of 58

DEDGYYWITGR at the positions 506-516 with an ion score 64

Spot No 603 was also matched as acetate-CoA ligase from other organisms as *Hahella chejuensis* KCTC 2336 with a score of 136 and from *Nitrobacter hamburgersis* X14 with a score of 52.

Acetyl-CoA ligase that is ATP-dependent AMP-binding enzyme family (also known as acetate-CoA synthetase and acetyl-activating enzyme) is a ubiquitous enzyme, found in both prokaryotes and eukaryotes, which catalyses the formation of acetyl-CoA from acetate, coenzyme A (CoA) and ATP [115].

The activity of this enzyme is crucial for maintaining the required levels of acetyl-CoA, a key intermediate in many important biosynthetic and catabolic processes. (some prokaryotic species can also activate acetate by either acetate kinase/phosphotransacetylase or by ADP-forming acetyl-CoA synthase).

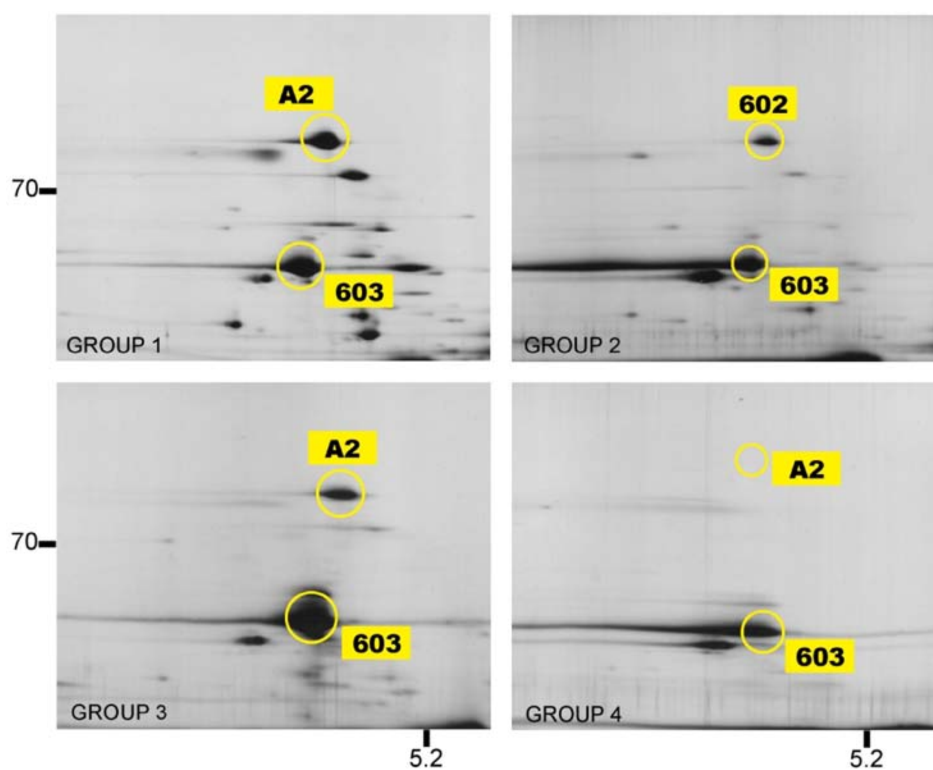

Figure 5.11 Zoomed silver stained 2-DE-gel representations of 4 groups of Isolate No 6 for spots A2, and 603.

5.5.4 DIHYDROLIPOAMIDE DEHYDROGENASE (Spot No 623)

Spot No 623 located near pH 5.6 and 64 kDa Mr value on group1, 2, 3 2-DE gels of Isolate No 6 seen in figure 5.12 but absent in group 4 gel. The expression level is higher on group 1 gel. Spot No 623 was analyzed by Nano LC-ESI MS-MS and

identified as dihydrolipoamide dehydrogenase. The protein was matched from *Chromohalobacter salexigens* DSM 3043 with the best score of 127, but also the same protein was matched from *Haemophilus influenzae* Rd KW20 and *Pseudomonas maendocina ymp* with scores of 51 and 50 respectively. The peptides fragmented by MS/MS were:

ADKFDVIVIGAGPGGYVAAIR at the positions 2-22 with an ion score of 74

ANGVTALEGTGK at the positions 112-123 with an ion score of 44

Dihydrolipoamide dehydrogenase belongs to disulfide oxidoreductase family and also has several other names as lipoamide dehydrogenase, lipoamide oxidoreductase, dehydrolipoate dehydrogenase and some others. The cellular component of the enzyme is cytoplasm It is the E3 component of dehydrogenase complexes for pyruvate, 2-oxoglutarate, 2-oxoisovalerate, and acetoin. It can also serve as the L protein of the glycine cleavage system. It takes part in isoleucine degradation I, valine degradation I, TCA cycle, glycine cleavage complex, and pyruvate dehydrogenase.

