Molecular characterization of the genes encoding acetohydroxy acid synthase in the cyanobacterium *Spirulina platensis*

A. MILANO, E. DE ROSSI, E. ZANARIA, L. BARBIERATO, O. CIFERRI and G. RICCARDI*

Dipartimento di Genetica e Microbiologia 'A. Buzzati Traverso', Via Abbiategrasso 207, 27100 Pavia, Italy

(Received 3 January 1992; revised 10 March 1992; accepted 16 March 1992)

The enzyme acetohydroxy acid synthase (AHS), which catalyses the first common step in the biosynthesis of isoleucine, leucine and valine, has been demonstrated to be present in *Spirulina platensis* in two isoenzymic forms. The complete nucleotide sequences of the genes *ilvX* and *ilvW* encoding these two enzymes have been determined. Sequence analysis revealed the presence of two open reading frames, of 1836 and 1737 nucleotides for *ilvX* and *ilvW*, respectively. The predicted amino acid sequences of the two isoenzymes, compared with the *Synechococcus* PCC 7942 AHS enzyme and the large subunits of the *Escherichia coli* AHS I, II, III isoenzymes, revealed a notable degree of similarity. A small subunit has not been identified for either of the *S. platensis* AHS isoenzymes. Analysis by Northern blot hybridization demonstrated that the *ilvX* and *ilvW* genes are transcribed to give mRNA species of approximately 2.15 kb and 1.95 kb, respectively.

Introduction

The study of amino acid biosynthesis, which has substantially contributed to our present knowledge of microbial metabolism, has been very limited in cyanobacteria (Riccardi *et al.*, 1989). We are studying the pathway of isoleucine, valine and leucine biosynthesis in *Spirulina platensis* as an approach to understanding the regulation of cyanobacterial metabolism (Riccardi *et al.*, 1990). Acetohydroxy acid synthase (AHS, EC 4.1.3.18) is the first common enzyme in the biosynthetic pathways to valine, isoleucine and leucine. AHS has recently been demonstrated to be the target of two new and structurally unrelated classes of herbicides, the sulphonylureas (Chaleff & Mauvais, 1984; Ray, 1984) and the imidazolinones (Shaner *et al.*, 1984).

Three AHS isoenzymes have been found in *Escherichia coli* and *Salmonella typhimurium* (De Felice *et al.*, 1982; Umbarger, 1983). The genes encoding these isoenzymes are organized in operons and include *ilvBN*, *ilvGM* and *ilvIH*, coding for isoenzymes I, II and III, respectively

(Wek *et al.*, 1985; Lawther *et al.*, 1981; Squires *et al.*, 1983). In these bacteria AHS is a tetramer composed of two large and two small subunits. The large subunits have a molecular mass of about 60 kDa, while the small subunits have molecular mass of 10 to 20 kDa (Eoyang & Silverman, 1988; Schloss & Van Dyk, 1988; Barak *et al.*, 1988). Only one gene encoding functional AHS lacking a small subunit was isolated from yeast (Falco & Dumas, 1985; Falco *et al.*, 1985). Genomic clones encoding AHS have been isolated and sequenced from the higher plants *Arabidopsis thaliana* and *Nicotiana tabacum* (Mazur *et al.*, 1987). In *A. thaliana* the gene codes for an AHS of molecular mass 72 593 Da. Friedberg & Seijffers (1990) isolated and sequenced a gene from *Synechococcus* PCC 7942 that encodes a protein of 612 amino acids showing high sequence similarity with the AHSIII large subunit of *E. coli* and the monomeric tobacco AHS.

Two isoforms of AHS were detected in cell-free extracts of *Spirulina platensis* and separated both by ion-exchange chromatography and by hydrophobic interaction (Forlani *et al.*, 1991). The isoforms differ in pH optimum, FAD requirement for both activity and stability, and heat lability (Forlani *et al.*, 1991). The corresponding genes, *ilvX* and *ilvW* (named *ilvY* in our previous papers), were isolated from a λ library of *S. platensis* genomic DNA. The 4.2 kb *ClaI* (*ilvX*) and the 3.2 kb *SalI/ClaI* (*ilvW*) fragments were successively subcloned in the plasmid vector pAT153 (Maniatis *et al.*,

* Author for correspondence. Tel. (0382) 391574; fax (0382) 528496.

Abbreviations: AHS, acetohydroxy acid synthase; RBS, ribosome-binding site.

The nucleotide sequence data reported in this paper have been submitted to GenBank and have been assigned the accession numbers M75906 for *ilvX* and M75907 for *ilvW*, respectively.

1982). The *ilvX* gene was able to complement a suitable mutant of *E. coli* while the *ilvW* gene supported poor growth of the same *E. coli* mutant (Riccardi *et al.*, 1991).

We here present the complete nucleotide sequence and a preliminary transcriptional analysis of the two genes (*ilvX*, *ilvW*) encoding AHS isolated from *S. platensis*. We also compare the deduced amino acid sequences of the two AHS isoenzymes to that of the large subunits of the *E. coli* AHS isoenzymes I, II and III and to the *Synechococcus* PCC 7942 enzyme.

Methods

Culture conditions, RNA isolation and Northern hybridization. Culture conditions for *S. platensis* C1 have been previously described (Riccardi *et al.*, 1981). RNA was extracted from 1 litre of *S. platensis* culture grown to mid-exponential phase and then harvested by filtration on paper discs. Cells were immediately frozen in liquid nitrogen, ground using a ceramic mortar and pestle, and resuspended in guanidine. HCl buffer (8 M-guanidine.HCl, 0.1 M-sodium acetate, 5 mM-dithiothreitol, 0.5% sodium lauryl sarcosinate, pH 5.2). The cell suspension was extracted with an equal volume of water-saturated phenol and the aqueous phase was re-extracted with phenol/chloroform/isoamyl alcohol prior to a final extraction with chloroform/isoamyl alcohol (Maniatis *et al.*, 1982). Sodium acetate (pH 5.2) was added to a final concentration of 0.3 M and RNA was precipitated by adding 2.5 vols ice-cold ethanol followed by storage at -20°C for at least 2 h. RNA was recovered by centrifugation at 10000 g for 10 min at 4°C . The pellet was washed with ice-cold 70% (v/v) ethanol, recentrifuged briefly, and redissolved in a small volume of water. Three volumes of ethanol were added, and the preparation was stored at -70°C until needed.

