Plant Physiol. (1995) 107: 703–708

Synechococcus sp. PCC7942 Transformed with *Escherichia coli bet* Genes Produces Glycine Betaine from Choline and Acquires Resistance to Salt Stress[1]

Mika Nomura, Manabu Ishitani, Teruhiro Takabe, Ashwani K. Rai[2], and Tetsuko Takabe*

BioScience Center, Nagoya University, Chikusa, Nagoya 464–01, Japan (M.N., M.I., A.K.R., Tetsuko Takabe); and Faculty of Science and Technology, Meijo University, Tenpaku, Nagoya 468, Japan (Teruhiro Takabe)

***Synechococcus* sp. PCC7942, a fresh water cyanobacterium, was transformed by a shuttle plasmid that contains a 9-kb fragment encoding the *Escherichia coli bet* gene cluster, i.e. *bet*A (choline dehydrogenase), *bet*B (betaine aldehyde dehydrogenase), *bet*I (a putative regulatory protein), and *bet*T (the choline transport system). The expression of these genes was demonstrated in the cyanobacterial cells (*bet*-containing cells) by northern blot analysis, as well as by the detection of glycine betaine by ^{1}H nuclear magnetic resonance in cells supplemented with choline. Endogenous choline was not detected in either control or *bet*-containing cells. Both control and *bet*-containing cyanobacterial cells were found to import choline in an energy-dependent process, although this import was restricted only to *bet*-containing cells in conditions of salt stress. Glycine betaine was found to accumulate to a concentration of 45 mM in *bet*-containing cyanobacterial cells, and this resulted in a stabilization of the photosynthetic activities of photosystems I and II, higher phycobilisome contents, and general protective effects against salt stress when compared to control cells. The growth of *bet*-containing cells was much faster in the presence of 0.375 M NaCl than that of control cells, indicating that the transformant acquired resistance to salt stress.**

Cyanobacteria, the oxygenic photosynthetic prokaryotes from which plastids of photosynthetic eukaryotes (algae and plants) derive, inhabit a variety of environments including those in which extreme conditions such as high temperatures and/or high salinity are found. Cyanobacteria that are able to grow in high-salt-concentration environments maintain their cell turgor by accumulation of potassium ions and by the synthesis and accumulation of low mol wt organic osmoprotectants. Thus, freshwater cyanobacteria accumulate disaccharides and glucosylglycerol in response to osmotic stress, whereas halotolerant forms accumulate Gly betaine (Blumwald et al., 1983; Mackay et al., 1984; Reed et al., 1986). Gly betaine is synthesized and accumulated by a wide range of organisms including bacteria, higher plants, and animals (Galinski and Truper, 1982; Csonka and Hanson, 1991; Garcia-Perez and Burg, 1991).

Escherichia coli synthesizes Gly betaine via a two-step reaction mediated by a membrane-bound oxygen-dependent choline dehydrogenase, which oxidizes choline to betaine aldehyde and the latter to Gly betaine. The second step is also catalyzed by a soluble NAD-dependent betaine aldehyde dehydrogenase (Landfald and Strøm, 1986; Andresen et al., 1988). The genes that encode the osmoregulatory choline-Gly betaine pathway (as well as the choline uptake system) are clustered in the *E. coli* chromosome (Lamark et al., 1991), and their expression is induced by osmotic shock and the presence of choline (Landfald and Strøm, 1986). Four open reading frames encoding choline dehydrogenase (*bet*A), betaine aldehyde dehydrogenase (*bet*B), an energy-dependent transport system for choline (*bet*T), and a putative regulatory protein (*bet*I) were identified in a 9-kb fragment. The *bet*T gene was located upstream of (and transcribed divergently to) the operon encoding the linked *bet*IBA genes (Andresen et al., 1988; Lamark et al., 1991).