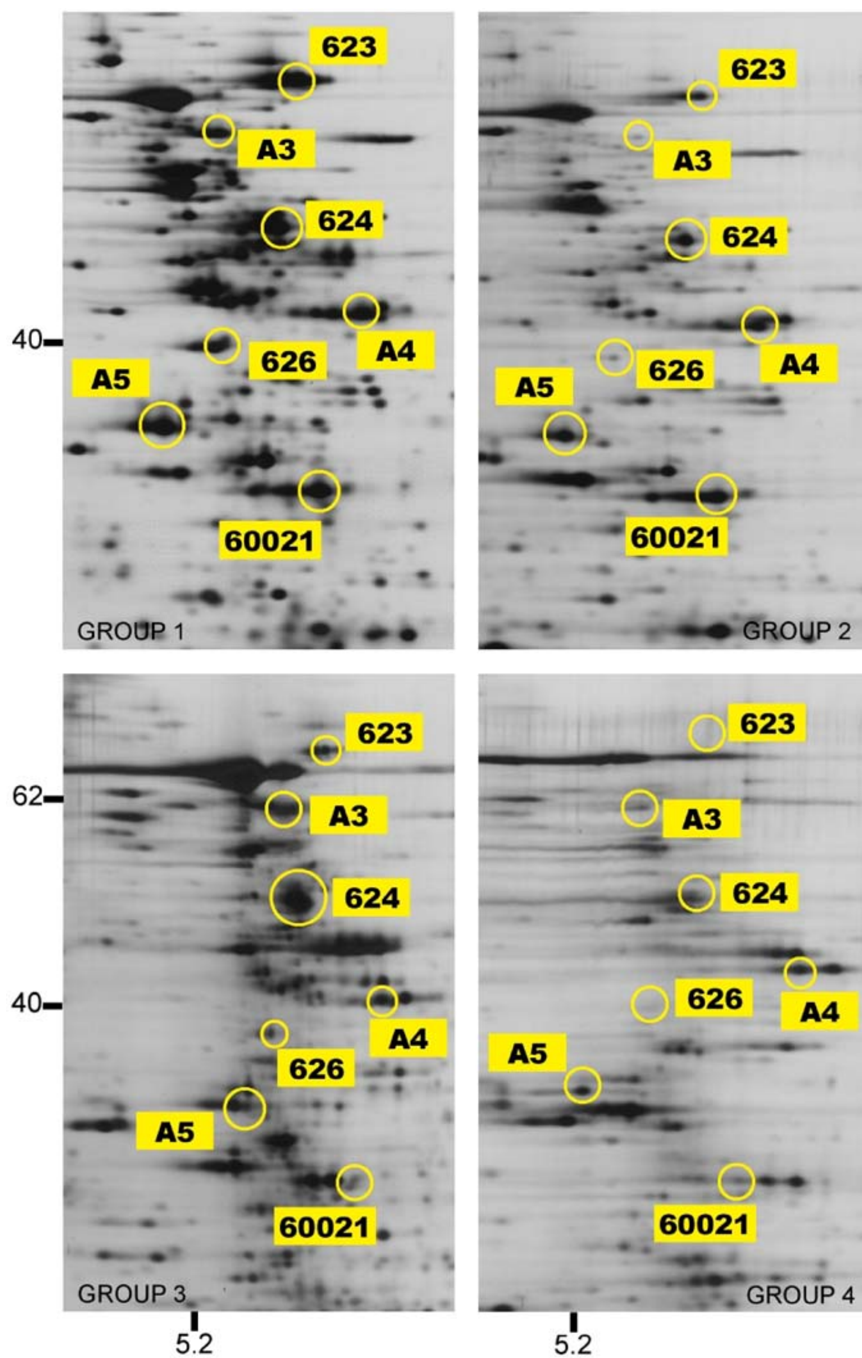

Figure 5.12 Zoomed silver stained 2-DE-gel representations of 4 groups of Isolate No 6 for spots A3, 623,624, A4, 626, A5, and 60021.

5.5.5 TRANSLATION ELONGATION FACTOR Tu (Spot No 624)

As shown in figure 5.12, spot No 624 is located near the area of pH 5.4 and 50 kDa Mr value on 2-DE gels of Isolate No 6. In group 4 spot 624 is weakly seen. The analysis method used was Nano LC-ESI MS-MS for spot 624 from group1 2-DE gel. This spot was identified as translation elongation factor Tu from *Chromohalobacter salexigens* DSM 3043 with a score of 817. The peptides fragmented by MS/MS were:

TTLTAALTR at the positions 26-34 with an ion score of 52

GITIATAHVEYQSEER at the positions 60-75 with an ion score of 86

NMITGAAQMDGAILVCSAADGPMPQTR at the positions 91-117 with an ion score 98 and oxidations at Met-2, Met-9, Met-23 and cysteine carbamidomethylation at Cys -16

ADMVDDEELLELVEMEVR at the positions 138-155 with an ion score 96 and oxidation at Met-3.

ELLNEYDFPGDDCPIITGSALMALEGK at the positions 156-182 with an ion score 100 and oxidation at Met-22 and cysteine carbamidomethylation at Cys-13

DDNGMGTTAVANLIK at the positions 183-197 with an ion score 90

ALDAYIPEPER at the positions 198-208 with an ion score 57

SGEEVEIVGLK at the positions 242-252 with an ion score 73

TTVTGVEMFR at the positions 257-266 with an ion score 61

AGENIGALLR at the positions 274-283 with an ion score 69

GYRPQFYFR at the positions 329-337 with an ion score 39.

For spot No 624, the same protein name translation elongation factor Tu was found from other organisms as *Reinekea sp*. MED297, *Oceanobacter sp*. RED65, *Marinomonas sp*. MED121, *Idiomarina loihiensis* L2TR, *Haemophilus influenzae* Rd KW20, *Geobacter metallireducens* GS-15, *Colwellia psychrerythraea* 34H, *Neisseria meningitides* MC58, *Alkalilimnicola ehrlichei* MLHE-1, marine gamma proteobacterium, *Saccharophagus degradans*, and *Desulfuromonas acetoxidans* DSM 684, with significant hits.