Aliquots containing 20 µg of the RNA were fractionated by formaldehyde-agarose gel electrophoresis (Maniatis *et al.*, 1982) and transferred by an alkali blotting procedure to Hybond-N⁺ filters (Amersham) according to the manufacturer's instructions. Filters were prehybridized and hybridized according to standard procedures (Maniatis *et al.*, 1982). The hybridized blots were washed twice for 10 min each in $2 \times \text{SSC}$ (g l^{-1} : 17.5 NaCl, 8.8 $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$, pH 7.2) and 0.1% (w/v) SDS at 50°C and exposed to X-Omat AR X-ray films at -70°C with an intensifying screen.

DNA sequence determination. Double-strand nucleotide sequencing of the two *ilv* genes from *S. platensis* was performed on both strands by the dideoxy-chain termination method (Sanger *et al.*, 1977), after subcloning overlapping restriction fragments, ranging from 200 to 500 bp, in the plasmid pGEM4Z (Promega Corp.). A sequencing kit was used (Pharmacia, LKB); reactions were carried out according to the manufacturer's instructions. SP6 and T7 promoter sequences (Promega Corp.) were used as primers. To overcome secondary structures in G + C-rich stretches, 7-deaza-dGTP was used rather than dGTP in the sequencing reactions. DNA sequences were analysed with

the DNA and Protein Sequence Analysis Programs of D. W. Mount and B. Conrad, University of Arizona. Protein sequence alignments were generated with the CLUSTAL program (Higgins & Sharp, 1989). Possible secondary structures downstream of *ilv* genes were identified using the RSEC program (Lang & Burger, 1986). Free energy of formation for these structures was also calculated using the RSEC program.

Results and Discussion

Sequencing of the *S. platensis* genes encoding AHS

In a previous study, a *S. platensis* genomic library was shown to contain a 4.2 kb *Cla*I fragment and a 3.2 kb *Cla*I/*Sal*I fragment carrying the presumptive genes encoding AHS (Riccardi *et al.*, 1991). The sequences of these two genes and of their deduced polypeptides are shown in Fig. 1(a, b). A total of 3373 and 3170 nucleotides including respectively the entire *ilvX* and *ilvW* genes and their 5' and 3' flanking regions were sequenced. The coding region of *S. platensis ilvX* includes 1836 nucleotides, corresponding to 612 amino acids, while that of the *ilvW* gene has 1737 nucleotides, corresponding to 579 amino acids. The molecular masses predicted by the sequences are about 67 kDa for *ilvX* and 63 kDa for *ilvW*. In *ilvX*, GTG appears to be the initiation codon. The same codon was found in the corresponding gene of *Synechococcus* PCC 7942 (Friedberg & Seijffers, 1990), as well as in other cyanobacterial genes (Reddy *et al.*, 1988; Yasui *et al.*, 1988). GTG is also a translation start codon in other organisms (Kozak, 1983). Each gene is preceded by a polypurine tract which resembles the sequences proposed by Shine & Dalgarno (1974) for a ribosome-binding site (RBS). The *ilvX* gene is preceded by the sequence 5' GAGGAG 3', 6 to 11 nucleotides upstream from the apparent translation start codon. The *ilvW* gene is preceded by the sequence 5' GAGGAA 3', 5 to 10 nucleotides upstream from the apparent translation start signal. The sequence of the 16S rRNA from the cyanobacterium *Anacystis nidulans* (Tomioka & Sugiura, 1983) is very similar to that of the 16S rRNA from *E. coli*, suggesting that analogous RBS sequences are likely to occur in both organisms. Indeed, sequences such as AAGG, GGAG have been found upstream from most of the cyanobacterial genes sequenced so far (Tandeau de Marsac & Houmard, 1987).

AHS in other bacteria studied is composed of two large and two small subunits whose genes are organized in

Fig. 1 (on following five pages). Nucleotide and deduced amino acid sequences of the *ilvX* (a) and *ilvW* (b) genes. Nucleotides are numbered starting from the *Hinc*II site for *ilvX* and from the *Sal*I site for *ilvW* (Riccardi *et al.*, 1991). A Shine-Dalgarno box and potential promoter regions are indicated by underlining. Palindromic sequences in the 3' non-coding region are indicated by arrows below the sequence. The dyad symmetry found in the upstream sequences of *ilvX* and *ilvW* is indicated by paired arrows above the sequence.