We reported previously the protective role afforded in vitro by Gly betaine against elevated salt concentrations on some cyanobacterial enzymes (Incharoensakdi et al., 1986). Protective effects of Gly betaine on various components (membranes, proteins) have also been reported under various stress conditions (Paleg et al., 1981; Coughlan and Heber, 1982; Jolivet et al., 1982; Mamedov et al., 1991; Papageorgiou et al., 1991). In the present work, we have introduced the *E. coli bet* genes into the freshwater cyanobacterium *Synechococcus* sp. PCC7942 to study the physiological role of Gly betaine in photosynthetic organisms growing under salt stress conditions.

MATERIALS AND METHODS

Growth of Cyanobacteria

Synechococcus sp. PCC7942 was grown at 30°C in BG11 liquid medium containing in addition 20 mM Hepes-KOH

[1] This work was supported by a grant-in-aid for Scientific Research on Priority Areas (No. 04273103) from the Ministry of Education, Science, and Culture of Japan and by a grant from the Bio-Media Project of the Japanese Ministry of Agriculture, Forestry, and Fisheries (BMP94-III-1-1).

[2] Present address: Department of Botany, Banaras Hindu University, Vanarasi, 221005, India.

* Corresponding author; e-mail i45200a@nucc.cc.nagoya-u.ac.jp; fax 81–52–789–4282.

Abbreviations: DAD, 2,3,5,6-tetramethyl-β-phenylenediamine; FCCP, *p*-trifluoromethoxyphenyl hydrazone; MV, methylviologen; PBQ, phenyl-1,4-benzoquinone; QAC, quaternary ammonium compound.

(pH 8.0), under illumination with continuous fluorescent white light (Kuhlemeier and Arkel, 1987). After transformation with shuttle plasmids that confer streptomycin resistance, the cyanobacterial cells were grown in the presence of 10 $\mu g\ mL^{-1}$ antibiotic. Cell growth was followed by measuring A_{730}, and cell number was determined in a Coulter counter (Hialeah, FL). Cyanobacteria were also grown in solid medium by adding 1% agar and 1 mM sodium thiosulfate to the liquid medium described above (Allen, 1968).

Plasmids Used in This Study

Plasmid pBET is a pUC119 derivative containing a 9-kb *Bam*HI fragment carrying the *Escherichia coli bet* genes. Plasmid pUC303 is an *E. coli*/*Synechococcus* shuttle vector that carries a streptomycin-resistance gene for its selection in the latter host (Kuhlemeier and Arkel, 1987). Plasmid pUC303-Bm was kindly donated by Dr. N. Murata (National Institute for Basic Biology, Okazaki, Japan); in this plasmid a *Bam*HI site replaces the *Eco*RI site of pUC303. *E. coli* DH5α was used in this study as a host for plasmid construction and maintenance.

Construction of a *bet* Gene Expression Vector for *Synechococcus* sp. PCC7942 Cells

The 9-kb *Bam*HI fragment from pBET was ligated into the *Bam*HI site of pUC303-Bm (Fig. 1), producing the 20-kb plasmid pCBET, which was used to transform *Synechococcus* cells following the method of Kuhlemeier et al. (1987).

Northern Analysis

Total RNA was extracted from *Synechococcus* cells as described by Aiba et al. (1981), and northern hybridization analysis was carried out by the method of Yang et al. (1993).

Choline Uptake Assay

Synechococcus cells transformed either by vector plasmid pUC303-Bm or *bet*-containing plasmid pCBET were grown to mid-log phase in BG11 medium with or without 200 mM NaCl. Choline transport activity was measured at 25°C using the radiochemical filtration method described by Lamark et al. (1991) with 10 μM [^{14}C]choline (58.5 mCi/mmol). When indicated, FCCP was added to the cells in a final concentration of 5 μM.