This protein, translation elongation factor Tu is a member of the G- protein superfamily clan, EF-Tu/EF-1A subfamily. Elongation factors belong to a family of proteins that promote the GTP-dependent binding of aminoacyl tRNA to the A site of

ribosomes during protein biosynthesis, and catalyze the translocation of the synthesized protein chain from the A to the P site.

This protein promotes the GTP-dependent binding of aminoacyl-tRNA to the A-site of ribosomes during protein biosynthesis. EF1A (or EF-Tu) is responsible for the selection and binding of the cognate aminoacyl-tRNA to the A-site (acceptor site) of the ribosome. EF2 (or EF-G) is responsible for the translocation of the peptidyl-tRNA from the A-site to the P-site (peptidyl-tRNA site) of the ribosome, thereby freeing the A-site for the next aminoacyl-tRNA to bind. Elongation factors are responsible for achieving accuracy of translation and both EF1A and EF2 are remarkably conserved throughout evolution.

5.5.6 ASPARTATE SEMIALDEHYDE DEHYDROGENASE (Spot No 626)

Spot No 626 is located near pH 5.2 and 40 kDa Mr value on 2-DE gel of Group 1 of Isolate No 6. Probable spot No 626, is weakly present at group 3 but very weakly present at group 2 and no spot seen at the same position in group 4 gels. (figure 5.12). The analysis of spot No 626 from group 1 gel was done by Nano LC-ESI MS-MS. The spot was identified as aspartate semialdehyde dehydrogenase from various microorganisms as *Chromohalobacter salexigens*, *Chromobacterium violaceum* ATCC 12472, *Vibrio cholerae, Marinomonas sp*. MED121, *Pseudomonas mendocina ymp, Marinobacter aquaeolei* VT8 and *Methylophilus methyltrophus* with significant scores. The best score was 111 and from *Chromobacterium violaceum.* The 2 peptides fragmented by MS/MS are :

GMVGSVLMQR at the positions 11-20 with an ion score of 54 and oxidation at Met-2

SHSQAFTIK at the positions 274-282 with an ion score of 46.

Aspartate semialdehyde dehydrogenase, which is found in the cytoplasm, belongs to the oxidoreductase family. Other known names of the enzyme are aspartic semialdehyde dehydrogenase, and L-aspartate-beta-semialdehyde dehydrogenase. It takes role in lysine biosynthesis I, homoserine biosynthesis, and ectoine biosynthesis. It has an oxidoreductase activity, acting on the aldehyde or oxo group of donors.

5.5.7 SUCCINYL –CoA LIGASE [ADP-FORMING] SUBUNIT ALPHA (Spot No 60021)

As seen in figure 5.12, spot 60021 was detected near pH 5.6 and 30 kDa Mr value on 2-DE gel of group 1. The corresponding spot on group 2 positioned at the same

location but less dense, also in group 3 but shifted to the basic site at group 4 gel. The N-Terminal sequencing was applied and the sequence determined was "SILIDKNTKVI". The Blast hit score was 38 bits (81) with a threshold value 0.047. This spot was found to be succinyl-CoA ligase [ADP-forming] subunit alpha with an average molecular weight of 29.77 kDa, and theoretical pI value of 56 in the similarity search, Blast. This protein was matched with the same score from different bacteria.

Succinyl-CoA ligase [ADP-forming] subunit alpha belongs to the succinate/malate CoA ligase alpha subunit family. Succinyl-CoA ligase (ADP-forming) is a bacterial enzyme that during aerobic metabolism functions in the TCA cycle, coupling the hydrolysis of succinyl-CoA to the synthesis of ATP.

5.5.8 SUPEROXIDE DISMUTASE (Spot No 60013)

Spot 60013 is detected near pH 5.3 and 22 kDa Mr value on 2-DE gel of group1. As seen in figure 5.13, the spot's locations on 2-DE gels are similar but only on group 4 gel, the spot seems to be located more in the acidic area. With the N-terminal sequence analysis the sequence of 10 amino acids was determined as "AFELPALPYE". This sequence gave matches to superoxide dismutase from various bacteria with highest score of 36 bits (77) in the similarity search Blast. Theoretical average molecular weight of this protein is 21.64 kDa and pI is 4.89 and shows similarity to iron/manganese superoxide dismutase family. These enzymes destroy radicals which are normally produced within the cells and which are toxic to biological systems. SODs (Superoxide dismutases) catalyse the conversion of superoxide radicals to molecular oxygen. Fe/Mn SODs are ubiquitous enzymes that are responsible for the majority of SOD activity in prokaryotes.