(a) Sequence of *ilvX*

- 878 AACC CGG CCA CTG ATC GAC ATAG CGA TCG TCT CAAG GCG TGG ATA AGA AACT CCC ACC ACTAC - 816

- 815 TTCC CC CAG GTG GG GAG AGG CTT CAT GGC CGG GA AATA ATCG CGG AAA AGCG TTT AAA ATT GCT TCTA - 753

- 752 GGT TCT TTT TCT CCG TGT GCG GATA CGA ATA ATTT GCT CAA CTGG AGAG ACA AATAT TTTT ACC GT - 690

- 689 CTCCA ATTTT CAC CTGTAC GAG AGG CCG ATA CGA TTTT TCT TACA ACC ATATCA ACGT GCT CAT - 627

- 626 CTT CGA CCACA ATTTT CAC CTGTG AGT TTTT GTAG GA AACTCA ACTGT ATATT CCGA ACC CCG AT - 564

- 563 AAC GCTC AGTCTG ACC CTTCT GGC GGC GCA AAC CCG CGA AACTT CCG AAA CTGT CATTCCA ACAA - 501

- 500 TGCC AGC ATTG ACC AGG GCA ATCTT GACTT CATCA AGTTT AAA TGG CCGA ATA ATGGG CTTCT - 438

- 437 ATCT TTTT TCA AGG TTTT CACT CCTA ACG CTTACT GTTT GACA ATCCTAC ACTTGGC AGTTGGG - 375

- 374 TTTACT GCCTG CTGATC AGGTCTT CCGAG GCAATCG GTCGA AACTTTG TTTGAG ATCAG CTCACC - 312

- 311 AGCTATCTAAGATACTACCAGTTCGATGCAAATCTCCCCAGGGAGTAGACATTCGACCCCGAT - 249

- 248 TTATTCAGCCATACATAGCCCCAAACCATTGATATCACTATCAAAGCCCCGCAATTGGTAAAT - 186

- 185 CGTGATACAATTGCAGGAAAAGCCTCGACCCCTAACCTATCGGAGCCAAACCGTTAGCTTAAT - 122

- 121 AGTGGTGGCTGAACAATGTAAAATCTTGTTAAACCCCTAACCGTCCAGTCCCCTTAAACAAAA - 59

- 58 TCCTTATCTAAAATAGAGGTTTTAACTCAACAATATAGTTAAATTAGAGGAGCGATCGTGCAA 6
M Q

7 CTTCAA AACTAAA ATTGCTGCCAAGCGTGCAACAGGTGCGTTTCGCCTCATTGATAGTCTTAAA 69
L Q T K I A A K R A T G A F R L I D S L K

70 CGTCATGGCGTACAGCACATTTTTGGCTATCCTGGTGGAGCCATTCTCCCCATTTATGATGAA 132
R H G V Q H I F G Y F G G A I L P I Y D E

133 CTGTATCGAGCCGAAGCCGAGGGCGATATACAGCATATCCTAGTTTCGCCATGAACAGGGGGCA 195
L Y R A E A E G D I Q H I L V R H E Q G A

196 TCCCACGCGGCGGATGGGTATGCCAGAGCTACCGGACGAGTGGGAGTGTGCTTTGGTACATCA 258
S H A A D G Y A R A T G R V G V C F G T S

259 GGACCAGGAGCCACTAACCTAGTGA CTGGCATTGCCACCGCCACATGGACTCTATACCAATG 321
G F G A T N L V T G I A T A H M D S I P M

322 GTAATCATTACCGGACAGGTAGCGCGCCCGCCATTGGTACGGATGCCTTCCAGGATTTCAGAT 384
V I I T G Q V A R P A I G T D A F Q D S D

385 ATTTTCGGCATTACCCTACCGATAGTCAAACATTCTATGTGGTTTCGTGAACCGGGTGACATG 447
I F G I T L P I V K H S Y V V R E F G D M

448 GCTCGCATTGTGCGAGAAGCCTTCCATATTGCCAGCACAGGCCGTCCCGGTCCGGTACTCATA 510
A R I V A E A F H I A S T G R P G P V L I

511 GACGTTCCCAAAGACGTAGGTTTGAAGAATTTGACTATATTCCCGTTAATCCAGGAGAGGTT 573
D V F K D V G L E E F D Y I F V N F G E V

574 TCTCTACCTGGCTATCGTCCCACGGTTAAAGGCAATGTTCCGCCAAATCAACCAAGCCATTAAA 636
S L P G Y R P T V K G N V R Q I N Q A I K

637 CTTATAGAAGAAGCAGAACGTCCCCTGATGTATGTGGTGGTGGGGCGATTTCGGGCCACAGCC 699
L I E E A E R P L M Y V G G G A I S A T A

700	CATGCAGAAATTGCGGAATTGGCAGAGCTTTTTCAGATTCGCGTAACTACCACATTAATGGGG H A E I A E L A E L F Q I P V T T T L M G	762
763	AAAGGATCTTTTGTATGAAAAGCATCCCTATCTGTGGGAATGTTGGGGATGCACGGAACAGCC K G S E D E K H F L S V G M L G M H G T A	825
826	TATGCTAACTTTGCTGTTAGTGAATGTGATTTCCTCATAGCTGTCGGGGCTAGGTTTGACGAT Y A N F A V S E C D F L I A V G A R F D D	888
889	CGCGTTACCGGGAAACTTGATGAATTTGGATCTAGGGCGAAGGTGATTCATATTGACATTGAC R V T G K L D E F G S R A K V I H I D I D	951
952	CCGGCGGAAGTTGGTAAAAACCGGAACCCCGGAAGTGCCAATTGTGGGAGATGTTGCCAGGTG P A E V G K N R T F E V F I V G D V R Q V	1014
1015	TTGATACACCTGTTGCGCCGCTGCCGAGAAATTGGAGATGTCGGGAACGATAATCAAACCTCAA L I H L L R R C R E I G D V G N D N Q T Q	1077
1078	TCGTGGTTAGAGCGGATTAACCGTTGGCCGGAAGATTATCCTCTGGTGGTTCCCTCTTATTCT S W L E R I N R W P E D Y F L V V F S Y S	1140
1141	GATAGCCTCTCTCCCCAAGAGGTGATCGCAGAATTAGGAAAAATGGCACCGGATGGCTACTAT D S L S P Q E V I A E L G K M A F D G Y Y	1203
1204	ACCACGGATGTGGGTCAGCACCAAATGTGGGCGGCTCAATTTTTGAAAAATGGCCCCCGTCAG T T D V G Q H Q M W A A Q F L K N G F R Q	1266
1267	TGGATATCTAGCGCTGGGTTAGGAACTATGGGCTATGGTATCCCGTCGGCTATGGGAGCTAAA W I S S A G L G T M G Y G I F S A M G A K	1329
1330	GTGGCTTTGGAGCGATCGCAGGTGATTTGTATTGCTGGGGATGCCAGTGTGCAAATGAACATC V A L E R S Q V I C I A G D A S V Q M N I	1391
1392	CAAGAACTCGGAACCATAGCACAAATATGGCATAAATGTCAAGACCGTAATTATTAATAACGGT Q E L G T I A Q Y G I N V K T V I I N N G	1455
1456	TGGCAAGGTATGGTCCGCCAGTGGCAACAAGCCTTTTATGGTGAGCGTTATTCCGCTTCTAAC W Q G M V R Q W Q Q A F Y G E R Y S A S N	1518
1519	ATGGAAATCGGAATGCCAGATTTTGAAATGTTAGCACGTTCCCTATGGCGTAAAAGGCATGGTC M E I G M P D F E M L A R S Y G V K G M V	1581
1582	GTTAAGAGTCGTGACGAATTACACCAAGCACTAGCAGAAATGTTGGCTTATGATGGTCCTGTG V K S R D E L H Q A L A E M L A Y D G P V	1644
1645	CTGATGGATGTTTCATGTTACCAAGGATGAAAATTGTTATCCTATGGTAGCACCAGGAAGGAGT L M D V H V T K D E N C Y P M V A F G R S	1707
1708	AATGCTCAGATGATAGGATTACCTGAACGCCGCCAGTTAGAAAAAGCGGTTGAGTTAATCTAT N A Q M I G L F E R R Q L E K A V E L I Y	1770
1771	TGCAGTAATTGTGGCGCGAAAAATGTCGCGAGTAACAATTTCTGTCTGAGTGTGGCACTAAA C S N C G A K N V A S N N F C F E C G D K	1833
1834	CTATAAACACTCCTCTATTACCTGTCTAGACATCAGGAATGATTCCGGTGGTGCTTTGATTAGTC L	1896
1897	GATAAGTAGAGACAGGGTTGGTCTTGCTCTACTGTGTCAATTCTCGATGAGGGGTTATGCTG <u>L</u>	1959
1960	ACACCTAGCAGTCAAATTAATAGGGGAGAAGCGGTAAACCTCCCGTCGGAATAGAAAAATCAGAT	2022
2023	GGTCAGCCAAATCTGGATTTTTTAAGAGTAGCTGATATTGGGTGGGTGCAAACCTTAATCACTT	2085

