List of Figures

Fig. 1.1: Schematic diagram of cyanobacterial cell constituents 2
Fig. 1.2: Endosymbiotic origin of plastids 4
Fig. 1.3: Typical structure of a phycobilisome 7
Fig. 1.4: Purified phycobiliproteins: (1) Allophycocyanin, (2) Phycocyanin, (3) Phycoerythrin 8
Fig. 1.5: Phycobiliprotein chromophore groups: (a) phycocyanobilin; (b) phycoerythrobilin 9
Fig. 1.6: Typical phycobilisome structure depicting all the type of linker proteins 10
Fig. 1.7: Orderly process for phycobilisome degradation 15
Fig. 1.8: The assembly of a typical phycobilisome in response to red and green lights, respectively 16
Fig. 1.9: Crystal structure of R-phycoerythrin from Polysiphonia urceolata at 2.8 Å resolution (ILIA) 19
Fig. 2.1: Fluorescence emission spectra of intact phycobilisomes. (a) Lyngbya sp. A09DM, (b) Phormidium sp. A27DM, (c) Nostoc sp., (d) Halomicronema sp. A32DM 40
Fig. 2.2: Comparative fluorescence spectra of intact PBS when excited at 545 nm, 590 nm, 610 nm and 650 nm. (a) Lyngbya sp. A09DM, (b) Phormidium sp. A27DM, (c) Halomicronema sp. A32DM, (d) Nostoc sp. 41
Fig. 2.3: Fluorescence emission spectra of dissociated PBS when excited at 545 nm. (a) Lyngbya sp. A09DM, (b) Phormidium sp. A27DM, (c) Halomicronema sp. A32DM, (d) Nostoc sp. 42
Fig. 2.4: Comparative fluorescence spectra of intact and dissociated PBS(a) Lyngbya sp. A09DM, (b) Phormidium sp. A27DM, (c) Halomicronema sp. A32DM, (d) Nostoc sp. 43
Fig. 2.5: Fluorescence emission spectra of GPC eluates of intact PBS. (a) Phormidium sp. A27DM, (b) Nostoc sp. 44
Fig. 2.6: Absorption spectra of intact PBS. (a) Lyngbya sp. A09DM, (b) Phormidium sp. A27DM, (c) Halomicronema sp. A32DM, (d) Nostoc sp. 45
Fig. 2.7: Absorption spectra of dissociated PBS. (a) Lyngbya sp. A09DM, (b) Phormidium sp. A27DM, (c) Halomicronema sp. A32DM, (d) Nostoc sp. 46
Fig. 2.8: Overlay absorption spectra of Lyngbya sp. A09DM (red), Phormidium sp.
A27DM (pink), Halomicronema sp. A32DM (green), Nostoc sp. (blue). (a) intact and dissociated PBS, (b) dissociated PBS

Fig. 2.9: Absorption spectra of GPC eluates of intact PBS. (a) Phormidium sp. A27DM, (b) Nostoc sp.

Fig. 3.1: UV-Visible absorption overlay spectra of C-APC from Geitlerinema sp. A28DM at each step of purification. Crude extract (red); Ethodin treated extract (green); Gel permeation chromatography eluate (blue)

Fig. 3.2: Silver stained 10% Native gel electrophoresis of pure C-APC from Geitlerinema sp. A28DM. Presence of only one band indicated the homogeneity of C-APC

Fig. 3.3: Silver stained 15% SDS gel electrophoresis at each stage of purification of C-APC from Geitlerinema sp. A28DM. Lanes: (1) Protein molecular mass standard, (2) Crude extract, (3) Ethodin treated extract, (4) Pure allophycocyanin eluate from gel permeation chromatography. Pure allophycocyanin showed two bands of 15 and 17.5 kDa molecular masses corresponding to characteristic α and β subunits

Fig. 3.4: Detection of biliprotein of Geitlerinema sp. A28DM by zinc-assisted fluorescence enhancement method as observed under IP/ light. 10% native-PAGE of purified C-APC showed only one band suggesting the homogeneity and integrity of subunits