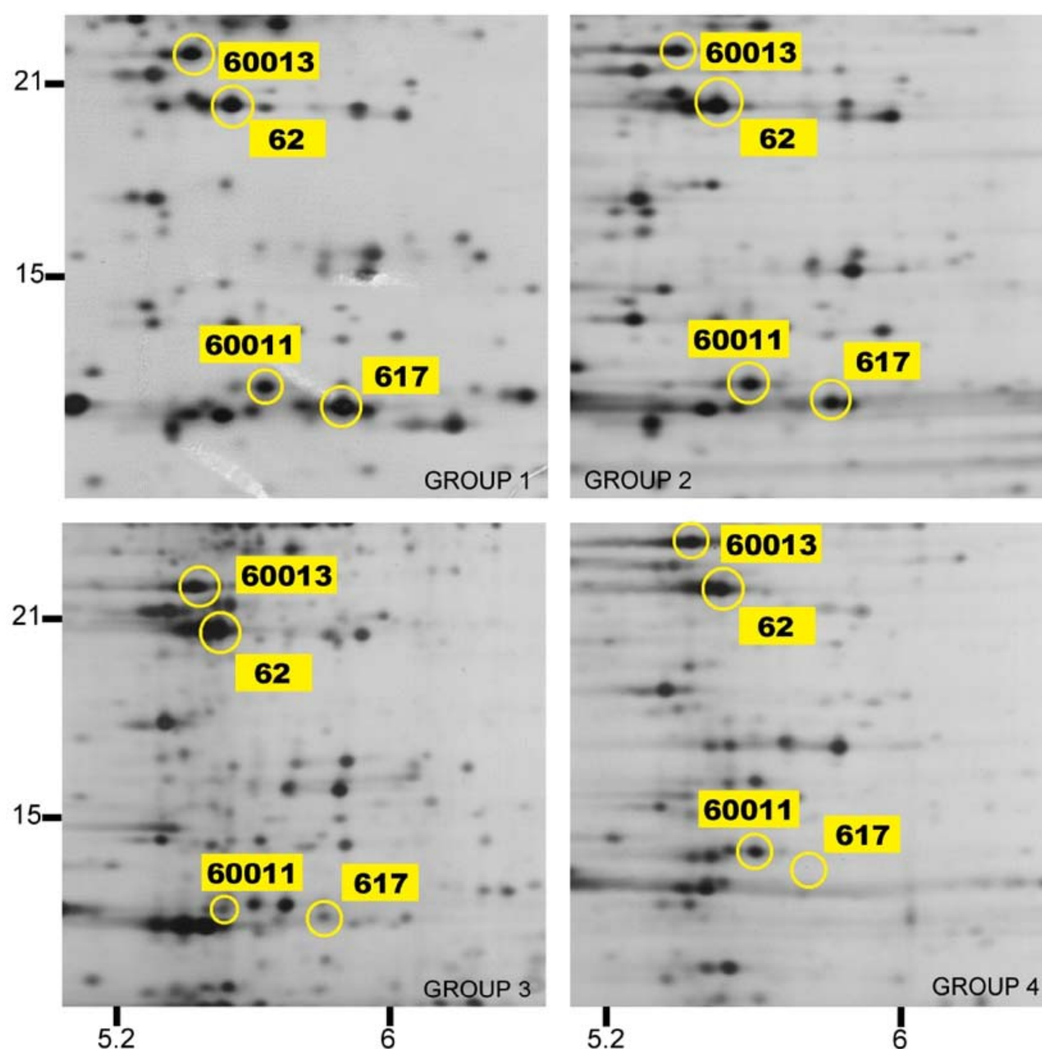

Fig 5.13 Zoomed silver stained 2-DE-gel representations of 4 groups of Isolate No 6 for spots 60013, 62, 60011 and 617.

5.5.9 ALKYL HYDROPEROXIDE REDUCTASE (Spot No 62)

As seen in figure 5.13, spot No 62 detected near 5.5-pH value and 20 kDa Mr value on large gel 2-DE gels. In group 2, 3, gels similar spot is found in the same localization and in group 4 gel it s seen shifted to more acidic region. The protein spot was subjected to automate Edman sequence analysis from the blotted membrane of group 1 gel. The resultant sequence was SVLVGRQAPDFEA, which showed high homology to alkyl hydroperoxide reductase from *Chromohalobacter salexigens* DSMZ 3043, and also various bacteria and achaea in the blast search. The blast search score was 40 bits (86) with the threshold value 0.011. The theoretical molecular weight of the protein is 22.01 kDa with a pI value of 5.03.

Also from group 2 gels, the numbered spot 62 was also studied with Edmann sequencing and the sequence obtained was “ALRLGDIAPDF”. This sequence was

searched in blast and gave homology to alkyl peroxide reductase / Thiol specific antioxidant from *Rastonia metallidurans* with a score of 37.5 bits (81) with a threshold value 0.08.

Alkyl hydroperoxide reductase (AhpC) is responsible for directly reducing organic hyperoxides in its reduced dithiol form. Thiol specific antioxidant (TSA) is a physiologically important antioxidant which constitutes an enzymatic defense against suphur-containing radicals. It is known that it is induced by heat shock, salt stress, oxidative stress and glucose limitation.

5.5.10 L- ECTOINE SYNTHASE (FRAGMENT) (Spot No 60011)

Spot 60011 was detected near pH 5.6 and 13 Mr value on DE gel of group 1 2-DE gel of Isolate No 6. On the same location there is also clear, probable spot 60011, on group 2 3 and 4 gels, but less dense on group 3 gel as seen in figure 5.13. The protein spot of group 1 gel is subjected to N-terminal sequencing and the resultant sequence was MIVRNLEEA. This sequence showed a high homology to L-ectoine synthase (fragment) from various halophilic bacteria and achaea. The highest score was 33 bits (77) from *Halomonas elongata*. From the SwissProt entry for this protein O52251, the theoretical Mr value is 7.62 kDa and pI value is 5.