2086	CGTAATCAAGAGTTTTTAAGGAATTTTTTCGGCCAGGGTAAAAAGGAAGTTTCCGGGTTAGACA	2148
2149	AGTTGAATTGATAAATCTCTGGGGTATCTTGTCTAATTAAAAATTGTATCGTCCAATTCCTCGTA	2211
2212	AATTATGGTGCATGACTGTCTGAATGTTATTGGTGGTAAATTGTACCCCTTTCAAAGGGTCCGT	2274
2275	CACCGGGTTCGCGATTAAATGCGATCGCATATTTTTTCACCTCCCCCAAATAGATACAAACCCCT	2337
2338	GTATTAAACCGGTCCGATAAATATAAGCACCCCGATAATTATCAGGAATAGCAGTAATAATAG	2400
2401	CGGGGAGATTAGGGTGATCTTGTAACATTCCCAAAAAGAATATTTTGAGAAATGGAACCAGCCA	2463
2464	CCCGCCAATTTTGGAACAAAGATTGAACAGTTAAC	2498

(b) Sequence of *ilvW*

- 522	TCGACCTGGCGATGCAACGGATCGCGAACTTTAGAAGTAGCCGAAGGGTAACGGACCCGTCCTC	- 460
- 459	CGATCCTTAGCAACGAGCCTTGCGAAAGCCCTGCAAAATTTCTACCCAGAAATAGCCGAATTT	- 397
- 396	CAACTGCGCGATTATAAAGTGCGGATTTTAGATGGTAAAGCCGGAACCTTCGTCCAAAACCTCGT	- 334
- 333	GTATTGGTAGAGTCTAGTAATGGTCATCAGCGTTGGACTACCGTAGAGTCTCTACTAACATCT	- 271
- 270	TAGAGCCTACCTATCAAGCCGTGCTAGAAGGTTTAGAATATGGCTTAATGTTGCAAACAGCAA	- 208
- 207	ACCCCATCCAATTCAGTTATATTAGCGGAATCCTCACTGTAAAATAGCAGTGATAATCAGCTA	- 145
- 144	GGGATGGCATTATTGGCATAGCATAAAATGGCAATAATGGCAAGAGTATGAATCAGAAATTGT	- 82
- 81	CACAAATATAAAAAATTGATGACAATTGCTCAGGTAATCCCAACCATAATTTATCCTAGGCAT	- 19
- 18	CAGAGATGGAGAATGTTATGCAGGACCAAAAACAAGCCGTAAAAAGAGTAACAGGAGCCTTTG	46
	M Q D Q K Q A V K R V T G A F	
47	CCCTGATTGATAGCCTGCGCCGCCACGGTGTGCAGCATATTTTTGGCTATCCAGGAGGTTCAA	109
	A L I D S L R R H G V Q H I F G Y P G G S	
110	ACTTACCCATTTACGACGAAATATATAGAGCCGAACAAGCCGGGGAAATCAAACATTATCTGG	172
	N L F I Y D E I Y R A E Q A G E I K H Y L	
173	TGCGGCACGAACAAGGAGCGGCCACGCTGCCGATGGATATGCCCGGAGTACCGGAAAGGTGG	235
	V R H E Q G A A H A A D G Y A R S T G K V	
236	GGGTATGTCTAGCCACTTCTGGACCGGGAGCCACCAATTTAGTGACAGGTCTAGCCACTGCTT	298
	G V C L A T S G P G A T N L V T G L A T A	
299	ATCTCGATTCTGTCCCGGTGTTAGCCATTACCGGACAAGTCCCCCGCAGCGCCTTGGGAACAG	361
	Y L D S V P V L A I T G Q V P R S A L G T	
362	ATGCGTTTCAGGAAATTGATATTTTTGGGATTACCCTACCCATTGTTAAACATTCTATCTAG	424
	D A F Q E I D I F G I T L P I V K H S Y L	
425	TCCGCGAACCTTCGGAATTACCGAGAATTGTGGTCGAGGCATTTACCTAGCCATGAGTGGCA	487
	V R E P S E L P R I V V E A F H L A M S G	
488	GACCGGGACCTGTGTTAATTGACATTCCCAAAGATGTGGGTAATGCTCAAATTGATTATATTC	550
	R P G P V L I D I P K D V G N A Q I D Y I	
551	CGGTAGAACCAGGGTCAGTCAGACGGGTGGGATATCGTCCACGGAGCGGGGAAATCCTCGAC	613
	P V E P G S V R R V G Y R P T E R G N F R	