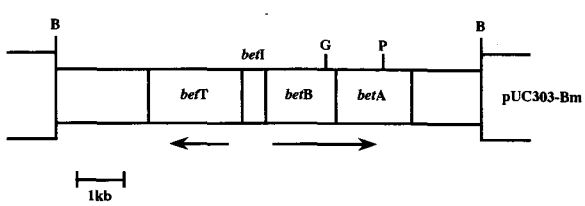

Figure 1. Construction of a plasmid expressing *E. coli bet* genes in *Synechococcus*. The *Bam*HI fragment of *E. coli* chromosomal DNA containing *bet* genes was cloned into shuttle vector pUC303-Bm to generate plasmid pCBET, as described in "Materials and Methods." *bet*A, Choline dehydrogenase; *bet*B, betaine aldehyde dehydrogenase; *bet*T, choline transport system; *bet*I, putative regulatory gene. The arrows indicate the directions of transcription. Restriction enzyme sites: B, *Bam*HI; P, *Pst*I; G, *Bgl*II. The *Bgl*II and *Pst*I fragment was used as a probe in the present study.

Quantification of Gly Betaine

Addition of choline at concentrations higher than 1 mM inhibited the growth of *Synechococcus* sp. PCC7942 cells under nonstressed and salt-stressed conditions. Therefore, we added 100 μM choline to produce Gly betaine in the *bet*-containing cells. Actually, at this level choline did not affect growth of control cells under various salinity conditions. *Synechococcus* cells grown in BG11 medium containing 100 μM choline (BG11-choline medium) for 2 d (to mid-log phase) were transferred to the same medium with NaCl of various concentrations. After incubation was continued for an additional 3 d, QACs were extracted from the cells with 1 N H_2SO_4 and precipitated as their periodides (Wall et al., 1960). Pellets were dissolved with 600 μL of distilled water containing *t*-butanol as an internal standard. Analysis of QACs was performed by ^{1}H-NMR spectroscopy using a JEOL JMN-500 Fourier transform NMR spectrometer. Cell volume was determined by electron spin resonance spectroscopy according to the method of Blumwald et al. (1983). Concentrated cells were treated with 1 mM freely permeable nitroxide spin probe TEMPONE (2,2,6,6,-tetramethylpiperidone-*N*-oxyl), in the presence of the membrane-impermeable paramagnetic quenching agents $Na_3Fe(CN)_6$ (20 mM) and $Na_2MnEDTA$ (75 mM) to elicit the electron spin resonance signal of the intracellular spin probe. Cell volume was also estimated by using ^3H_2O and [^{14}C]sorbitol as reported previously (Incharoensakdi and Takabe, 1988).

Measurements of Photosynthetic Activities

Cells grown in BG11-choline medium for 2 d (mid-log phase) were transferred to the same medium, 200 mM NaCl was added, and growth was continued for 4 d. Photosynthetic oxygen evolution and the activities of PSI and PSII were measured after the cells were transferred to nonstressed conditions for 1 d, in a Clark-type oxygen electrode. The reaction medium contained 100 μM DCMU, 1 mM sodium ascorbate, 500 μM DAD, and 400 μM MV for the measurements of PSI electron transport activities or 1 mM PBQ for the measurements of PSII electron transport activities. Photosynthetic activities were also measured immediately after the cells were subjected to osmotic stress in the presence of 200 mM NaCl.

Other Methods

Chl was determined by the method of Mackinney (1941), and protein was determined by the method of Lowry et al. (1951) with BSA as the standard. DNA manipulations were carried out as described by Sambrook et al. (1989).

RESULTS AND DISCUSSION

Transformation of *Synechococcus* sp. PCC7942 with *E. coli bet* Genes

The cyanobacterial cells were transformed by plasmid pCBET, which contains *E. coli bet* genes (Fig. 1), and selected for streptomycin resistance in agar plates. The resulting colonies were screened for the presence of the *bet* genes by Southern hybridization with a ^{32}P-labeled *bet* probe (Fig. 1), and a positive cyanobacterial colony was selected for further analysis.

Northern Blot Analysis

To confirm the expression of the *bet* genes in the *Synechococcus* cells transformed by pCBET, northern blot analysis was carried out using *Bgl*II and *Pst*I fragments (Fig. 1) as a probe. As shown in Figure 2, a transcript of approximately 9 kb was detected in these cells grown for 2 d without NaCl and an additional 5 d with 200 mM NaCl on a BG11-choline medium. This transcript could not be detected in cells transformed by the plasmid vector pUC303-Bm (Fig. 2). Based on the size of this transcript, it seems that the *bet*IBA operon was transcribed in the cyanobacterial cells and that the transcription runs over the *Bam*HI site of the shuttle vector pUC303-Bm.