This protein to L-ectoine synthase (fragment) belongs to ectoine synthase family, which takes role in amine and polyamine biosynthesis, ectoine biosynthesis, L-ectoine from L-aspartate 4-semialdehyde pathways. It catalyzes the circularization of gamma-N-actyl-alpha, gamma-diaminobutric acid to ectoine (1,4,5,6-tetrahdro-2-methyl-4-pyrimidine carboxylic acid), which is an excellent osmoprotectant.

5.5.11 UspA [Csal 2397] (Spot No 617)

As given in figure 5.13, spot 617 is located near pH value 5.9 and Mr value 13 kDa on 2-DE gels of Isolate No 6. This spot is weakly present in group 3 gel and nearly can not be seen in group 4. N-terminal sequencing was applied to this spot and the sequence obtained was “SNEY(R)(H) VLVAV” which gave only one homology result as universal stress protein A from *Chromohalobacter salexigens* DSMZ 3043. The individual score was 32 bits (69). The theoretical Mr value of this protein is 16.37 kDa and the pI value is 5.11. The universal stress protein UspA is a small cytoplasmic bacterial protein whose expression is enhanced when the cell is exposed to stress agents. UspA enhances the rate of cell survival during prolonged exposure to such conditions, and may provide a general "stress endurance" activity.

5.6 2-DE GEL COMPARISON OF 4 GROUPS OF *HALOMONAS SALINA*

4 groups of *Halomonas salina* were compared within each other according to their 2-DE migration profiles. The spots which showed migration or expression differences between the 4 groups of the same microorganism, were numbered on group 1 gels and the spots which correspond to orientation of those spots on the other groups gels, were also numbered. The numbered spots on group 1 gels and spot 2821 both from group 1 and group 2 gels, were studied with different identification methods as Nano LC-ESI MS-MS, and MALDI-TOF.

5.6.1 EXTRACELLULAR SOLUTE-BINDING PROTEIN, FAMILY 5 (Spot 292)

Spot 292 was detected near 62 kDa and pH 4 area on 2-DE gel of group 1 of *Halomonas salina*. In group 2 and group 3 gels the corresponding spot appears at the same location but less dense. In group 4, there is a very weak spot at the same location but a bit at more acidic site, seen in figure 5.14. The analysis was made by Nano LC-ESI MS-MS. The protein matched was extracellular binding protein family 5 from *Chromohalobacter salexigens* DSM 3043. The score was 58, which is in the acceptable limit. The peptide fragmented further was:

VEAFQDPR with an individual score 48 at the positions 291-298.

Bacterial high affinity transport systems are involved in active transport of solutes across the cytoplasmic membrane. The protein components of these traffic systems include one or two transmembrane protein components, one or two membrane-associated ATP-binding proteins and a high affinity periplasmic solute-binding protein. In addition, some solute-binding proteins function in the initiation of sensory transduction pathways. Family 5 includes periplasmic oligopeptide-binding proteins (oppA) of gram-negative bacteria.