614	AAATTAATCAGGCTTTACAGTTGATTTCCGAAGCTACTAAACCCCTCCTATATGTGGGGGGAG	676
	Q I N Q A L Q L I S E A T K P L L Y V G G	
677	GTGCAATTATGGCGGGGGCTCATGCTGAAATCGCCGAATTGTGCGAACGCTTCCAAATACCAG	739
	G A I M A G A H A E I A E L S E R F Q I P	
740	TTACTAGCACTTTGATGGGAAAAGGTCGTTTTGATGAAAATCACCCCTATCTCTGGGAATCG	802
	V T S T L M G K G R F D E N H P L S L G I	
803	TGGGAATGCTGGGAATGCACGGAACCGCCTATGCTAATTTTGCGGTGATGGAATTGGATTTG	865
	V G M L G M H G T A Y A N F A V M E L D F	
866	TGATCGCGGTGGGGGTTGCTTTGATGACCGGGTGGCGGGTACTGGAGATCAATTCGCCATA	928
	V I A V G V R F D D R V A G T G D Q F A H	
929	GCGCTAAGGTGATTCATATTGATATTGACCGGGCGGAAGTGGGCAAAAATCGCAGTACTGATG	991
	S A K V I H I D I D P A E V G K N R S T D	
992	TTCCTATTGTGGGAGATGTGCGACAGGTTTTGGGGGATATGCTACAACGTACGTATCATTGGG	1052
	V P I V G D V R Q V L G D M L Q R T Y H W	
1055	AACGGAAATTAAGCCGGAACAAACCCAGGAATGGCACTGACCTTAATCAATTGCGTGAACCAA	1117
	E R K L S R N K P R N G T D L N Q L R E F	
1118	TACCCCTGACGGTTCCTCACCCGGAAGATGGTATCTCTCCTCAAGATGGTGATTGGGAACTTA	1180
	I P L T V P H P E D G I S P Q D G D W E L	
1181	GCCATCAATGTCTGATGCTTTCTATACTACTGATGTGGGTGAGCATCAAATGTGGGCGGGCC	1243
	S H Q C P D A F Y T T D V G Q H Q M W A G	
1244	AATTCGTCCAAAATGGCCCCGACGTTGGATGACTAGCGCGGTTTAGGGACTATGGGTTACG	1306
	Q F V Q N G P R R W M T S G G L G T M G Y	
1307	GTTTACCTGCTGCTGTGGGGGTTAAGGTGGCTCATCCTCATGATACGGTTACCTGTATTAGCG	1369
	G L P A A V G V K V A H P H D T V T C I S	
1370	GTGATGGTAGTTTCCAAATGAATATGCAAGAATTGGGAACTATTGCTCAGTATGGTATTGGGG	1432
	G D G S F Q M N M Q E L G T I A Q Y G I G	
1431	TTAAGGTGATTATTCTCAATAATGGCTGGTTGGGAATGGTGCGCCAGTGGCAACATATGTTTT	1495
	V K V I I L N N G W L G M V R Q W Q H M F	
1496	ATAATGACCGCTATGAGGCTACTAACCTGGAAGATGGAACCCAGAAATTTGCCCGGTTAGCTG	1558
	Y N D R Y E A T N L E D G T P E F A R L A	
1559	ATGTTTATGGACTTGAGGCTATGAACGTCCGCCAACGGAAGATTTACCAAAGGCGACTGCCTA	1621
	D V Y G L E A M N V R Q R K I Y Q R R L P	
1622	AAGCTCTATCTCACAAGGGTCCGATGATCCTAGATGTTCCGGTTACCGTGATGAGGACTGTT	1684
	K A L S H K G P M I L D V R V T R D E D C	
1685	ACCCGATGGTAGCCCCCGGTCACGATAACAGCGACATGATGGGTTTATCCAGTTAGTTGCTCT	1747
	Y P M V A P G H D N S D M M G L S S	
1748	AACCCCTCACCCCCCTTAGTGCTTTATGGGGGGGTATTCTGTCTGAGAGCAAATCTTACATT	1810
1811	AACCTGAAAAAACGCCATATTGATTTACAAAAATTCGCAAAAATTAATAATGTTTTGTTTGAT	1873
1874	TAATCAGGTGTAAAATTGGTGACAAAATATCCTAAATTAATGGTAGTTCACTCTCAATCCC	1936
1937	TAACCGGAATGATCTATCCTAATATGAGCCGGAGACTAAGGGAAATCGAGAGCAGCAAAGTCC	1999
2000	CACGGTATCCCGGTTGTGAATTGTTGAAAAATCTGGATTTTCCAAGAGATGATTTCTCGTTC	2062

2063	AAAAAATCACAATTGCCACCGTGTATAATTATTGATTTATCCGGTTTCAGGAGATCGTCAGA	2125
2126	TGACAAATAATGTTGCATCAGAAAACCGTAATGTGCGGATTGTGGCCCCCTATTTGAGCGGCA	2188
2189	AAACAGCGCTGCTAGAAAGTCTGCTGAAAGCGACGGGAACGATTACCCGTAAAGGTACGGCTA	2251
2252	AAGAAGGCAATCGGATCGGGGATAGCGTACTGGAGGCTCGCGATCGCAATATGAGTGTAGAAA	2314
2315	TCAATGCCCGCTTATCATGAATATGGCGGCATTACATTTCACTTCCTCGCATGTCCAGGTTG	2377
2378	GTAGATATTGCCCAAGACACATTACAACGCCCTGTAGGGTGCAGGTTAGCATAAGTAGGTTTG	2440
2441	TGCCAAGCCCAATTACAGCTAATTTTTGGATGATTGGGAAATTCCCATATTGTGTTTCATTAA	2503
2504	TAAATGGATCGGGCTTTCTCTGATGAAGAAGGCAGCTCCCTCAACTTTTTAGAGTTATTA	2566
2567	TGCCCTGAAAACGTATCGAGTCGCCCCATAGTTCCCCAAGAATATCCGATCGCCAAAGGTTTC	2629
2630	AGAATTAATTGGTTTTATCG	2649

operons (Umbarger, 1983). Attempts to find additional open reading frames in the 3' flanking regions of both *ilvX* and *ilvW* were unsuccessful. We hypothesize that the AHS small subunit either is not present in *S. platensis*, similar to the situation in *Arabidopsis thaliana* and *Nicotiana tabacum* (Mazur *et al.*, 1987), *Brassica napus* (Wiersma *et al.*, 1989), yeast (Falco *et al.*, 1985) and *Synechococcus* PCC 7942 (Friedberg & Seijffers, 1990), or is not organized in an operon with *ilvX* or *ilvW*. However, the existence of a small subunit in the *S. platensis* AHS isoenzymes cannot be excluded. We intend to purify the *S. platensis ilvX* and *ilvW* gene products from recombinant *E. coli* and to prepare antibodies against them in order to purify the isoenzymes from *S. platensis* by affinity chromatography. This approach could further elucidate the subunit structure of the cyanobacterial AHS.

Amino acid sequences encoded by *ilvX* and *ilvW*

The deduced amino acid sequences of these genes can be aligned with *Synechococcus* PCC 7942 and *E. coli* AHS proteins as shown in Fig. 2. The six AHS isoenzymes show close amino acid sequence similarity. Previously, a comparison of the *ilvG*, *ilvB* and *ilvI* gene products revealed three regions of similarity (Wek *et al.*, 1985). These three regions of similarity can also be identified in the *ilvX* and *ilvW* gene products. The overall degree of similarity is not equally distributed over the entire lengths of the six polypeptides: several of these blocks extend for 20 to 30 amino acids with few mismatches and other regions show much less similarity. However, the degree of overall identity indicates that these genes encode a family of related polypeptides. In addition to

these regions there is conserved similarity between the carboxyl termini of the *ilvX* and *ilvW* gene products and the *ilvB* and *ilvG* gene products that is not shared with the *ilvI* product.

Comparison of the translated ORFs revealed that the two *S. platensis* AHS isoenzymes are 66% identical. The deduced amino acid sequences of *ilvX* and *ilvW* are respectively 70% and 61% identical with that of *Synechococcus* PCC 7942; 48% and 50% with that of *ilvG*; 42% and 44% with that of *ilvB*; and 46% and 44% with that of *ilvI*.