Fig. 3.5: Detection of biliproteins of Geitlerinema sp. A28DM by zinc-assisted fluorescence enhancement method at each step of purification as observed under UV light. SDS electrophoresis was run in 15% gel. Lanes: (1) Protein molecular mass marker, (2) Crude extract, (3) Ethodin treated extract, (4) Pure C-APC eluate from gel permeation chromatography

Fig. 4.1: Silver stained 15% SDS-PAGE. Lanes: (1) Protein molecular mass standard in kDa, (2) Intact PE with two subunits, (3) Truncated PE with single subunit

Fig. 4.2: (a) UV-Visible absorption overlay spectra of intact PE with both the subunits at each step of purification, (b) UV-Visible absorption overlay spectra of truncated PE with single subunit fragment at each step of purification [Steps for (a) and (b): crude extract ▶; 70% ASC ▼; purified PE ▼], (c) UV-Visible absorption overlay spectra of purified intact PE ▼ and purified truncated PE ▼, and (d) Fluorescence emission overlay spectra of purified intact PE ▼ and purified truncated PE ▼

Fig. 4.3: Silver stained 15% SDS-PAGE of PE purified from Phormidium sp. A27DM grown in (a) static condition with medium replenishment (Lanes: 1, Protein molecular mass standard in kDa; 2, PE purified after 10 days; 3, PE purified after 20 days; 4, PE purified after 30 days; 5, PE purified after 40 days; 6, PE purified after 50 days; 7, PE purified after 60 days; 8, PE purified after 70 days), (b) static condition without medium replenishment (Lanes: 1, Protein molecular mass standard in kDa; 2, PE purified after 10 days; 3, PE purified after 20 days; 4, PE purified after 30 days; 5, PE purified after 40 days), (c) sparging condition with medium replenishment (Lanes: 1, Protein molecular mass standard in kDa; 2, PE purified after 10 days; 3,
Fig. 4.4: Silver stained 15% SDS-PAGE of PE purified from *Phormidium* sp. A27DM grown in medium of different pH. Lanes: (1) Protein molecular mass standard in kDa, (2) PE purified from medium with pH 6, (3) PE purified from medium with pH 8, (4) PE purified from medium with pH 10.

Fig. 4.5: Silver stained 15% SDS-PAGE of PE purified from *Phormidium* sp. A27DM grown in medium without sodium nitrate. Lanes: (1) Protein molecular mass standard in kDa, (2) PE purified after 3 days, (3) PE purified after 6 days, (4) PE purified after 9 days, (5) PE purified after 12 days.

Fig. 4.6: Silver stained 15% SDS-PAGE (a) of PE purified from *Phormidium* sp. A27DM grown in medium with different proteases (Lanes: 1, Protein molecular mass standard in kDa; 2, PE purified from medium with trypsin; 3, PE purified from medium with chymotrypsin; 4, PE purified from medium with pepsin; 5, Control - PE purified from medium without any proteases); (b) of PE after incubation with different proteases (Lanes: 1, Protein molecular mass standard in kDa; 2, purified PE incubated with trypsin; 3, purified PE incubated with chymotrypsin; 4, purified PE incubated with pepsin), (c) of PE purified from *Phormidium* sp. A27DM grown in medium with different protease inhibitors (Lanes: 1, Protein molecular mass standard in kDa; 2, PE purified from medium with sodium azide; 3, PE purified from medium with DTT; 4, PE purified from medium with EDTA), (d) of PE incubated with glacial acetic acid (Lanes: 1, Protein molecular mass standard in kDa; 2, PE with 5% glacial acetic acid; 3, PE with 15% glacial acetic acid; 4, Control - PE with no glacial acetic acid).

Fig. 4.7: Absorbance spectra of (a) intact and (b) truncated C-PE in the presence and absence of GdmCl at pH 7.0 and 25°C.

