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Short Communication

Cloning and sequence analysis of the glucose-6-phosphate dehydrogenase gene from the cyanobacterium *Synechococcus* PCC 7942

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Abstract

The glucose-6-phosphate dehydrogenase (EC 1.1.1.49) gene (*zwf*) of the cyanobacterium *Synechococcus* PCC 7942 was cloned on a 2.8 kb *Hin*d III fragment. Sequence analysis revealed an ORF of 1572 nucleotides encoding a polypeptide of 524 amino acids which exhibited 41% identity with the glucose-6-phosphate dehydrogenase of *Escherichia coli*.

In cyanobacteria the dissimilation of fixed carbon occurs predominantly via the oxidative pentose phosphate pathway [11]. Glucose-6-phosphate dehydrogenase (G-6-PD, EC 1.1.1.49), apart from being the key enzyme for the entry of fixed carbon into this pathway [see 14], has also been implicated in the supply of reductant to nitrogenase [1]. Because of the central importance of this enzyme in the transition from phototrophic metabolism to heterotrophic metabolism in the dark, the regulation of its activity has been the focus of much attention. To date there are no reports of the characterization, at the nucleotide sequence level, of the G-6-PD gene (*zwf*) from any cyanobacterium or, indeed, from any oxygen-evolving photosynthetic organism. Recently, however, the *zwf* genes from several non-photosynthetic bacterial species [3, 10, 13] have been sequenced and found to exhibit considerable homology both with each other and with G-6-PD genes from eukaryotic organisms. Consequently, we utilized the approach of heterologous hybridization to isolate and characterize a cyanobacterial *zwf* gene.

Chromosomal DNA from *Synechococcus* PCC 7942 was partially digested with *Sau* 3A and used to construct a library in λ charon 35. A 1.9 kb *Kpn* I-*Xho* I fragment from the plasmid pTC117 which is derived from pTC111 [3] and carries the *Zymomonas mobilis* G-6-PD (*zwf*) gene was used as a hybridization probe to screen the library. One clone from the library hybridized strongly with the 1.9 kb *Kpn* I-*Xho* I fragment and contained a 2.8 kb *Hin*d III fragment which also hy-

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number X64768.