Haughn *et al.* (1988) have shown that in *A. thaliana*, a single C to T transition within codon 197, resulting in proline being replaced by serine, is responsible for the mutant chlorsulphuron-resistant phenotype. Lee *et al.* (1988) have also shown that in the *N. tabacum* gene encoding AHS, the equivalent proline may be replaced by glutamine or alanine to give resistant forms of the enzyme. As shown in Fig. 2, motifs homologous to those of *Brassica napus*, *A. thaliana* and *N. tabacum* (AITGQVPRRMIGT; Wiersma *et al.*, 1989) are also present in *ilvX* and *ilvW*. It is noteworthy that proline is replaced by alanine in the *ilvX* protein. Since *S. platensis* is resistant to 0.4 mM-chlorsulphuron (data not shown), this may be due to the presence of a resistant form of AHS. This result was confirmed in the *E. coli* mutant complemented with *S. platensis ilvX* (data not shown).

Transcriptional analysis

RNA isolated from *S. platensis*, grown in minimal medium, was subjected to Northern blot analyses using, respectively, the 1.5 kb *EcoRV* fragment from *ilvX* (nucleotides -215 to 1270, Fig. 1a) and the 0.66 kb

A MASSGTTSTRKRETFGAEFIVHFELEQQGIKIVTGIFGGSILPVDALS---QSTQIRHIL
 B MN-----GAQWVVHALRAQGVNTVEGYPGGAIMPVYDALY---DGG-VEHLL
 C MEM-----LSGAEMVVRSLIDQGVKQVFGYFGGAVLDIYDALH---TVGGIDHVL
 D MQLQ-TVTIQQRATGAYALIDSLCQHGVKHFEGYFGGAILPIYDELHRAEAAGGEVGHIL
 E MQLQ-TKIAAKRATGAFLIDSLKRHGVQHIFEGYFGGAILPIYDELYRAEAEGD-IQHIL
 F MQ-D-QKQAVKRVTFGAFLIDSLRRHGVQHIFEGYFGGSNLPYDELYRAEQAGE-IKHIL
 * * * * *

A ARHEQGAGFIAQGMARTDGKPAVCMACSGFGATNLVTAIADARLDSIPLICITGQVFSAM
 B CRHEQGAAMAAIGYARATGKTGVCIATSGFGATNLITGLADALLDSIPVVAITGQVSAFF
 C VRHEQAAVHMADGLARATGEVGVVLTSGFGATNAITGIARTAYMDSIPLVVLSSGQVATSL
 D VRHEQFAVHAADAYSRAATGEVGVCFATSGFGATNLVTGIATAQMDSIPIVVTGQVFRTA
 E VRHEQGASHAADGYARATGEVGVCFATSGFGATNLVTGIATAHMDSIPIVVIITGQVAFPA
 F VRHEQGAHAADGYARSTGKVGVCATSGFGATNLVTGLATAYLDSVPVLAITGQVFRSA
 *** * * * * *

A IGTDAFQEVDYTGISIFITKHNYLVRHIEELPQVMSDAFRIAQSGRFGFVWIDIPKDVQT
 B IGTEAFQEVDVLGLSLACTKHSEFLVQSLLELPRIMAEAFDVACSGRFGFVLVDIPKDIQL
 C IGYDAFQECMDVGISRFVVKHSEFLVKQTED-PQVLKKAFLAASGRFGFVVDLPFKDILM
 D IGTDAFQE-DIYGITLPIVKHSYVVRDPDMARIVAEAFHIAQSGRFGFVLIDVPKDVGT
 E IGTDAFQSDIFGITLPIVKHSYVVRDPDMARIVAEAFHIASTGRFGFVLIDVPKDVGL
 F LGTDAFQEIDIFGITLPIVKHSYLVREPSSELPRIVVEAFHLAMSGRFGFVLIDIPKDVGN
 * * * * *

A AVFEIETQFAMAEEKAAFAESES-----IRDAAAMINAARKRPVLYLGGGVIMAF--R
 B ASGDLE--PWETTVEVEVTEPHAE-----VEQARQMLAKAQKPMLYVGGGYGMAQAVPR
 C FANKLPYV--WFE3VSMESYNPTTTGHKGQIKRALQSVVAVKKEFVVYVGGGAIAGVCHQ
 D E--EFDYVPVAFGDIRLPGYRPTTRGNPRQI-QAIALVKVAREFLLYVGGGAIAGAHAE
 E E--EFDYIPVNPGEVSLPGYRPTVKGNVRQINQAIKLIEEAERPLMYVGGGAIAGAHAE
 F A--QIDYIPVEPGSVRRVGYRPTTRGNPRQINQALQISEATKPLLYVGGGAIMAGAHAE
 * * * *

A VRELAEKAQLPTTMTLMALGMLPKAHFLSLG---MLGMHGVRESTMYILQEADLLIV-LGA
 B LREFLAATKMPETCTLKGLGAVEADYPYLG---MLGMHGTAKAMFAVQECDDLIAAVGA
 C LKETVEALNLPVVCSLMGLGAFFATHR---QVLGMLGMHGTYEAMTMHND-VIFAVGV
 D LSFAAERFQLPVTTLTMGKGADEFHFLALG---MLGMHGTAYANFAVSECDELI-AVGA
 E IAEALAEFLQIPVTTLTMGKGSFDEKHLFSVG---MLGMHGTAYANFAVSECDELI-AVGA
 F IAELSERFQIPVTSTLMGKGFEDENHPLSLGIVGMLGMHGTAYANFAVMELDFVI-AVG
 * * * *

A REDDRAIGKTEQFCFNA-KIIHVIDIDRAELGKIKQPHVAIQADVDDVLAQLIP---LVEA
 B REDDRVGTGLNTEAFPHR-SVIHMDIDPAEMMKLRQAHVALLQGDNLALLP---A---LQQP
 C REVDRRTNNLAKYCPN-ATVLHIDIDPTSISKTVTADIPVGDARQVLEQMLE---LLSQE
 D REDDRVGTGLDQFA-SKAQVIHVIDIDPTSISKTVVPEVPLVGDVRQVNLNELLARA---EEQ
 E REDDRVGTGLDEFGTSRAKVIHVIDIDPAEVGKNRTEPEVPIVGDVRQVLIHLRLRC---RE-
 F REDDRVAGTGDDQFAHS-AKVIHVIDIDPAEVGKNRSTDPVPIVGDVRQVLDGMLQRTYHWR
 ** ** * * * *

A QPRAEWHQLVADLQRE-----EPCFIFKACDPLSHYGLIMAVAACVDDNAIITTDVGQH
 B LNQYDQHQHCAQLRDEHSWR--YDHEGDAIYAPL---LLKQLSDEKFPADCVVTTDVGQH
 C SAHQPLDEIRDWWQQIEQWRARQCLKYDTHSEKIKFQAVIETLWRLTKGDAYDTSVGQH
 D ISADDATRTQPLDRIAYWKREYFLQIPYADVLSPPQVHSLGE-AAPDAYFTTDVGQH
 E IGDVGNDNQSWLERINRWPEYPLVVPSSYSDLSPPQVIAELGK-MAPDGYTTDVGQH
 F KLSRNKPRNGTDLNQL---REPIPLTVPHPEDGISPDGDWELSH-QCPDAFYTTDVGQH
 * * * *

