CONTENTS

CHAPTER 1: INTRODUCTION
1.1 Unique features of Archaea ................................................................. 2
1.1.1 Typically eubacterial or eukaryotic features of Archaea ................. 3
1.2 Haloarchaea ......................................................................................... 4
1.2.1 Taxonomy of HaloarchAEA ............................................................. 4
1.3 Cellular features of Haloarchaea .......................................................... 5
1.3.1 Cell Envelopes of Haloarchaea ......................................................... 5
1.3.2 Haloarchael Cytoplasmic Membranes ............................................. 6
1.3.3 Purple Membrane and other Rhodopsins ....................................... 7
1.3.4 Genome of Haloarchaea .............................................................. 8
1.4 Dependence of Haloarchaal cellular architecture on sodium chloride 8
1.5 Strategies of osmotic balance in Haloarchaea ..................................... 14
1.6 Halophilic eubacteria ............................................................................ 14
1.6.1 Adaptation mechanisms of halophilic eubacteria to sodium chloride 15
1.6.2 Adaptations in cellular Proteins / Lipids of halophilic eubacteria .... 16
1.7 Response of microorganisms to heavy metals ................................... 19
1.7.1 Mechanisms of resistance of Cu^{2+} and Zn^{2+} .............................. 20

AIM & SCOPE

CHAPTER 2 HALOARCHAEAL CHARACTERISTICS OF HALOBACTERIM STRAIN R, MTCC 3265
Methodology ......................................................................................... 23
2.1 Culture ................................................................................................. 23
2.1.1 Source and Maintenance ............................................................... 23
2.1.2 Preparation of seed culture of HR ................................................. 23
2.2 Growth of HR .................................................................................... 24
2.2.1 Optimisation of physico-chemical conditions for growth of HR .... 24
2.2.2 Preparation of resting cells of HR ............................................... 24
2.3 Determination of Cellular characteristics of HR ................................ 25
2.3.1 Gram reaction of HR ..................................................................... 25
2.3.2 Stability of whole cells of HR in water .......................................... 25
2.3.3 Detection of Diaminopimelic acid (DAP) in cells of HR
2.3.4 Determination of hydrophobicity of whole cells of HR
2.3.5 Whole cell acid methanolysis
2.3.6 Analysis of pigment of HR
2.3.7 Isolation and characterisation of lipids of HR

Results

2.4 Growth of HR in NTYE medium
2.5 Optimum physico-chemical conditions for growth of HR
2.5.1 Effect of varying concentration of solar salt on growth of HR
2.5.2 Effect of pH of medium on growth of HR
2.5.3 Effect of incubation temperature on growth of HR
2.6 Haloarchaeal cellular characteristics of HR
2.6.1 Morphological characteristics
2.6.2 Lysis of HR in water
2.6.3 DAP analysis
2.6.4 Surface hydrophobicity
2.6.5 Presence of glycerol diether moieties
2.6.6 Pigment analysis
2.6.7 Lipid analysis

Discussion

CHAPTER 3 CELLULAR ADAPTATION OF HR TO SINGLE STEP FLUCTUATION IN PHYSICO-CHEMICAL CONDITIONS OF GROWTH
Methodology

3.1 Determination of the effect of growth conditions on cellular features of HR
3.2 SDS – PolyAcrylamide Gel Electrophoresis of whole cells of HR
3.2.1 Preparation of HR cell lysates
3.2.2 Preparation of cell envelopes of HR
3.2.3 Isolation of the cell membrane of HR
3.2.4 Electrophoresis and visualization
3.3 Response of HR to elevated temperatures
3.3.1 Preparation of cell suspension
3.3.2 Exposure of HR to elevated temperatures ........................................... 48
3.3.3 2,3,5 Triphenyl Tetrazolium Chloride (TTC) dye reduction assay .......... 48
Results ........................................................................................................... 48
3.4 Response of HR to one point fluctuation in consecutive growth cycles .... 48
3.4.1 Proteins ................................................................................................. 49
3.4.2 NaCl dependent stability of whole cell proteins of HR ......................... 50
3.4.3 Susceptibility to water ......................................................................... 51
3.4.4 Cell surface hydrophobicity ................................................................. 52
3.4.5 Pigmentation ......................................................................................... 52
3.4.6 Lipids .................................................................................................... 54
3.5 Response of resting cells of HR to elevated temperatures .................... 55
3.5.1 Influence of growth temperature on response of HR to elevated
temperatures .............................................................................................. 55
Discussion .................................................................................................... 56
CHAPTER 4 RESPONSE OF HR TO Zn\(^{2+}\) AND Cu\(^{2+}\)
Methodology .................................................................................................. 62
4.1 Determination of Growth of HR in mineral salts medium containing glucose
as SOLE SOURCE of carbon .......................................................................... 62
4.1.1 Effect of Cu\(^{2+}\) / Zn\(^{2+}\) on growth of HR in NGSM ......................... 62
4.1.2 Effect of continuous subculture in Cu\(^{2+}\) / Zn\(^{2+}\) on growth of HR .......... 63
4.2 Determination of chemical composition of cell envelopes and membranes
of HR grown in presence of metal ions ......................................................... 63
4.2.1 Isolation of cell envelopes and cell membranes of HR grown in NGSM with
and without Cu\(^{2+}\) / Zn\(^{2+}\) ........................................................................ 63
4.2.2 Protein .................................................................................................. 63
4.2.3 Lipid .................................................................................................... 64
4.2.4 Total Carbohydrate ............................................................................. 64
4.3.4 Hexosamine ....................................................................................... 64
4.3.5 Total Sulphate ................................................................................... 65
4.3 Determination of Cu\(^{2+}\) / Zn\(^{2+}\) ions in cells of HR ................................. 65
4.3.1 Incubation of HR with metal ions ......................................................... 65
4.3.2 Atomic absorption spectrophotometric analysis of Cu$^{2+}$/Zn$^{2+}$ in cell digests

4.4 Screening for presence of Plasmid in HR

4.4.1 Alkali-lysis method

4.4.2 Slot lysis method

Results

4.5 Growth of HR with glucose as sole source of carbon

4.6 Tolerance to Zn$^{2+}$ during growth of HR

4.7 Tolerance to Cu$^{2+}$ during growth of HR

4.8 Response of HR to continuous subculture in Cu$^{2+}$/Zn$^{2+}$

4.9 Effect of metal ions on pigmentation of HR

4.10 Changes in Cell surface hydrophobicity of HR grown with Zn$^{2+}$/Cu$^{2+}$

4.11 Changes in cellular lipids of HR grown with Zn$^{2+}$/Cu$^{2+}$

4.12 Changes in chemical composition of cell envelope and plasma membrane of HR grown with Zn$^{2+}$/Cu$^{2+}$

4.13 Accumulation of Zn$^{2+}$/Cu$^{2+}$ by resting cells of HR

4.14 Occurrence of Plasmid in HR

Discussion

CHAPTER 5 SUMMARY

BIBLIOGRAPHY

APPENDICES