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       10                  30                  50                  70                  90
ATGACTCCCAAACTGCTTGAGAACCCGCTTCGCATTGGACTCCGCCAAGACAAAGTCCCTGAACCGCAAATCCTCGTCATCTTTGGGGCC
M  T  P  K  L  L  E  N  P  L  R  I  G  L  R  Q  D  K  V  P  E  P  Q  I  L  V  I  F  G  A
                  110                 130                 150                 170
ACCGGCGACTTAACCCAGCGCAAACTGGTGCCTGCCATCTACGAGATGCACCTCGAACGGCGTTTGCCGCCAGAACTGACGATCGTGGGG
T  G  D  L  T  Q  R  K  L  V  P  A  I  Y  E  M  H  L  E  R  R  L  P  P  E  L  T  I  V  G
       190                 210                 230                 250                 270
GTGGCTCGGCGCGACTGGAGCGATGACTATTTCCGAGAGCACCTTCGCCAAGGGGTTGAACAGTTTGGCGGCGGCATTCAAGCAGAGGAA
V  A  R  R  D  W  S  D  D  Y  F  R  E  H  L  R  Q  G  V  E  Q  F  G  G  G  I  Q  A  E  E
                  290                 310                 330                 350
GTTTGGAACACCTTTGCACAAGGCCTCTTCTTTGCGCCGGGCAACATTGATGACCCCCAGTTTTATCAAACCCTTCGCGATCGCCTTGCG
V  W  N  T  F  A  Q  G  L  F  F  A  P  G  N  I  D  D  P  Q  F  Y  Q  T  L  R  D  R  L  A
       370                 390                 410                 430                 450
AATCTGGATGAGCTGCGCGGCACGCGGGGCAATCGCACTTTTTACCTCTCGGTCGCACCCCGTTTCTTTGGTGAAGCTGCAAAACAACTC
N  L  D  E  L  R  G  T  R  G  N  R  T  F  Y  L  S  V  A  P  R  F  F  G  E  A  A  K  Q  L
                  470                 490                 510                 530
GGGGCAGCCGGAATGCTTGCCGATCCAGCTAAAACGCGGCTGGTCGTCGAAAAACCTTTTGGCCGCGATCTCAGCTCCGCTCAGGTGCTG
G  A  A  G  M  L  A  D  P  A  K  T  R  L  V  V  E  K  P  F  G  R  D  L  S  S  A  Q  V  L
       550                 570                 590                 610                 630
AATGCCATCTTGCAGAACGTTTGCCGCGAAAGCCAGATCTATCGGATTGACCATTACCTCGGCAAAGAAACAGTTCAAAACCTCTTAGTT
N  A  I  L  Q  N  V  C  R  E  S  Q  I  Y  R  I  D  H  Y  L  G  K  E  T  V  Q  N  L  L  V
                  650                 670                 690                 710
TTCCGGTTTGCCAATGCCATTTTTGAGCCGCTCTGGAACCGGCAATACATTGACCATGTCCAAATCACGGTGGCTGAAACTGTGGGGTTG
F  R  F  A  N  A  I  F  E  P  L  W  N  R  Q  Y  I  D  H  V  Q  I  T  V  A  E  T  V  G  L
       730                 750                 770                 790                 810
GAAGGGCGAGCTGGCTACTACGAAACTGCTGGTGCTCTGCGGGATATGGTGCAAAACCACTTGATGCAGCTCTTCAGCCTGACGGCGATG
E  G  R  A  G  Y  Y  E  T  A  G  A  L  R  D  M  V  Q  N  H  L  M  Q  L  F  S  L  T  A  M