Fig. 4.8: GdmCl-induced denaturation of (a) intact and (b) truncated C-PE. Absorbance studies at 567 nm as a function of GdmCl concentration at pH 7.0 and 25°C.

Fig. 4.9: GdmCl-induced denaturation of (a) intact and (b) truncated C-PE. Fluorescence intensity at 320 nm as a function of GdmCl concentration at pH 7.0 and 25°C.

Fig. 4.10: GdmCl-induced denaturation of (a) intact and (b) truncated C-PE. Fluorescence intensity at 573 nm as a function of GdmCl concentration at pH 7.0 and 25°C.

Fig. 4.11: Normalized denaturation curves of phycoerythrin. Curves were calculated from the results of change in absorbance at 567 nm and fluorescence at 320 nm and 573 nm as a function of GdmCl concentrations at pH 7.0 and 25°C.

Fig. 5.1: UV-Visible absorption overlay spectra of PE from *Phormidium* sp. A27DM young (a) and old (d) cultures, *Lyngbya* sp. A09DM young (b) and old (e) cultures, *Halomicronema* sp. A32DM young (c) and old (f) cultures, at each step of purification. Steps: crude extract ——; 70% ASC ——; purified PE ——.
Fig. 5.2: Silver stained 10% Native gel electrophoresis of pure PE from *Phormidium* sp. A27DM young (a) and old (d) cultures, *Lyngbya* sp. A09DM young (b) and old cultures (c), *Halomicronema* sp. A32DM young (c) and old (f) cultures.------------------ 103

Fig. 5.3: Silver stained 15% SDS gel electrophoresis at each stage of purification of PE from *Phormidium* sp. A27DM young (a) and old (d) cultures, *Lyngbya* sp. A09DM young (b) and old (e) cultures, *Halomicronema* sp. A32DM young (c) and old (f) cultures. Lanes: 1, Protein molecular mass standard; 2, Crude extract; 3, 70% ASC; 4, Pure phycoerythrin eluate from gel permeation chromatography.---------------- 106

Fig. 5.4: Detection of biliproteins of (a) *Phormidium* sp. A27DM, (b) *Lyngbya* sp. A09DM, (c) *Halomicronema* sp. A32DM, by zinc-assisted fluorescence enhancement method at each step of purification as observed under UV light. Native gel electrophoresis was run in 10% gel. Lanes: 1, Protein molecular mass marker; 2, Crude extract; 3, 70% ASC; 4, Pure PE eluate from gel permeation chromatography.------------------ 107

Fig. 5.5: UV-Visible absorption overlay spectra of purified PE from (a) young (20 days) and old (around 70 days) cultures of *Phormidium* sp. A27DM, (b) young (20 days) and old (around 200 days) cultures of *Lyngbya* sp. A09DM, (c) young (20 days) and old (around 200 days) cultures of *Halomicronema* sp. A32DM. Young culture ———; old culture ———. The amount of PE used was approximately 0.75 mg. The excitation and emission slits were 1.5 nm and the fluorescent measurements were taken at 24°C.------------------------------------------------------------------------------------- 109

Fig. 5.6: Fluorescence emission overlay spectra of purified PE from (a) young (20 days) and old culture (around 70 days) of *Phormidium* sp. A27DM, (b) young (20 days) and old culture (around 200 days) of *Lyngbya* sp. A09DM, (c) young (20 days) and old culture (around 200 days) of *Halomicronema* sp. A32DM, young culture ———; old culture ———. The amount of PE used was approximately 0.75 mg. The excitation and emission slits were 1.5 nm and the fluorescent measurements were taken at 24°C.------------------------------------------------------------------------------------- 110

Fig. 5.7: The total protein content in the three cultures when grown in different lights.------------------ 112

Fig. 5.8: UV-visible overlay spectra of phycobiliproteins purified from cultures, (a) *Lyngbya* sp. A09DM, (b) *Phormidium* sp. A27DM, (c) *Halomicronema* sp. A32DM, at different days in different light conditions (White ———, Red ———, Yellow ——— , Green ———). 114