Choline Uptake by *Synechococcus* Cells

We examined the ability of control (pUC303-Bm transformed) and *bet*-containing (pCBET transformed) cyanobacterial cells to import exogenously added choline. Endogenous choline was not detected in the cyanobacterial cells when growing in the absence of this compound (data not shown). As shown in Figure 3A, uptake of choline proceeded in both control and *bet*-containing cells under nonstressed conditions, although both the initial rate and cellular content after 30 min was higher (up to 40%) in cells bearing plasmid pCBET. This difference may result from the functional expression of the *bet*T gene present in pCBET, which encodes a choline transport system active in *E. coli* (Lamark et al., 1991). Choline uptake was strongly inhibited in all cases by the addition of the uncoupler FCCP (Fig. 3), suggesting the presence of an energy-dependent transport system in *Synechococcus*.

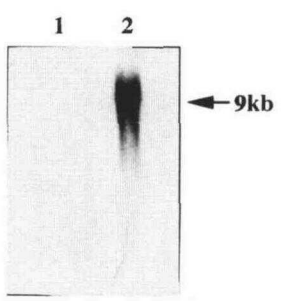

Figure 2. Expression of *E. coli bet* genes in *Synechococcus*. pUC303-Bm- (lane 1) and pCBET-transformed cyanobacterial (lane 2) cells, respectively, were grown in BG11-choline medium for 2 d and in the same medium with 200 mM NaCl for 5 d. RNA extraction and northern blot analysis were carried out as described in "Materials and Methods," using 30 μg of RNA in each lane.

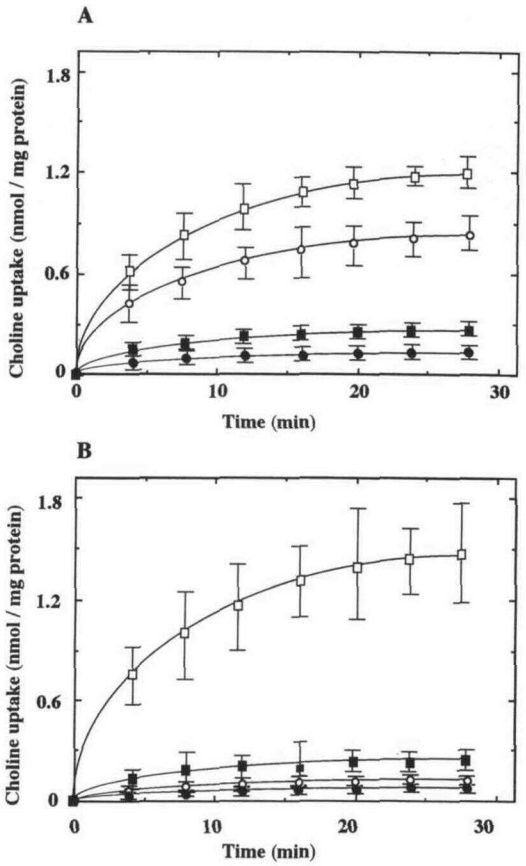

Figure 3. Choline uptake in control and *bet*-containing *Synechococcus* cells. The uptake of choline in the presence of 10 μM [^{14}C]choline in the medium was followed in BG11 medium in the absence (A) or in the presence (B) of 200 mM NaCl, as described in "Materials and Methods." ○, pUC303-Bm-transformed cells; □, pCBET-containing cells. The uptake measured in these cells in the presence of 5 μM FCCP is indicated by the filled symbols. The mean and the SD of three independent measurements are indicated.