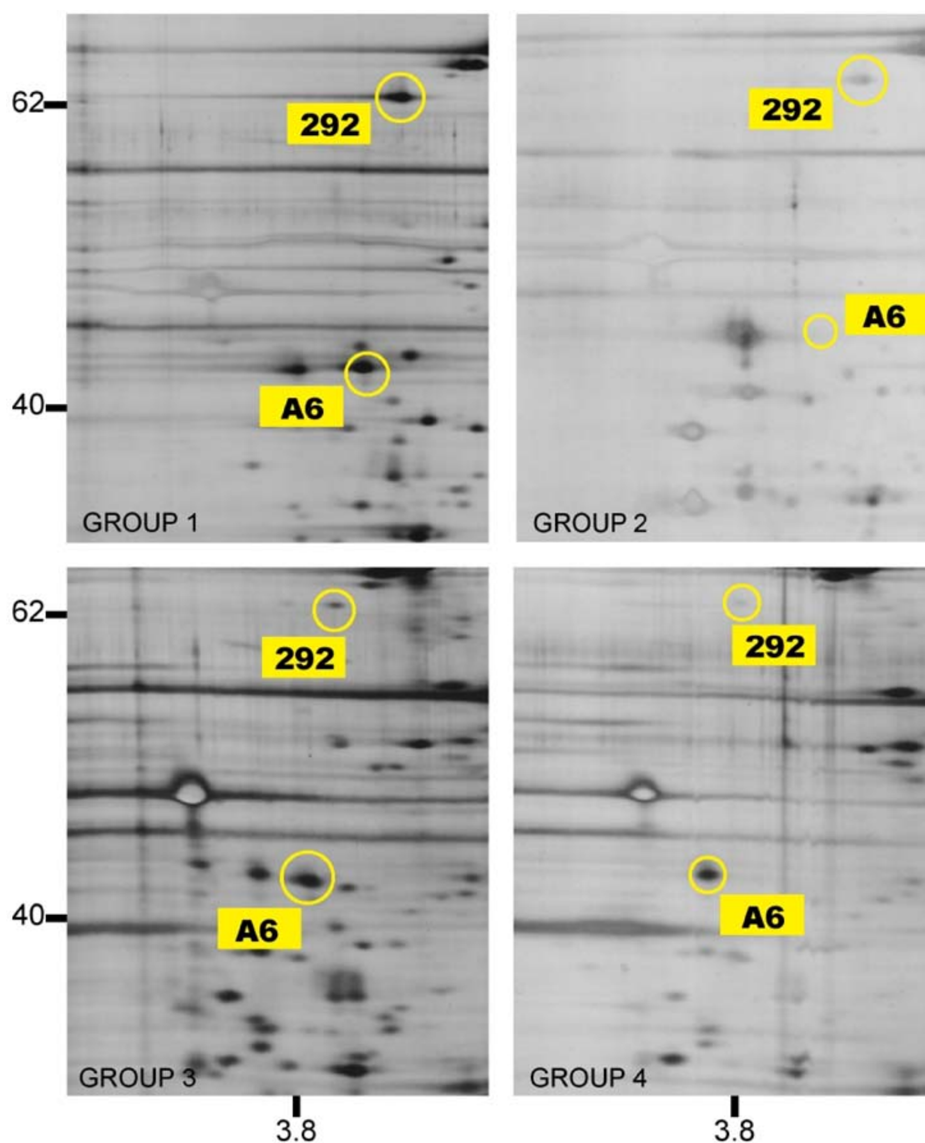

Fig.5.14 Zoomed silver stained 2-DE-gel representations of 4 groups of *Halomonas salina* for spots 292 and A6.

5.6.2 PUTATIVE COBALT-PRECORRIN-6A SYNTHASE (Spot 2816)

As given in figure 5.15, spot 2816 is clearly seen in group 1 of *Halomonas salina*, corresponding spot in group 2 is less dense but not so easily seen in group 3 and 4 of 2-DE gels of *Halomonas salina*. Spot 2816 from group 1 gel of *Halomonas salina* was identified by MALDI-TOF. The matched protein was putative cobalt-precorrin -6A synthase [deacetylating] with a score of 59, which was above the minimum acceptable limit. The matched 8 peptides were:

IDPVNNFK at the positions 2-9

IPEEWIAR at the positions 10-17

KTVDSVSVPTPVGLR at the positions 66-80

SSPGRAVVK at the positions 88-96

GAEVTILIPEGER at the positions 170-182

CTDKVVLTTGR at the positions 224-234 with cysteine carbamidomethylation at Cys-1

VVLTTGRIGMK at the positions 328-338

DGSVLMDSK at the positions 328-336 with an oxidation at Met-6

Putative cobalt-precorrin -6A synthase may catalyze the methylation of C-1 in cobalt-precorrin-5 and the subsequent extrusion of acetic acid from the resulting intermediate to form cobalt-precorrin-6A. It takes role mainly in cofactor biosynthesis, and adenosylcobalmin biosynthesis.

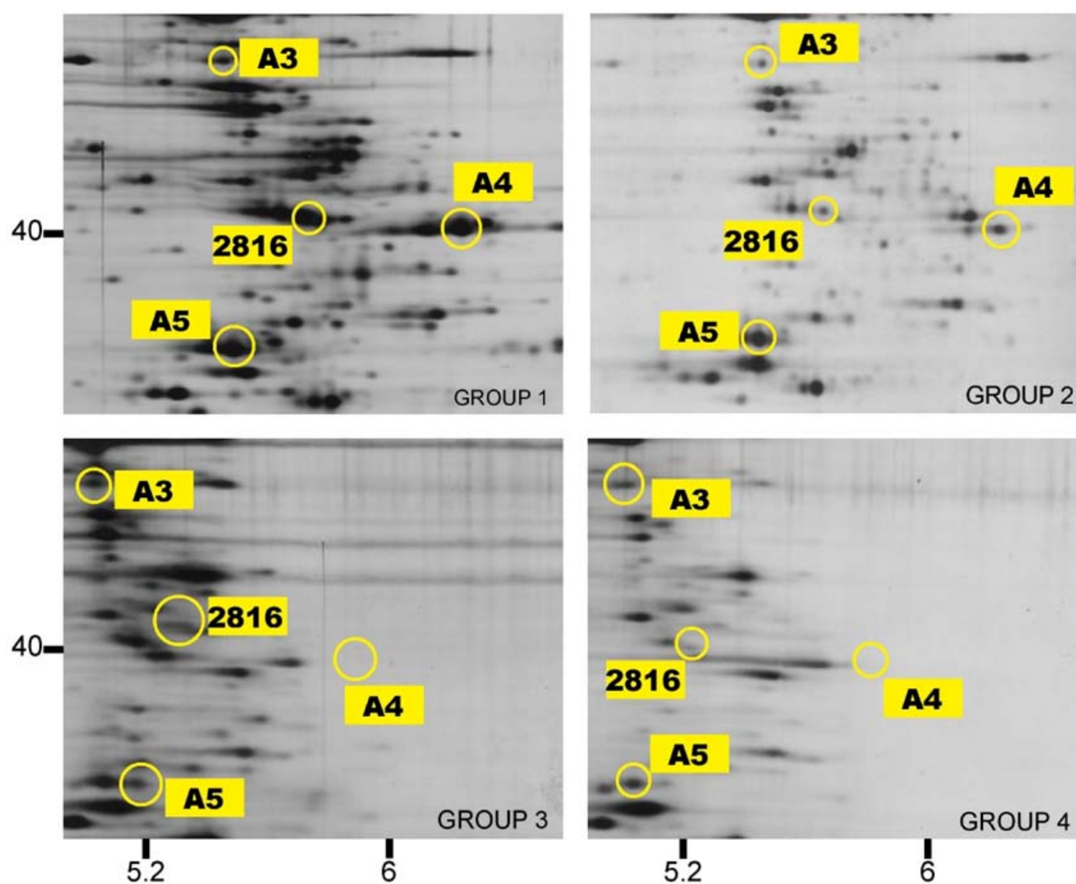

Figure 5.15 Zoomed silver stained 2-DE-gel representations of 4 groups of *Halomonas salina* for spots A3, 2816, A4 and A5.