                  830                 850                 870                 890
GAGCCGCCAAACTCTCTAGGTGCTGACGGTATCCGTAACGAAAAGGTCAAGGTGGTGCAAGCCACACGGCTGGCGGATATCGACGATCTC
E  P  P  N  S  L  G  A  D  G  I  R  N  E  K  V  K  V  V  Q  A  T  R  L  A  D  I  D  D  L
       910                 930                 950                 970                 990
AGTTTGTCTGCGGTGCGGGGGCAGTACAAAGCGGGCTGGATGAATGGCCGCTCTGTGCCCGCCTATCGGGATGAGGAGGGAGCGGATCCC
S  L  S  A  V  R  G  Q  Y  K  A  G  W  M  N  G  R  S  V  P  A  Y  R  D  E  E  G  A  D  P
                 1010                1030                1050                1070
CAGTCGTTTACGCCCACCTATGTCGCCATGAAATTGCTGGTCGACAACTGGCGCTGGCAGGGAGTGCCGTTCTATCTACGGACGGGTAAA
Q  S  F  T  P  T  Y  V  A  M  K  L  L  V  D  N  W  R  W  Q  G  V  P  F  Y  L  R  T  G  K
      1090                1110                1130                1150                1170
CGGATGCCCAAAAAGGTGACGGAGATTGCCATTCAGTTCAAAACCGTGCCGCACTTGATGTTCCAGTCAGCCACCCAAAAAGTGAATAGT
R  M  P  K  K  V  T  E  I  A  I  Q  F  K  T  V  P  H  L  M  F  Q  S  A  T  Q  K  V  N  S
                 1190                1210                1230                1250
CCCAACGTCTTAGTGCTGCGGATTCAGCCCAATGAAGGCGTGTCCTTGCGCTTTGAAGTGAAAACACCGGGTTCCTCGCAACGGACGCGA
P  N  V  L  V  L  R  I  Q  P  N  E  G  V  S  L  R  F  E  V  K  T  P  G  S  S  Q  R  T  R
      1270                1290                1310                1330                1350
TCGGTGGATATGGACTTCCGCTACGACACGGCTTTTGGCTCCCCCACCCAAGAGGCCTATAGCCGCCTGCTGGTGGACTGCATGCTCGGC
S  V  D  M  D  F  R  Y  D  T  A  F  G  S  P  T  Q  E  A  Y  S  R  L  L  V  D  C  M  L  G
                 1370                1390                1410                1430
GATCAGACGCTGTTCACCCGCGCTGATGAGGTTGAAGCGTCTTGGCGGGTTGTGACGCCGTTACTCGAATCTTGGGATGACCCGCGCCAA
D  Q  T  L  F  T  R  A  D  E  V  E  A  S  W  R  V  V  T  P  L  L  E  S  W  D  D  P  R  Q
      1450                1470                1490                1510                1530
GCCGCTGGCATTTCTTTTTACGAAGCTGGCACTTGGGAGCCGGCAGAGGCGGAGCAGTTGATCAACCGTGATGGTGCCGTTGGCGTCGTC
A  A  G  I  S  F  Y  E  A  G  T  W  E  P  A  E  A  E  Q  L  I  N  R  D  G  A  V  G  V  V
                 1550                1570
TCTAGGATCCCTGCAACCCAGCTCAATTCTTCTGGAGATGTTTGATGA
S  R  I  P  A  T  Q  L  N  S  S  G  D  V  *  *
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Fig. 1. Nucleotide sequence and derived amino acid sequence of the 1572 kb ORF encoded on the 2.8 kb *Hin*d III fragment from *Synechococcus* PCC 7942.