Interestingly, under salt-stress conditions choline was actively taken up only by *bet*-containing cyanobacterial cells (Fig. 3B). As a possible explanation for these results, it could be postulated that an energy-dependent transport system for choline present in the cyanobacterial plasma membrane is altered in the presence of high salt concentrations in the medium and that the production of Gly betaine (see below) exerts a stabilizing effect on the membrane that allows transport to proceed under these conditions. Alternatively, the uptake of choline may proceed in high salt conditions as a consequence of the functional expression of *bet*T, present in pCBET. In that case utilization of choline in Gly betaine synthesis might alleviate the transport system from feedback regulation by choline in the *bet*-containing cyanobacterial cells (cf. Lamark et al., 1991). Further work is required to distinguish between these alternatives.

Gly Betaine Production by the Transformed *Synechococcus* Cells

Enzyme activity of neither choline dehydrogenase nor betaine aldehyde dehydrogenase was detected in *Synechococcus* sp. 7942 (data not shown), and the wild-type cells did not accumulate Gly betaine at all under salinity conditions. Gly betaine content was determined in control and *bet*-containing cyanobacterial cells by ^{1}H-NMR. As shown in Figure 4, only *bet*-transformed cells synthesized and accumulated Gly betaine. Interestingly, the levels of Gly betaine in these cells changed in response to the salt concentration of the culture medium, ranging from approximately 3 mM under nonstressed conditions to 45 mM when the NaCl concentration reached 375 mM, as shown in Table I. These levels of Gly betaine provide enough protection for various cellular functions (Genard et al., 1991; Rhodes and Hanson, 1993), a result also demonstrated elsewhere in this work.

Table I. *Accumulation of Gly betaine in bet-containing cells under various NaCl concentrations*

Control and *bet*-containing cells were grown in BG11-choline medium for 2 d, transferred to the same medium with NaCl of various concentrations, and grown for an additional 3 d. The quantification of Gly betaine was carried out as described in "Materials and Methods."

NaCl in Culture Medium	Gly Betaine
M	mM
0	3.2 ± 0.4
0.1	4.5 ± 0.6
0.2	8.8 ± 0.7
0.3	24.1 ± 2.0
0.375	45.1 ± 1.9

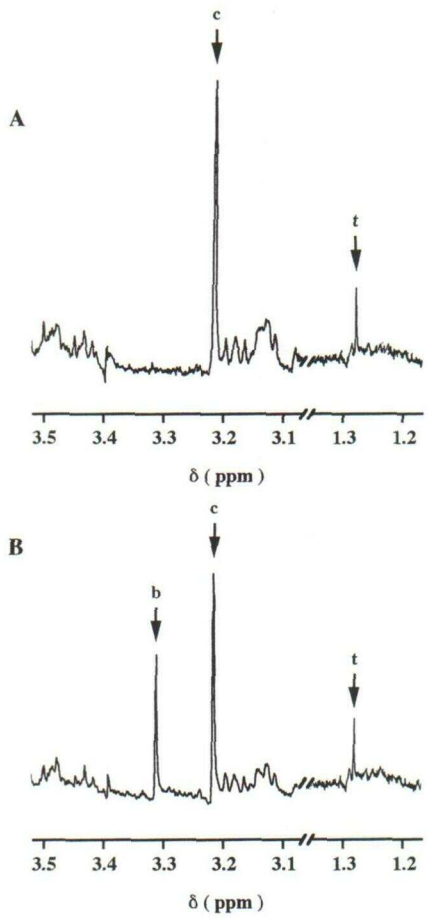

Figure 4. ^{1}H-NMR spectra of QACs derived from control (A) or *bet* gene-containing (B) cells. The cells were grown for 2 d in BG11-choline medium and then transferred to the same medium with or without 200 mM NaCl and grown for an additional 5 d. QACs were extracted and processed as described in "Materials and Methods." Peaks b and c represent Gly betaine and choline, respectively. ^{1}H-NMR response positions (δ) for *N*-methylprotons of Gly betaine and choline are 3.31 and 3.23 ppm, respectively, with 100 μM *t*-butanol (t) at 1.28 ppm as a reference.