Fig. 5.9: UV-visible overlay spectra of phycobiliproteins purified from all the cultures (*Phormidium* sp. A27DM ———, *Lyngbya* sp. A09DM ——— , *Halomicronema* sp. A32DM ———) at different days in different light conditions [(a) white light, (b) yellow light, (c) red light and (d) green light].--------------------------------------- 115

Fig. 5.10: Silver stained 15% SDS-PAGE of phycobiliproteins purified from cultures (a) *Phormidium* sp. A27DM, (b) *Lyngbya* sp. A09DM, (c) *Halomicronema* sp. A32DM: Lanes 1, 6, 11, 16 – Molecular mass standard; Lanes 2, 7, 12, 17 – phycobiliproteins purified from cultures growing in white light at 30, 40, 50 and 60 days, respectively; Lanes 3, 8, 13, 18 – phycobiliproteins purified from cultures growing in red light at 30, 40, 50 and 60 days, respectively; Lanes 4, 9, 14, 19 – phycobiliproteins purified from cultures growing in yellow light at 30, 40, 50 and 60 days, respectively; Lanes 5, 10, 15, 20 – phycobiliproteins purified from cultures growing in green light at 30, 40, 50 and 60 days, respectively.--------------------------------------- 118

Fig. 6.1: Crystals of (a) truncated PE from *Phormidium* sp. A27DM, (b) intact PE.
Fig. 6.2: (a) Sequence comparison of F-αC-PE from Phormidium sp. A27DM with αC-PE from Synechococcus sp. (WH7803), Fremyella diplosiphon, Pseuomabaena sp. (PCC7409) and Synechocystis sp. (PCC6701). The sequence identities are given in parentheses. The residues that are different were indicated in blue colour. Cysteine linking the chromophores covalently is shown in yellow colour and the residues involved in non-covalent interaction with chromophores PEB1 and PEB2 are shown in red and green colours, respectively. (b) The sequence comparison of F-αC-PE from Phormidium sp. A27DM (2G9M) with αR-PEs from Polysiphonia urceolata (1LIA), Gracilaria chilensis (1EYX) and Griffithsia monilis (1B8D). The cylinders indicate helices and the bold lines represent loops. The differences are shown in blue. The cysteines linked covalently with chromophores are in yellow colour. The hydrogen bonded residues to chromophore are shown in red.

Fig. 6.3: Sequence comparison of complete C-PE from Lyngbya sp. A09DM with PE from other red algal species whose structures are known (a) α-subunit, (b) β-subunit.

Fig. 6.4: Overall structure of F-αC-PE for two crystallographically independent molecules A and B drawn as a ribbon diagram using PYMOL. The dotted lines indicate ionic and hydrogen bonded interactions between molecules A and B. The covalently linked chromophores to protein via Cys82 and Cys139 are indicated in yellow color. The α-helices are named according to standard scheme of phycobiliprotein nomenclature.

Fig. 6.5: (A) The superposition of Cα atoms of αC-PE from Phormidium sp. A27DM molecule A (green) on the Cα atoms of molecule A (red). (B) superposition of PEB1 over PEB2 of molecule A and PEB1 over PEB2 of B, respectively. (C) superposition of PEB1 of F-αC-PE over PEB1 of Polysiphonia urceolata R-PE and PEB2 of αC-PE over PEB2 of Polysiphonia urceolata R-PE. (D) superposition of PEB1 over PEB1 and PEB2 over PEB2 of molecule A and molecule B.

Fig. 6.6: The representation of (A) α-subunit, (B) β-subunit of Lyngbya sp. A09DM drawn as a ribbon diagram using PYMOL.

Fig. 6.7: View of the two hexamers of C-PE from Lyngbya sp. A09DM as seen along the C-axis.

Fig. 6.8: The hydrogen bonded interactions in C-PE from Phormidium sp. A27DM between protein atoms and two chromophore atoms: (A) PEB1, (B) PEB2.