5.6.3 ACETOACETATE SYNTHASE SMALL SUBUNIT (Spot 285)

Spot 285 was detected near 21 kDa at pH 5.8 on 2-DE gel of group1 of *Halomonas salina*. When group 1 gel is correlated to group 2, 3, and 4, there is a similar spot in group 2 but no clear spot in group 3 and 4 (figure 5.16). The spot 285 from group 1 of *Halomonas salina* was analyzed by Nano LC-ESI MS-MS and was found as electron transfer flavoprotein beta-subunit from *Chromohalobacter salexigens* DSM 3043 and *Pseudomonas aeruginosa* PAO1, *Marinobacter aquaeolei* VT8 also with the same score 91. Peptide fragmented by MS/MS is given as:

MAMNPFCEIAVEEAVR at the positions 33-48 with an ion score of 91 and oxidations at Met-1, Met-3 and cysteine carbamidomethylation at Cys-7

Electron transfer flavoproteins (ETFs) serve as specific electron acceptors for primary dehydrogenases, transferring the electrons to terminal respiratory systems. Group II ETFs produced by some prokaryotes under specific growth conditions, receiving electrons only from the oxidation of specific substrates [116]. ETFs are

heterodimeric proteins containing an FAD cofactor and AMP [117-119] FAD is bound in a cleft between domains II and III, while domain III binds the AMP molecule. Interactions between domains I and III stabilizes the protein.

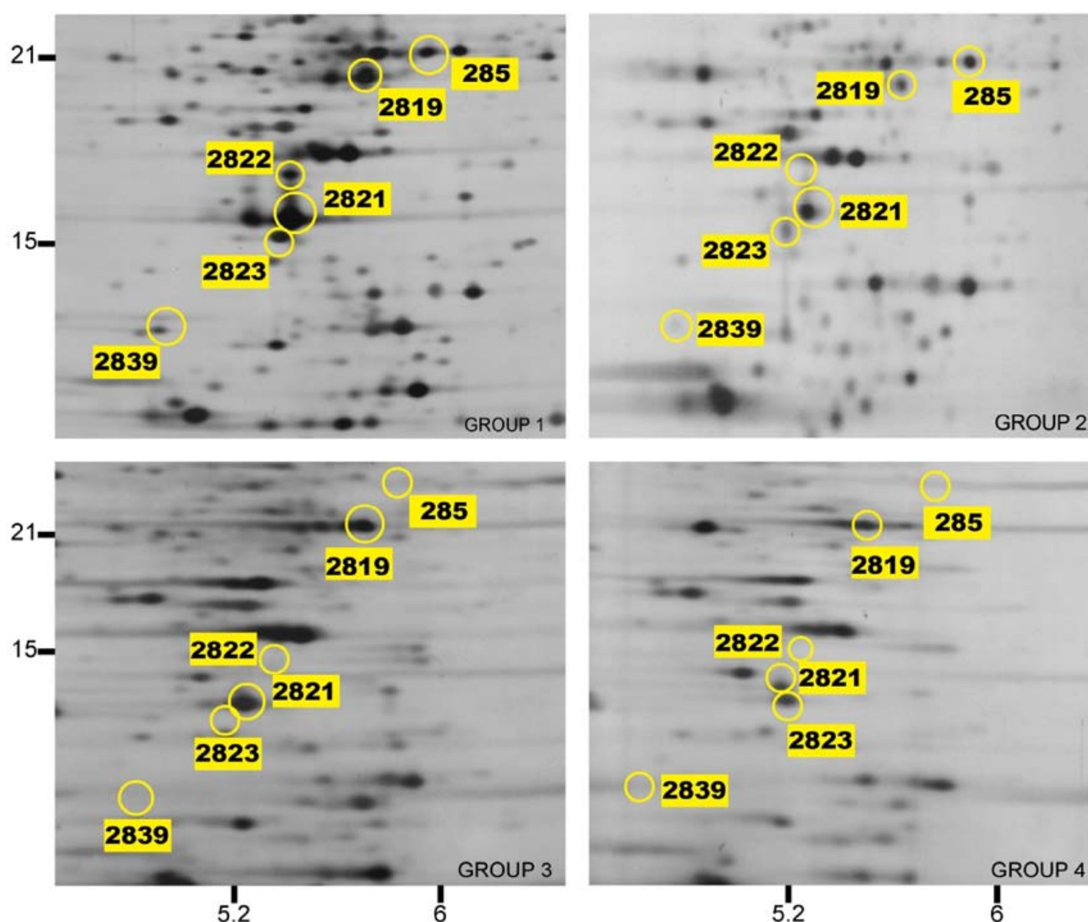

Figure 5.16 Zoomed silver stained 2-DE-gel representations of 4 groups of *Halomonas salina* for spots 285, 2819, 2821, 2823 and 2839.