A QMWTAQAYPLNRPRQWLTSGGLGTMGFGLPAAIGAALANFDRKVLCSGSGDGLMMNIQEM
 B QMWAQAHIAHTRFENFISSGLGTMGFGLPAAVGAQVARENDTVVCISGSGSFMNNVQEL
 C QMFAALYYPFDKPRRWIMSGGLGSMGFGLPAAALGVKMAFPEETVVCVTGSGSIQMMNIQEL
 D QMWAQ-FIKNGPRAWISSAGLGTMGFGMFAAMGAQMALEDDTVICVAGDASILMMNIQEL
 E QMWAQ-FLQNGPRQWISSAGLGTMGYGIPSAMGAQVALEFSQVICIAGDASVQMMNIQEL
 F QMWAGQ-FVQNGPRRWMTSGGLGTMGYGLPAAVGVKVAHPHDTVTCTISGSGSFMNNVQEL
 * * * * *

A ATASENQLDVKIILMNNEALGLVHQQSSLEYEQGVFAATYF-GKINFMQIAAGFLETCD
 B GTVKKKQLPLQIVLLDNQRLGMVRQWQQLFFQERYSETTLT-DNFDELMLASAFGIHQGH
 C STALQYELFVLVVMNNRYLGMVKQWQDMYSGRHSQSYM-QSLPDEVREGA-YGHVGIQ
 D GTLAQYNIFIKVNVVNNGWQGMVRQWQEAIFYDEYNSNMMERGMPDEVKLAEAFGIKGM
 E GTIAQYGINVKTVIINNGWQGMVRQWQAFYGERYSASNMEIGMPDEMLASRYGVKGMV
 F GTIAQYIGVVKVILNNGWLGMRVQWQHMFYNDRYEATNLEDGTPEFARLADVYGLEAMN
 * * * * *


```

A  LNNEADPEQASLQEIINRFGEPLIHVRIDAEKVVYPMVFPFGAANTEMVG-----E-----
B  ITRKDQVEAALDTMLNSDGPYLLHVSIDELEMVWFLVFPFGASNSEMLE-----KLS-----
C  ISHPHGWKANLARRWN-----RCALIAVCLMLPMSMAASTS-----
D  VSHFDELQAAIAEMLAFDGFVFLDAIVKRDENCYFMVPSGMSNAQLMGLPKDETLDLDA
E  VKSRDELHQALAEMLAYDGFVLMVDVHTKDENCYFMVAFGRSNAQMIGLFERRQLEKAVE
F  VRQRKIYQRRLPKALSHKGPMILDVREVTREDCYFMVAFGHDNSDMMGLSS-----

A  -----
B  -----
C  -TECRFAGAEMW-----KCG---
D  IATCSSCGAKTLFESHKFCFDCGAKL
E  LIYCSNCGAKNVASNNFCFECGDKL
F  -----

```

Fig. 2. Comparison of some known AHS amino acid sequences. The deduced amino acid sequences of genes encoding AHS are shown for: A, *E. coli* isoenzyme I; B, *E. coli* isoenzyme II; C, *E. coli* isoenzyme III; D, *Synechococcus* PCC 7942 enzyme; E, *Spirulina platensis* isoenzyme I; F, *S. platensis* isoenzyme II. The overlined sequences indicate blocks of similarity associated with sulphonylurea-resistant phenotypes. Identical residues are indicated by asterisks, and conservative amino acid substitutions by dots. Dashes represent gaps inserted to optimize the protein alignment.

Fig. 3. Northern blot analysis of *ilvX* and *ilvW* transcripts in *S. platensis*. Total RNA extracted from *S. platensis* cells grown in minimal medium was fractioned on formaldehyde-agarose gel, transferred to a Hybond-N⁺ filter and hybridized with the 1.5 kb *EcoRV* fragment from *ilvX* (A) and the 0.66 kb *EcoRV/HaeIII* fragment from *ilvW* (B) probes.

EcoRV/HaeIII fragment from *ilvW* (nucleotides 580 to 1240, Fig. 1b) as internal probes (Fig. 3). The preponderant message species was about 2.15 kb for *ilvX* and

1.95 kb for *ilvW*, corresponding roughly to the coding length required for the genes (1836 kb for *ilvX* and 1737 kb *ilvW*, Fig. 1).

The *ilvBN* and *ilvGM* operons are both regulated by transcription attenuation (Hauser & Hatfield, 1983; Lawther & Hatfield, 1980). The presence of a leader region (encoding a polypeptide rich in branched-chain amino acids) was not apparent in the 5' flanking sequences of either *ilvX* or *ilvW*. Analysis of the 5' non-coding region of *ilvX* and *ilvW* disclosed putative promoter sequences homologous to known cyanobacterial promoters (Tandeu de Marsac & Houmard, 1987). Some hypothetical -10 and -35 regions of *ilvX* have resemblances to the *E. coli* consensus promoter sequences (TTGACA, TATAAT). This could be an explanation for the difference between the two *S. platensis* genes in the complementation of the *E. coli* mutant (Riccardi *et al.*, 1991). To overcome this problem we are trying to place the *ilvW* coding region under the control of an *E. coli* promoter. The ability to express this cyanobacterial enzyme in *E. coli* offers the opportunity to take advantage of the fast growth of *E. coli* cells to study a protein whose analysis is difficult in the organism of origin, essentially due to its high lability and to the presence of large amounts of pigments. It is noteworthy that the 5' flanking regions of *ilvX* and *ilvW* contain, near a hypothetical -10 region, an inverted-repeat sequence that could play a role in the expression of these genes (operator?). Downstream from the *S. platensis ilvX* and *ilvW* genes are, respectively, 30 bp and 28 bp sequences (underlined converging arrows in Fig. 1) which can code for an RNA capable of forming a stable stem and loop structure. The free energy of formation of such a conformation from these sequences is approximately -25.8 kcal mol⁻¹ (1 cal = 4.184 J) in *ilvX* and -22.7 kcal mol⁻¹ in *ilvW*. Whether these structures act as a transcription terminator and/or as a barrier against

3' exonuclease degradation remains to be determined. The nucleotide sequences of *ilvX* and *ilvW* provide the starting point for further studies on the regulation of cyanobacterial AHS.

The authors would like to thank Professor M. De Felice for helpful advice.

The present work was supported by MURST 60% and CNR Progetto Finalizzato PB no. 90.01304.14.