Photosynthetic Activities of *Synechococcus* Cells under Salt Stress

Although *Synechococcus* can grow in culture media containing NaCl concentrations as high as 400 mM, the cells turn pale yellow after growing for 4 d in the presence of 300 mM NaCl (Fig. 5). Interestingly, this effect was not observed in *bet*-containing cyanobacterial cells, which remained green (Fig. 5). As expected, absorption spectra indicated drastic decreases in phycobilisome (C-phycocyanin; absorption at 620–630 nm) and Chl content in the control cells under salt stress when compared to those of

Figure 5. Effect of salt stress on control and *bet*-containing *Synechococcus* cells. Cells were grown in BG11-choline medium for 2 d and transferred to the same medium containing 300 mM NaCl and grown for an additional 4 d. Control cells (left) were bleached, whereas *bet*-containing cells (right) remained green.

Table II. *Effect of salt stress on the photosynthetic electron transport activities*

Control and *bet*-containing cells were grown in BG11-choline medium for 2 d, transferred to the same medium with 200 mM NaCl, and grown for an additional 4 d. Since the photosynthetic activities of these cells were low, the cells were transferred to fresh BG11-choline medium without NaCl for 1 d before analysis. The average of three different measurements is indicated (SE were within 5%).

Reaction	Oxygen Evolution or Absorption	
	Control (pUC303-Bm) cells	*Bet*-containing cells
	μmol O_2 mg^{-1} Chl h^{-1}	
Total ($H_2O \rightarrow CO_2$)	81 (100)	98 (121[a])
PSI ($DADH_2 \rightarrow MV$)	145 (100)	165 (114)
PSII ($H_2O \rightarrow PBQ$)	156 (100)	217 (139)

[a] Percentage of control.

bet-containing cells (data not shown). The activities associated with PSI and PSII, as well as the photosynthetic oxygen evolution, were determined after growing the cells for 2 d without NaCl, for 3 d in culture medium containing 200 mM NaCl, and then for 1 d in the absence of NaCl (Table II). In all cases, *bet*-containing cells exhibited activities higher than those found in control cells.

Table III shows the photosynthetic activities obtained after transferring cells grown under nonstressed conditions to a high concentration of salt (200 mM NaCl). As shown in the Table III, the activities in all cases (and especially those of PSI) decreased almost instantaneously in control cells when compared to the values obtained in the absence of salt stress, whereas this reduction was less pronounced in *bet*-containing cells. These observations show that Gly betaine production generates a stabilizing effect on phycobilisome and photosystem complexes under salt stress. It must be noted that these results coincide with the previously reported protective effect of Gly betaine on photosystem complexes evidenced by in vitro experiments (Mamedov et al., 1991; Papageorgiou et al., 1991). However, Gly betaine at concentrations in the hundred-millimolar range was tested for those in vitro experiments. Further analysis is required to determine whether Gly betaine exerts any stabilization effect on gene expression.

