

Sl. No.	CONTENTS	Page No.
1.	INTRODUCTION	1
2.	REVIEW OF LITERATURE	5
3.	MATERIALS AND METHODS	31
4.	RESULTS	48
5.	DISCUSSION	129
6.	SUMMARY AND CONCLUSION	143
7.	REFERENCES	146
8.	PUBLICATIONS	

LIST OF FIGURES

Figure No.	Title	Page No.
1.	Phylogenetic tree depicting the relationship between microorganisms	2
2.	Types of halophilic bacteria	5
3.	High salt-in strategy	7
4.	Salt-out strategy	8
5.	The chloride regulon of <i>Halobacillus halophilus</i>	8
6.	Structure of carotenoids in halophilic bacteria	21
7.	Map showing study area Marakkanam taluk	32
8.	Collection of soil samples at the sampling site	33
9.	Pigment producing bacteria isolated from Marakkanam salt pan	50
10.	Pure cultures of pigment producing halophilic bacteria	51
11.	Distribution of halophilic bacteria isolated from the Marakkanam salt pan in the month of January 2014	52
12.	Distribution of halophilic bacteria isolated from the Marakkanam salt pan in the month of May 2014	52
13.	Pigment producing halophilic bacteria cultured in halophilic broth for pigment extraction	57
14.	Pigments extracted from pigment producing halophilic bacteria from Marakkanam saltern sediment sample	58
15.	Antioxidant activity of pigment extract of halophilic bacteria isolated from Marakkanam salt pan.	59
16.	Cytotoxic activity of pigment extracts showing morphological changes in HeLa cell lines after 48 hours of incubation	60

17.	Cytotoxic activity of pigment extracts from <i>Chromohalobacter</i> sp. S2 against HeLa cell lines after 48 hours of incubation	61
18.	Cytotoxic activity of pigment extracts from <i>Chromohalobacter</i> sp. S6 against HeLa cell lines after 48 hours of incubation	61
19.	Cytotoxic activity of pigment extracts from <i>Oceanobacillus</i> sp. S12 against HeLa cell lines after 48 hours of incubation	62
20.	Cytotoxic activity of pigment extracts from <i>Bacillus</i> sp.S15 against HeLa cell lines after 48 hours of incubation	62
21.	Cytotoxic activity of pigment extracts from <i>Brevundimonas</i> sp. S17 against HeLa cell lines after 48 hours of incubation	63
22.	Genomic DNA of selected pigment producing halophilic bacteria	64
23.	Amplified PCR products of halophilic bacterial isolates	64
24.	NCBI home page showing the sequences submitted in NCBI	66
25.	16S rRNA gene sequence of <i>Chromohalobacter salexigens</i> S2 in NCBI data base	67
26.	16S rRNA gene sequence of <i>Chromohalobacter israelensis</i> S6 in NCBI data base	68
27.	16S rRNA gene sequence of <i>Oceanobacillus manasiensis</i> S11 in NCBI data base	69
28.	16S rRNA gene sequence of <i>Bacillus licheniformis</i> S15 in NCBI data base	70
29.	16S rRNA gene sequence of <i>Brevundimonas viscosa</i> S17 in NCBI data base	71
30.	Phylogenetic tree showing the evolutionary relationship of <i>Chromohalobacter salexigens</i> S2	72
31.	Phylogenetic tree showing the evolutionary relationship of <i>Chromohalobacter israelensis</i> S6	72
32.	Phylogenetic tree showing the evolutionary relationship of <i>Oceanobacillus manasiensis</i> S12	73