5.6.4 SINGLE-STRAND BINDING PROTEIN (Spot 2819)

Spot 2819 is located near pH 5.4 and 21 kDa on 2-DE gel of group 1 of *Halomonas salina*. The possible same spot was seen on other groups' gels. But as can be seen in figure 5.16, in group 4 2-DE gel, this probable spot is more like a smear. The identification method applied to spot No 2819 of group 1 of *Halomonas salina* was Nano LC-ESI MS-MS. The protein matched from this analysis was single-strand binding protein from *Chromohalobacter salexigens* with a hit of 128 and also 86 as the second good hit the same protein from *Ehrlichia ruminantium*. The peptides fragmented by MS/MS, were:

VILIGNLGQDPEVR at the positions 8-21 with an ion score 86

YSTEIVANDMQMLDSR at the positions 97-112 with an ion score 41

Single-strand binding protein is also known as the helix-destabilizing protein. It binds tightly, as a homotetramer, to single-stranded DNA (ss-DNA) and plays an important role in DNA replication, recombination and repair.

5.6.5 HYPOTHETICAL PROTEIN ECA3428 (Spot 2821)

As seen in figure 5.16, spot 2821 is found near pH 5.1 and Mr 16 on the group 1 2-DE gel of *Halomonas salina*. The corresponding spot in group 2 gel is less dense and also the same in group 3 and 4 2-DE gels of *Halomonas salina*. For spot 2821 of group 1 and group 2 gels the identification was done by Nano LC-ESI MS-MS and the protein matched for spot 2821 was hypothetical protein ECA 3428 from *Erwina carotovora* for both 2821 spots from different group gels. For spot 2821 from group 1, hypothetical protein ECA 3428 was hit with a score of 194. The peptides fragmented by MS/MS were given as:

KIDWEHTVAGTSGSDDWR at the positions 150-167 with an individual score of 110

IDWEHTVAGTSGSDDWR at the positions 151-167 with an ion score 84

For spot 2821 from group 2, hypothetical protein ECA 3428 was hit with a score of 105 from the same source organism, *Erwina carotovora*.

The peptides fragmented by MS/MS were given as:

KIDWEHTVAGTSGSDDWR at the positions 150-167 with an individual score of 91

IDWEHTVAGTSGSDDWR at the positions 151-167 with an ion score 14

The hypothetical protein ECA 3428 has a nominal mass (Mr) 19.11kDa and has calculated pI value of 5.13.

5.6.6 HYPOTHETICAL PROTEIN SSO0826 (Spot 2822)

Spot 2822 can be seen on group 1 gel of *Halomonas salina* but the corresponding spot on group 2, 3 and 4 2-DE gels do not have similar spot on the same location near pH 5 and Mw 14 (figure 5.16). Spot 2822 of group 1 gel was analyzed by MALDI-TOF and the protein matched was found as hypothetical protein SSO0826

from archaeal strain *Sulfolobus solfataricus P2* with a score of 69 which was higher then the acceptable minimum limit. The matched peptides were:

MQIIYRLSK at the positions 1-9

LSKGSEIEK at the positions 7-15

GSEIEKFLR at the positions 10-18

KPSDDICK at the positions 37-44

LNFYDILKVINNPYAEDR at the positions 45-62

NFILAIASYFPFTLRK at the positions 65-80

IYNFRVK at the positions 86-92

The theoretical pH value of the protein is 10.04 where in 2-DE gel this protein was seen at location near pH 5.

5.6.7 NUCLEOSIDE DIPHOSPHATE KINASE (Spot No 2823)

Spot 2823 is placed just under spot 2821 in group 1 of 2-DE gel of *Halomonas salina* as seen in figure 5.16. At the same location in group 2 2-DE gel, there is also a spot but less dense. Also group 3 and 4 have a spot at the same location but with a little shift. Spot No 2823 from group 1 2-DE gel of *Halomonas salina* was identified by Nano LC-ESI MS-MS as nucleoside diphosphate kinase with a score of 92 from *Halomonas sp*. #593, *Marine gamma proteobacterium* HTCC2207, *Chromohalobacter salexigens* DSM3043 and *Alcanivorax borkumensis* SK2. The peptides fragmented by MS/MS analysis were:

TLSIIKPDAVAK at the positions 6-17 with an individual ion score of 59

DLMGATNPK at the positions 88-96 with an individual ion score of 32

Nucleoside diphosphate kinases catalysis the reaction: ATP + nucleoside diphosphate = ADP + nucleoside triphosphate.

Nucleoside diphosphate kinases (NDK) are enzymes required for the synthesis of nucleoside triphosphates (NTP) other than ATP. They provide NTPs for nucleic acid synthesis, CTP for lipid synthesis, UTP for polysaccharide synthesis and GTP for protein elongation, signal transduction and microtubule polymerization.

5.6.8 30S RIBOSOMAL PROTEIN S20 (Spot No 2839)

As seen in figure 5.16, spot No 2839 was detected near pH value 4.5 and Mr value 12 on 2-DE gel of Group 1 of *Halomonas salina*. The corresponding spot on group 2 2-DE gel is present but as a weaker spot and in group 3 and 4, corresponding spots are not detectable. Spot No 2839 was identified from group 1 2-DE gel and MALDI-TOF was used for identification. The spot was identified as 30S ribosomal protein S20 from *Chromobacterium violaceum* with a score of 45 just below the significant score minimum limit. The matched peptides were:

QRAHNASLR at the positions 17-25

AHNASLRTAFR at the positions 19-29

VLKAIEAGDK at the positions 35-44

AIEAGDKAAAR at the positions 38-48

AAARVVFQASEK at the positions 45-56

IADKGVFHK at the positions 61-69

SRLSAQIK at the positions 77-84

The theoretical pI value of this protein was calculated as 11.85 although on 2-DE gel it was found near area pH 4.5.