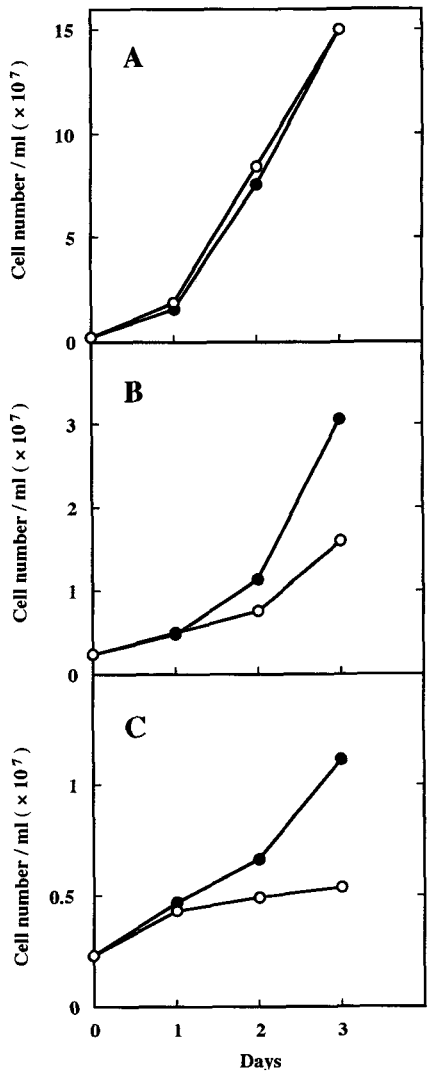

Figure 6. Growth curves of *Synechococcus* cells under salt- stress. Control and *bet*-containing cells were grown in BG11-choline medium for 2 d and transferred to the same medium containing NaCl of various concentrations (A, 0.1 M NaCl; B, 0.375 M NaCl; C, 0.4 M NaCl) and their growth was followed. ○, Control cells; ●, *bet*-containing cells. The initial cell density of each culture was kept constant. SE values were within 7%, $n = 3$.

Table III. *Effect of salt shock on the photosynthetic electron transport activities*

Control and *bet*-containing *Synechococcus* cells were grown for 4 d in BG11-choline medium in the absence of NaCl. Immediately before measurements, cells were transferred to BG11-choline medium containing 200 mM NaCl. The average of three different measurements is indicated (SE were within 5%).

Reaction	Oxygen Evolution or Absorption			
	Control (pUC303-Bm) cells		*Bet*-containing cells	
	−NaCl	+0.2 M NaCl	−NaCl	+0.2 M NaCl
	μmol O_2 mg^{-1} Chl h^{-1}			
Total ($H_2O \rightarrow CO_2$)	57 (100)	40 (68[a])	52 (100)	39 (74[a])
PSI ($DADH_2 \rightarrow MV$)	152 (100)	96 (63)	217 (100)	177 (82)
PSII ($H_2O \rightarrow PBQ$)	71 (100)	60 (85)	78 (100)	77 (98)

[a] Percentage of nonstressed cells.

Growth under Salt Stress

After control and *bet*-containing cells were grown in BG11-choline medium for 2 d, growth was examined in the same fresh medium with NaCl of various concentrations (Fig. 6). Control and *bet*-containing cells showed almost the same growth rate up to 0.3 M NaCl. However, at NaCl concentrations greater than 0.3 M growth rates of *bet*-containing cells were much faster than those of control cells, indicating that *bet*-containing cells acquire resistance to salt stress (Fig. 6, B and C). The overall results indicate that the *bet* genes present in plasmid pCBET were expressed and generated functional proteins in the *Synechococcus* cells. Moreover, the Gly betaine produced by the cells had a general beneficial effect on the cyanobacterial cells under salt-stress conditions. Further studies using this system may help to elucidate the protective role(s) played by Gly betaine to the stress imposed on cells growing in high saline environments. Tarczynski et al. (1993) reported stress protection of transgenic tobacco by production of the osmolyte mannitol. However, to our knowledge the present study is the first report of protective effects and acquisition of salinity tolerance by production of Gly betaine in photosynthetic organisms.

ACKNOWLEDGMENT

We thank Dr. A. Viale for his useful discussion.

Received July 18, 1994; accepted November 29, 1994.
Copyright Clearance Center: 0032-0889/95/107/0703/06.

LITERATURE CITED

Aiba H, Adhya S, de Crombrugghe B (1981) Evidence for two functional *gal* promoters in intact *Escherichia coli* cells. J Biol Chem **256:** 11905–11910

Allen MM (1968) Simple conditions for growth of unicellular blue-green algae on plates. J Phycol **4:** 1–4

Andresen PA, Kaasen I, Styrvold OB, Boulnois G, Strøm AR (1988) Molecular cloning, physical mapping and expression of the *bet* genes governing the osmoregulatory choline-glycine betaine pathway of *Escherichia coli*. J Gen Microbiol **134:** 1737–1746

Blumwald E, Mehlhorn RJ, Packer L (1983) Studies of osmoregulation in salt adaptation of cyanobacteria with ESR spin-probe techniques. Proc Natl Acad Sci USA **80:** 2599–2602

Coughlan SJ, Heber U (1982) The role of glycinebetaine in the protection of spinach thylakoids against freezing stress. Planta **156:** 62–69

Csonka LN, Hanson AD (1991) Prokaryotic osmoregulation: genetics and physiology. Annu Rev Microbiol **45:** 569–606