Fig. 6.9: (a) Orientation of α and β chains showing interacting residues, (b) Dimeric interfacial interactions between α and β chains, of C-PE of Lyngbya sp. A09DM.

Fig. 6.10: The superposition of Cα atoms of F-αC-PE from Phormidium sp. A27DM (green) on the Cα R-PE from Polysiphonia urceolata (blue).

Fig. 6.11: Superposition of the Cα atoms of C-PE from Lyngbya sp. A09DM (green) on the Cα R-PE from Polysiphonia urceolata (red): (a) α-subunit, (b) β-subunit.

Fig. 7.1: The graph represents the mean difference in body weights of control and C-PE (A09DM, A27DM and A32DM) treated male rats. P>0.05=ns, P<0.05=*

IX
Fig. 7.2: The graph represents food intake of control and C-PE (A09DM, A27DM and A32DM) treated male rats. P>0.05=ns, P<0.05=*, P<0.01=**, P<0.001=***------ 165

Fig. 7.3: Histology photographs of control and C-PE treated groups. (a) Liver, (b) Kidney--------------------------------------------------------------------------------------------- 169

Fig. 7.4: Viability of cells treated with C-PE purified from Lyngbya sp. A09DM, Phormidium sp. A27DM and Halomicronema sp. A32DM in comparison to control and DMSO treated cells-------------------------------------------------------------------------- 170

Fig. 7.5: DNA Fragmentation. Lanes: (1) DNA Ladder (100 bp), (2) DNA of control cells, (3) DNA of cells treated with C-PE from Lyngbya sp. A09DM, (4) DNA of cells treated with C-PE from Phormidium sp. A27DM, (5) DNA of cells treated with C-PE from Halomicronema sp. A32DM----------------------------------------------------- 171

Fig. 7.6: Graph showing serum glucose concentration, (A) before and (B) after, induction of diabetes (n=10)--------------------------------------------------------------------- 172

Fig. 7.7: Mean body weight difference at the beginning and at the end of the C-PE treatment period. *P value < 0.05 for control v/s DM; ns for control v/s A09DM and DM v/s A09DM (n=5)--------------------------------------------- 173

Fig. 7.8: Average food intake per day during the C-PE treatment period. ns - p value > 0.05 for control v/s DM; for control v/s A09DM and DM v/s A09DM (n=5)------ 173

Fig. 7.9: OGTT profile at the end of the C-PE treatment period (n=5)----------------------------------------------- 174

Fig. 7.10: Insulin level at the end of the C-PE treatment period. ***P value< 0.05 for control v/s A09DM and DM v/s A09DM; ns for control v/s DM (n=5)--------------------------------------------- 175

Fig. 7.11: Total cholesterol level at the end of C-PE treatment period. ns - p value > 0.05 for control v/s DM; for control v/s A09DM and DM v/s A09DM (n=5)-------------------------- 175

Fig. 7.12: HDL-Cholesterol level at the end of the C-PE treatment period. ns - p value > 0.05 for control v/s DM; for control v/s A09DM and DM v/s A09DM (n=5)-------------------------- 175

Fig. 7.13: Triglyceride concentration at the end of the C-PE treatment period. *P value < 0.05 for control v/s DM; ns for control v/s A09DM and DM v/s A09DM (n=5)------------------------------------- 176

Fig. 7.14: Glycosylated Hb at the end of the C-PE treatment period. ns - p value > 0.05 for control v/s DM; for control v/s A09DM and DM v/s A09DM (n=3)------------------------------------- 177

Fig. 7.15: Specific activity of Hepatic Glucose-6-Phosphatase at the end of the C-PE treatment period. *P value < 0.05 for control v/s A09DM and DM v/s A09DM (n=5)- 178

Fig. 7.16: Hepatic HMG CoA reductase activity at the end of the C-PE treatment period. ns - p value > 0.05 for control v/s DM; for control v/s A09DM and DM v/s A09DM (n=5)---------------------------- 178