33.	Phylogenetic tree showing the evolutionary relationship of <i>Bacillus licheniformis</i> S15	73
34.	Phylogenetic tree showing the evolutionary relationship of <i>Brevundimonas viscosa</i> S17	74
35.	Phylogenetic inference based on 16S rRNA gene analysis between five pigment producing halophilic isolates and other related	75
36.	Secondary structure of 16S rRNA sequence of <i>Chromohalobacter salexigens</i> S2	76
37.	Secondary structure of 16S rRNA sequence of <i>Chromohalobacter israelensis</i> S6	77
38.	Secondary structure of 16S rRNA sequence of <i>Oceanobacillus manasiensis</i> S12	78
39.	Secondary structure of 16S rRNA sequence of <i>Bacillus licheniformis</i> S15	79
40.	Secondary structure of 16S rRNA sequence of <i>Brevindimonas viscosa</i> S17	80
41.	<i>In silico</i> restriction site analysis for the 16S rRNA sequence of <i>Chromohalobacter salexigens</i> S2	81
42.	<i>In silico</i> restriction site analysis for the 16S rRNA sequence of <i>Chromohalobacter israelensis</i> S6	82
43.	<i>In silico</i> restriction site analysis for the 16S rRNA sequence of <i>Oceanobacillus manasiensis</i> S12	83
44.	<i>In silico</i> restriction site analysis for the 16S rRNA sequence of <i>Bacillus licheniformis</i> S15	84
45.	<i>In silico</i> restriction site analysis for the 16S rRNA sequence of <i>Brevundimonas viscosa</i> S17	85
46.	Growth curve of <i>Bacillus licheniformis</i> S15	87
47.	Optimization of pigment production with various carbon sources by <i>Bacillus licheniformis</i> S15	88
48.	Effect of various carbon sources on pigment production by <i>Bacillus licheniformis</i> S15	89

49.	Optimization of pigment production with various pH by <i>Bacillus licheniformis</i> S15 in halophilic broth	90
50.	Effect of varying pH on pigment production by <i>Bacillus licheniformis</i> S15	90
51.	Optimization of pigment production at different temperature by <i>Bacillus licheniformis</i> S15 in halophilic broth.	91
52.	Effect of temperature on pigment production by <i>Bacillus licheniformis</i> S15	92
53.	Effect of different metal ions on pigment production by <i>Bacillus licheniformis</i> S15	92
54.	Effect of various metal ions on pigment production by <i>Bacillus licheniformis</i> S15	93
55.	Experimental set up for RSM studies for production of pinkish orange pigment by <i>Bacillus licheniformis</i> S15	94
56.	3D surface plot for pigment production by <i>Bacillus licheniformis</i> S15 showing the interaction between sucrose and pH level	98
57.	Contour plot response surface for pigment production by <i>Bacillus licheniformis</i> S15 showing the interactive effect of the sucrose and	98
58.	3D surface plot for pigment production by <i>Bacillus licheniformis</i> S15 showing the interaction between sucrose and temperature	99
59.	Contour plot response surface for pigment production by <i>Bacillus licheniformis</i> S15 showing the interactive effect of the sucrose and temperature level	99
60.	3D surface plot for pigment production by <i>Bacillus licheniformis</i> S15 showing the interaction between temperature and pH level.	100
61.	Contour plot response surface for pigment production by <i>Bacillus licheniformis</i> S15 showing the interactive effect of the temperature and pH level	100
62.	Column chromatographic separation of pinkish orange pigment extracted from <i>Bacillus licheniformis</i> S15	102
63.	Fourier Transform - Infrared Spectrum (FT-IR) of pinkish orange pigment extracted from <i>Bacillus licheniformis</i> S15	104

64.	Mass spectrum of pinkish orange pigment produced by <i>Bacillus licheniformis</i> S15	105
65.	Mass spectrum of pinkish orange pigment produced by <i>Bacillus licheniformis</i> S15 (expansion 1)	106
66.	¹ H NMR Spectrum of purified pinkish orange pigment extracted from <i>Bacillus licheniformis</i> S15	108
67.	¹ H NMR expansion spectrum of purified pinkish orange pigment extracted from <i>Bacillus licheniformis</i> S15	109
68.	¹³ C NMR Spectrum of purified pinkish orange pigment extracted from <i>Bacillus licheniformis</i> S15	110
69.	¹³ C NMR Spectrum expansion of purified pinkish orange pigment extracted from <i>Bacillus licheniformis</i> S15	111
70.	DEPT 135 NMR Spectrum of pinkish orange pigment extracted from <i>Bacillus licheniformis</i> S15	112
71.	¹ H- ¹ H COSY NMR Spectrum of pinkish orange pigment extracted from <i>Bacillus licheniformis</i> S15	113
72.	¹ H- ¹ H COSY NMR Spectrum of pinkish orange pigment extracted from <i>Bacillus licheniformis</i> S15 (expansion 1)	114
73.	¹ H- ¹ H COSY NMR Spectrum of pinkish orange pigment extracted from <i>Bacillus licheniformis</i> S15 (expansion 2)	115
74.	HSQC NMR Spectrum of pinkish orange pigment extracted from <i>Bacillus licheniformis</i> S15	116
75.	HMBC NMR Spectrum of pinkish orange pigment extracted from <i>Bacillus licheniformis</i> S15 (expansion 1)	117
76.	HMBC NMR Spectrum of pinkish orange pigment extracted from <i>Bacillus licheniformis</i> S15 (expansion 2)	118
77.	HMBC NMR Spectrum of pinkish orange pigment extracted from <i>Bacillus licheniformis</i> S15 (expansion 3)	119
78.	Chemical structure of unique pinkish orange dinoxanthin derivative extracted from <i>Bacillus licheniformis</i> S15 based on 2DNMR studies	122