Galinski EA, Truper HG (1982) Betaine, a compatible solute in the extremely halophilic phototrophic bacterium *Ectothiorhodospira halochloris*. FEMS Microbiol Lett **13:** 357–360

Garcia-Perez A, Burg MB (1991) Renal medullary organic osmolytes. Physiol Rev **71:** 1081–1115

Genard H, Saos JL, Billard JP, Tremolieres A, Boucaud J (1991) Effect of salinity on lipid composition, glycine betaine content and photosynthetic activity in chloroplasts *Suaeda maritima*. Plant Physiol Biochem **29:** 421–427

Incharoensakdi A, Takabe T (1988) Determination of intracellular chloride ion concentration in a halotolerant cyanobacterium *Aphanothece halophytica*. Plant Cell Physiol **29:** 1073–1075

Incharoensakdi A, Takabe T, Akazawa T (1986) Effect of betaine on enzyme activity and subunit interaction of ribulose-1,5-bisphosphate carboxylase/oxygenase from *Aphanothece halophytica*. Plant Physiol **81:** 1044–1049

Jolivet Y, Larher F, Hamelin J (1982) Osmoregulation in halophylic higher plants: the protective effect of glycine betaine against the heat destabilization of membranes. Plant Sci Lett **25:** 193–201

Kuhlemeier CJ, Arkel GAV (1987) Host-vector systems for gene cloning in cyanobacteria. Methods Enzymol **153:** 199–243

Lamark T, Kaasen I, Eshoo MW, Falkenberg P, McDougall J, Strøm AR (1991) DNA sequence and analysis of the *bet* genes encoding the osmoregulatory choline-glycine betaine pathway of *Escherichia coli*. Mol Microbiol **5:** 1049–1064

Landfald B, Strøm AR (1986) Choline-glycine betaine pathway confers a high level of osmotic tolerance in *Escherichia coli*. J Bacteriol **165:** 849–855

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem **193:** 265–275

Mackay MA, Norton RS, Borowitzka LJ (1984) Organic osmoregulatory solutes in cyanobacteria. J Gen Microbiol **130:** 2177–2191

Mackinney G (1941) Absorption of light by chlorophyll solutions. J Biol Chem **140:** 315–322

Mamedov MD, Hayashi H, Wada H, Mohanty PS, Papageorgiou GC, Murata N (1991) Glycinebetaine enhances and stabilizes the evolution of oxygen and the synthsis of ATP by cyanobacterial thylakoid membranes. FEBS Lett **294:** 271–274

Paleg LG, Douglas TJ, Daal AV, Keech DV (1981) Proline, betaine and other organic solutes protect enzymes against heat inactivation. Aust J Plant Physiol **8:** 107–114

Papageorgiou GC, Fujimura Y, Murata N (1991) Protection of the oxygen-evolving photosystem II complex by glycinebetaine. Biochim Biophys Acta **1057:** 361–366

Reed RH, Borowitzka LJ, Mackay MA, Chudek JA, Foster R, Warr SRC, Moore DJ, Stewart WDP (1986) Organic solute accumulation in osmotically stresed cyanobacteria. FEMS Microbiol Rev **39:** 51–56

Rhodes D, Hanson AD (1993) Quaternary ammonium and tertiary sulfonium compounds in higher plants. Annu Rev Plant Physiol Plant Mol Biol **44:** 357–384

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning: A Laboratory Manual, Ed 2. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Tarczynski MC, Jensen RG, Bohnert HJ (1993) Stress protection of transgenic tobacco by production of the osmolyte mannitol. Science **259:** 508–510

Wall JS, Christianson DD, Dimler RJ, Senti FR (1960) Spectrophotometric determination of betaines and other quaternary nitrogen compounds as their periodides. Anal Chem **32:** 870–874

Yang H, McLeese J, Weisbart M, Dionne JL, Lemaire I, Aubin RA (1993) Simplified high throughput protocol for northern hybridization. Nucleic Acids Res **21:** 3337–3338