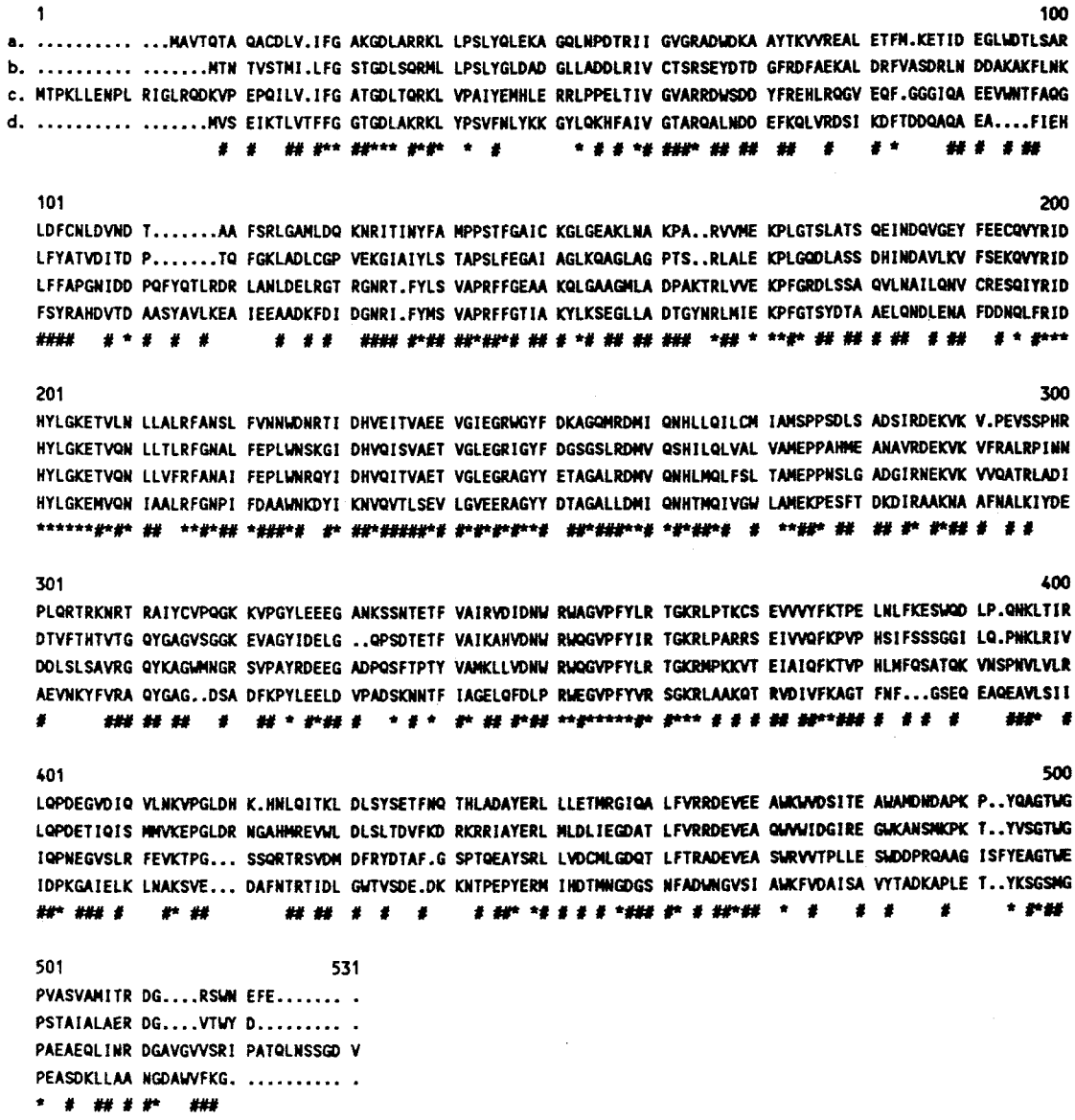

Fig. 2. Alignment of the amino acid sequences of glucose-6-phosphate dehydrogenases from (a) *Escherichia coli*, (b) *Zymomonas mobilis*, (c) *Synechococcus* PCC 7942 and (d) *Leuconostoc mesenteroides* obtained using the PILEUP program [6]. The * symbol indicates residues conserved in all four sequences and the # symbol indicates where a residue found in the *Synechococcus* PCC 7942 protein occurs in at least one of the other proteins.

bridized strongly with the probe. This 2.8 kb *Hind* III fragment was subcloned into pUC19 to yield pNUT1. The nucleotide sequence of the 2.8 kb fragment was determined by the dideoxy chain termination method following a combination of both random and directed subcloning into M13 mp18 and M13 mp10. Analysis of the nucleotide sequence revealed an ORF (524 amino acids) of 1572 nucleotides followed by two stop codons (Fig. 1). Alignment of the amino acid sequence of the predicted polypeptide encoded by this ORF with those of known G-6-PD from prokaryotic sources using the PILEUP program [6] revealed extensive homologies (Fig. 2) strongly

suggesting that this ORF represents the authentic *zwf* gene of *Synechococcus* PCC 7942. The cyanobacterial G-6-PD was estimated to be 60% similar (41% identical) to that of *Escherichia coli* using the GAP alignment program [6]. In addition, the predicted polypeptide includes the canonical sequence (DHYLGKE) of the active site of G-6-PD [9] as recognized in the PROSITE dictionary.

Numerous studies have been directed at analysing the regulation of activity of G-6-PD activity in cyanobacteria particularly in connection with the light-dependent control of activity. The metabolites implicated in regulation include NADPH [1, 12], ATP [8] and thioredoxin [5], the latter having a role in regulating G-6-PD activity in chloroplasts [2]. Thioredoxin can function as a protein disulphide reductase and has been shown to reduce the disulphides in certain proteins, such as the activation of chloroplast fructose-1,6-bisphosphatase [4]. In this context it is worth noting that the amino acid sequence reported here for the *Synechococcus* PCC 7942 enzyme contains only two cysteine residues and consequently both residues would be involved in such regulation. Neither of the two cysteines present in the cyanobacterial polypeptide are conserved residues in the other prokaryotic enzymes and one of the cysteines (Cys-188), which replaces a conserved phenylalanine in the other prokaryotic enzymes, lies immediately prior to the active site region defined by the sequence DHYLGKE.

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