3 MATERIALS

CHEMICALS

The chemicals used in this study were high quality grade. Especially, the chemicals used in electrophoresis were electrophoresis grade, chemicals used in sample preparation were molecular biology grade and the solvents used were ultra pure grade. The chemicals used in this study are given table 3.1 below.

Table 3.1 List of chemicals

Chemicals	**Supplier**
3 MM Filter Paper	Whatmann
Acetic acid	Carlo Erba
Acetonitrile (MeCN)	Carlo Erba
Acrylamide	Fluka Chemie AG
Agarose	Sigma Aldrich Chemie GmbH
Alcohol dehydrogenase (1nmol/vial)	Waters
Ammonium persulfate (APS)	Omni Life Science
Ammoniumsulfate	Merck Schuchardt OGH
Ampholyte 2-4	Fluka Chemie AG
Ampholyte 4-6	Fluka Chemie AG
Ampholyte 3-10	Fluka Chemie AG
Ampholyte 2-11	Serva Electrophoresis GmbH
Ampholyte 5-8	Fluka Chemie AG
Ampholyte 7-9	Fluka Chemie AG
Benzamidine	Sigma Aldrich Chemie GmbH
Calcium chloride ($CaCl_2$)	Merck Schuchardt OGH
CBB-G250	Merck Schuchardt OGH
α-cyano-4-hydroxycinnamic acid (CHCA)	Sigma Aldrich Chemie GmbH
DTT	Omni Life Science
EDTA	Merck Schuchardt OGH
Ethanol	Merck Schuchardt OGH
Ethylenediamine	Merck Schuchardt OGH
Formic acid	J.T. Baker
Formaldehyde 37%	Merck Schuchardt OGH

Glycerine	Omni Life Science
Glycine	Applichem
[Glu^1] –Fibrinogen 0.1 mg	Sigma
Glutaraldehyde 25%	Merck Schuchardt OGH
Isoproanol	Merck Schuchardt OGH
Hydrochloric acid	Merck Schuchardt OGH
Iodoacetamide	Sigma Aldrich Chemie GmbH
Leupeptine	Sigma Aldrich Chemie GmbH
Methanol	Merck Schuchardt OGH
N,N-methylen bis acrylamide	Omni Life Science
Potassiumchloride (KCl)	Merck Schuchardt OGH
Pepstatin	Sigma Aldrich Chemie GmbH
PMSF	Sigma Aldrich Chemie GmbH
Phosphoric acid (H_3PO_4)	Riedel-de Haën
Piperazine diacrylamide (PDA)	Sigma Aldrich Chemie GmbH
PVDF Membrane	Millipore
Sephadex G200 Super fine	Sigma Aldrich Chemie GmbH
Serdolit MB-1	Serva Electrophoresis GmbH
Silver nitrate	Omni Life Sience
Sodium acetate	Merck Schuchardt OGH
Sodium carbonate	Omni Life Sience
Sodium dodecylsulfate (SDS)	Merck Schuchardt OGH
Sodium hydrogencarbonate	Merck Schuchardt OGH
Sodium thiosulfate-pentahydrate	Merck Schuchardt OGH
TEMED	Merck Schuchardt OGH
Thimerosal	Sigma Aldrich Chemie GmbH
Trifluoroacetic acid (TFA)	Merck Schuchardt OGH
Tris-Base	Omni Life Sience
Tris-HCl	Amresco
Trypsin	Sigma Aldrich Chemie GmbH
Urea (Ultra pure)	Sigma Aldrich Chemie GmbH
Urea (Pearl form)	Merck Schuchardt OGH

SOLUTIONS AND BUFFERS

Ringer Solution: 0.9 % (w/v) NaCl, 0.042 % (w/v) KCl, 0.025 % (w/v) CaCl2

Sterile Stock Solution for Microorganisms: Sterilized 30 % (w/v) glycerol solution

Brown 1963 Culturing Media 1: 0.5 % (w/v) Yeast extract, 0.3 % (w/v) Tri sodium citrate, 2 % magnesium sulphate

heptahydrate, 0.2 % potassium chloride 5 % sodium chloride ; pH 7

Brown 1963 Culturing Media 2: 0.5 % (w/v) Yeast extract, 0.3 % (w/v) Tri sodiumcitrate, 2 % (w/v) magnesium sulphate heptahydrate, 0.2 % (w/v) potassium chloride 20 % (w/v) sodium chloride; pH 7

Protease Inhibitor Cocktail: 0.1 µM Pepstatin, 1 mM PMSF (PMSF stock solution was prepared in ultra pure ethanol), 0.08 % Benzamidine, 2.5 mM leupeptine and 1.5mM EDTA

Bradford Working Buffer: 0.01 % (w/v) Coomassie Brilliant Blue G-250, 5% (v/v) ethanol, 9 % (v/v) phosphoric acid

IEF Separation Gel Solution: 9 M urea, 5 % glycine, 3.5 % acrylamide, 0.3 % PDA, 0.06 % TEMED, 4 % ampholyte mix 3-7.5

IEF Cap Gel Solution: 9 M urea, 5 % glycine, 12 % acrylamide, 0.13 % PDA, 0.06 % TEMED, 4 % ampholyte mix 3-7.5

0.8% APS: 0.8 g ammonium persulfate prepared in 100 ml HPLC-H_2O as a stock solution and stored in - 80ºC in aliquotes