79.	Inhibition of α - amylase enzyme from purified pigment of <i>Bacillus licheniformis</i> S15 at varying concentrations	123
80.	Inhibition of α - glucosidase enzymes from purified pigment of <i>Bacillus licheniformis</i> S15 at varying concentrations	123
81.	Membrane stabilization activity of purified pigment from <i>Bacillus licheniformis</i> S15	124
82.	Nitric oxide scavenging ability of various concentration of purified pigment from <i>Bacillus licheniformis</i> S15	125
83.	Antibacterial activity of purified pigment against <i>Staphylococcus aureus</i>	126
84.	Antifungal activity of purified pigment against <i>Aspergillus niger</i>	127
85.	Antifungal activity of purified pigment against <i>Candida albicans</i>	128

LIST OF TABLES

Table No.	Title	Page No.
1.	Pigments reported from halophilic bacteria and their bioactivity	21
2.	Composition of halophilic agar medium	31
3.	Sequences of 16S primer	39
4.	Details of reaction mixture	39
5.	Colony morphology and Gram staining results of pigment producing halophilic bacteria (Samples collected in the month of January 2014)	53
6.	Colony morphology and Gram staining results of pigment producing halophilic bacteria (Samples collected in the month of May 2014)	54
7.	Biochemical characteristics of pigment producing halophilic bacteria	55
8.	Pigment producing halophilic bacterial isolates identified by conventional method.	56
9.	Restriction site analysis of 16S rRNA gene of pigment producing halophilic bacteria	86
10.	Effect of individual variable on pigment production by <i>Bacillus licheniformis</i> S15 studied using Box-Behnken design experiment	93
11.	ANOVA for pigment production by <i>Bacillus licheniformis</i> S15	95
12.	NMR chemical shifts for purified pinkish orange pigment extracted from <i>Bacillus licheniformis</i> S15	120
13.	Antibacterial activity of purified pigment from <i>Bacillus licheniformis</i> S15	126
14.	Antifungal activity of purified pigment against fungal pathogens	128

ABBREVIATIONS

%	Percentage
⁰ C	degree Celsius
ANOVA	Analysis of Variance
BLAST	Basic Local Alignment Search Tool
bp	base pairs
CDC1 ₃	deuterated chloroform
cm	centimeter
Da	Dalton
DNA	Deoxyribo Nucleic Acid
dNTP	deoxy Nucleotide tri phosphate
DW	Distilled water
Fig.	Figure
FT-IR Spectroscopy	Fourier Transform Infrared Spectroscopy
g	Gram
g/L	grams per liter
H	Hours
HPLC	High Performance Liquid Chromatography
Kb	Kilobase
KBr	Potassium bromide
LB	Luria Bertani
L	Liter
M	Molar
Mg	Milligram
mg/ml	milligram per milliliter
µg	microgram
µg/L	microgram per liter
µl	microliter
µm	micrometer
min.	Minutes
ml	Milliliter
Mm	millimeter

mM	milliMolar
NB	Nutrient Broth
NCBI	National Center for Biotechnology Information
Ng	nano gram
NJ	Neighbour joining
Nm	nanometer
NMR Spectroscopy	Nuclear Magnetic Resonance Spectroscopy
OD	Optical Density
PB	Plackett-Burman
PCR	Polymerase Chain Reaction
Rf	Retention factor
RNA	Ribonucleic acid
Rpm	revolutions per minute
Ppm	parts per million
rRNA	ribosomal RNA
RSM	Response Surface Methodology
sec.	Seconds
sp.	Species
TLC	Thin Layer Chromatography
U/ml	Units per milliliter
UV	Ultraviolet
±	plus or minus
FISH	Fluorescence in situ hybridization
ATCC	American Type Culture Collection
CFU	Colony Forming Unit
DMSO	Dimethyl Sulfoxide
f	Forward
r	Reverse
IR	Infrared
TMS	Tetramethylsilane
ppt	parts per thousand
RAPD	Random Amplified Polymorphic DNA