Cathodic Buffer for IEF: 9 M urea, 5 % (w/v) glycerol, 5 % (v/v) ethylenediamine

Anodic Buffer for IEF: 3 M urea, 7 % (v/v) phosphoric acid

Overlay Solution for IEF: 5 M urea, 2% (v/v) ampholyte 2-4, 6% (w/v) glycerol

Incubation solution for IEF: 125 mM Tris/H_3PO_4 (pH 6.8), 40 % (w/v) glycerol, 3 % (w/v) SDS, 65 mM DTT

SDS-PAGE Gel Solution: 15 % (w/v) acrylamide, 0.2 % (w/v) bis-acrylamide, 375 mM Tris/HCl (pH 8.8), 0.03 % (v/v) TEMED, 0.1 % (w/v) SDS, 0.08 % (w/v) ammonium persulphate

1.28 % APS: 12.8 g ammonium persulphate prepared in 1000 ml HPLC-H_2O as a stock solution and stored in - 80ºC in aliquotes

Overlay Solution for SDS-PAGE: 375 mM Tris/HCl (pH 8.8), 0.1 % (w/v) SDS

Agorose Gel Solution: 1 % (w/v) agarose, 0.1 % (w/v) SDS, 125 mM Tris/H_3PO_4 (pH 6.8)

SDS-PAGE Running Buffer: 192 mM glycine, 25 mM Tris, 0.1 % (w/v) SDS

Silver Staining Fixation Solution: 50 % (v/v) ethanol, 10 % (v/v) acetic acid

Silver Staining Incubation Solution: 0.5 M sodium acetate, 0.5 % (v/v) glutaraldehyde, 30 % (v/v) ethanol

Silver Staining Silver nitrate Solution: 0.1 % (w/v) silver nitrate, 0.01 % (v/v) formaldehyde

Silver Staining Development Solution: 2.5 % (w/v) sodium carbonate (pH 11.3), 0.05 mM sodium thiosulphate, 0.01 % (v/v) formaldehyde

Silver Staining Stopping Solution: 0.05 % (w/v) Na-EDTA, 0.02 % (w/v) thimerosal

Coomassie Brilliant Blue G-250 Fixation Solution: 50 % (v/v) ethanol, 2 % (v/v) phosphoric acid

Coomassie Brilliant Blue G-250 Washing Solution: 34 % (v/v) methanol, 17 % (w/v) ammonium sulphate, 2 % (v/v) phosphoric acid

Coomassie Brilliant Blue G-250 Staining Solution: 34 % (v/v) methanol, 17 % (w/v) ammonium sulphate, 2 % (v/v) phosphoric acid, 0.06 % (v/v) CBB G-250

Coomassie Brilliant Blue R-250 Staining Solution: 0.1 % (w/v) CBB-R250, 40 % methanol, 10 % acetic acid

Coomassie Brilliant Blue R-250 Destaining Solution: 40 % methanol, 10 % acetic acid

Towbin Buffer: 25 mM Tris-Base, 192 mM glycine, 3.5 mM SDS, 20 % methanol (pH 8.5)

Trypsin In-gel Digestion Shrinking Buffer: 50 % (v/v) 200 mM Tris/HCl pH 8.1, 50 % acetonitrile

Trypsin In-gel Digestion Quelle Buffer: 50 % (v/v) 200 mM Tris/HCl pH 8.1, 50 % HPLC-H_2O

Matrix Solution for MALDI-TOF MS: 10 mg/ml α-cyano–4 hydroxycinnamic acid (CHCA) prepared in 49,5 % ethanol, 49,5 % acetonitrile and 0.01 % TFA

LABWARE

Autoclave: SYSTEC (Model-3870 ELV)

Sterile Hood: (SAFE2010 0.9, HETO HOLTEN)

2 Dimensional Electrophoresis System: Big gel tank and equipments supplied from Wita GmbH, Teltow. Power supply used was from Apelex (Model PS 1006P)

Mass Spectrometers: MALDI TOF, MALDI Reflectron (M@LDI-R), Micromass UK Limited; ESI- MS/MS, Micromass UK Limited

Edman Sequencer: Applied Biosystems Model 492A Procise seqencer, Model 785A Detector and a Model 140C Microgradient systems

Ultrasonic Bath: Bandelin Sonorex

Centrifuge: Sigma Lab centrifuges (Modl 3K30)

Incubator: B. Braun Certomat (Model BS-1)

No frost Refrigerator: Arçelik

-80ºC Deep Freezer: Heto Holten Ultra Freeze (UF4420)

-20ºC Deep Freezer: Liebherr Comfort

Balance: Mettler Toledo (Models AB204 – S and PG403 – S)

Gel Dryer: Thermo Electron Corporation (Model SG 210 D SpeedGel)

Semi-Dry Blotting System: Bio - Rad

Pure Water System: ELGA (Models Pure Lab Prima and Pure Lab Maxima)

Orbital Shaker: Heidolph (Model Unimax 2010)

Automatic Pipettes: Finpipette

Spectrophotometer: Perkin Elmer (Lambda 35)

Vortex: Heidolph (REAX top)

96 Well Microplate: Abgene Protein Purification Plate, centrifuge compatible; Cat. No: AB-1151/C

KITS AND BACTERIAL TYPE STRAIN

Protein Extraction Kit: Sigma Total Prot

Moderately Halophilic Bacterial Type Strain: Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ No 5928)

Wild Type Moderately Halophiles: Isolated from soil samples supplied from Izmir Çamaltı saltern area

SOFTWARE, DATABASES, BIOINFORMATIC TOOLS

Mass Spectrometers' software: Masslynx, proteinlynx supplied from Micromass

Search Engine: Matrix science

Protein Identification Databases: NCBInr, SwissProt