References

- BARAK, Z., CALVO, J. M. & SCHLOSS, J. V. (1988). Acetolactate synthase isozyme III from *Escherichia coli*. *Methods in Enzymology* **166**, 455–458.
- CHALEFF, R. S. & MAUVAIS, C. J. (1984). Acetolactate synthase is the site of action of two sulfonylurea herbicides in higher plants. *Science* **224**, 1443–1445.
- DE FELICE, M., LAGO, C. T., SQUIRES, C. M. & CALVO, J. M. (1982). Acetohydroxy acid synthase isoenzymes of *Escherichia coli* K12 and *Salmonella typhimurium*. *Annales de Microbiologie* **133A**, 251–256.
- EOYANG, L. & SILVERMAN, P. M. (1988). Purification and assays of acetolactate synthase I from *Escherichia coli* K12. *Methods in Enzymology* **166**, 435–445.
- FALCO, S. C. & DUMAS, K. D. (1985). Genetic analysis of mutants of *Saccharomyces cerevisiae* resistant to the herbicide sulfometuron methyl. *Genetics* **109**, 21–35.
- FALCO, S. C., DUMAS, K. D. & LIVAK, K. J. (1985). Nucleotide sequence of the yeast *ILV2* gene which encodes acetolactate synthase. *Nucleic Acids Research* **13**, 4011–4027.
- FORLANI, G., RICCARDI, G., DE ROSSI, E. & DE FELICE, M. (1991). Biochemical evidence for multiple forms of acetohydroxy acid synthase in *Spirulina platensis*. *Archives of Microbiology* **155**, 298–302.
- FRIEDBERG, D. & SEIFFERS, J. (1990). Molecular characterization of genes coding for wild-type and sulfonylurea-resistant acetolactate synthase in the cyanobacterium *Synechococcus* PCC7942. *Zeitschrift für Naturforschung* **45c**, 538–543.
- HAUGHN, G. W., SMITH, J., MAZUR, B. & SOMERVILLE, C. (1988). Transformation with a mutant *Arabidopsis* acetolactate synthase gene renders tobacco resistant to sulfonylurea herbicides. *Molecular and General Genetics* **21**, 266–271.
- HAUSER, C. A. & HATFIELD, G. W. (1983). Nucleotide sequence of the *ilvB* multivalent attenuator of *Escherichia coli* K12. *Nucleic Acids Research* **11**, 127–139.
- HIGGINS, D. G. & SHARP, P. M. (1989). Clustal: a package for performing multiple sequence alignments on a microcomputer. *Gene* **73**, 237–244.
- KOZAK, M. (1983). Comparison of initiation of protein synthesis in Prokaryotes, Eucaryotes, and organelles. *Microbiological Reviews* **47**, 1–45.
- LANG, B. F. & BURGER, G. (1986). A collection of programs for nucleic acid and protein analysis, written in FORTRAN 77 for IBM-PC compatible microcomputers. *Nucleic Acids Research* **14**, 455–465.
- LAWTHER, R. P. & HATFIELD, G. W. (1980). Multivalent translation control of transcription termination at the attenuator of the *ilvGEDA* operon of *Escherichia coli* K-12. *Proceedings of the National Academy of Sciences of the United States of America* **77**, 1862–1866.
- LAWTHER, R. P., CALHOUN, D. M., ADAMS, C. W., HAUSER, C. A., GRAY, J. & HATFIELD, G. W. (1981). Molecular basis of valine resistance in *Escherichia coli* K12. *Proceedings of the National Academy of Sciences of the United States of America* **78**, 922–925.
- LEE, K. Y., TOWNSEND, J., TEPPERWAN, J., BLACK, M., CHUI, C. F., MAZUR, B., DUNSMUIR, P. & BEDBROOK, J. (1988). The molecular basis of sulfonylurea herbicide resistance in tobacco. *EMBO Journal* **7**, 1241–1248.
- MANIATIS, T., FRITSCH, E. F. & SAMBROOK, J. (1982). *Molecular Cloning. A Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- MAZUR, B. J., CHUI, C. F. & SMITH, J. K. (1987). Isolation and characterization of plant genes coding for acetolactate synthase, the target enzyme for two classes of herbicides. *Plant Physiology* **85**, 1110–1117.
- RAY, T. B. (1984). Site of action of chlorsulfuron: inhibition of valine and isoleucine biosynthesis in plants. *Plant Physiology* **75**, 827–831.
- REDDY, K. J., BULLERJAHN, G. S., SHERMAN, D. M. & SHERMAN, L. A. (1988). Cloning, nucleotide sequence, and mutagenesis of a gene (*irpA*) involved in iron-deficient growth of the cyanobacterium *Synechococcus* sp. strain PCC7942. *Journal of Bacteriology* **170**, 4466–4476.
- RICCARDI, G., SORA, S. & CIFERRI, O. (1981). Production of amino acids by analog resistant mutants of the cyanobacterium *Spirulina platensis*. *Journal of Bacteriology* **147**, 1002–1007.
- RICCARDI, G., DE ROSSI, E. & MILANO, A. (1989). Amino acid biosynthesis and its regulation in cyanobacteria. *Plant Science* **63**, 135–151.
- RICCARDI, G., MILANO, A., DE ROSSI, E., MALFATTI, C. & DE FELICE, M. (1990). Amino acid biosynthesis in *Spirulina*. In *Biosynthesis of Branched Chain Amino Acids*, pp. 193–197. Edited by Z. Barak, D. M. Chipman & J. V. Schloss. Weinheim: Verlag Chemie and Balaban Publishers.
- RICCARDI, G., DE ROSSI, E., MILANO, A., FORLANI, G. & DE FELICE, M. (1991). Molecular cloning and expression of *Spirulina platensis* acetohydroxy acid synthase genes in *Escherichia coli*. *Archives of Microbiology* **155**, 360–365.
- SANGER, F., NICKLEN, S. & COULSON, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences of the United States of America* **74**, 5463–5467.
- SCHLOSS, J. V. & VAN DYK, D. E. (1988). Acetolactate synthase isozyme from *Salmonella typhimurium*. *Methods in Enzymology* **166**, 445–454.
- SHANER, D. L., ANDERSON, P. C. & STIDHAM, M. A. (1984). Imidazolinones: potent inhibitors of acetohydroxy acid synthase. *Plant Physiology* **76**, 545–546.
- SHINE, J. & DALGARNO, L. (1974). The 3' terminal sequence of *Escherichia coli* 16S ribosomal RNA: complementarity to nonsense triplets and ribosome binding sites. *Proceedings of the National Academy of Sciences of the United States of America* **71**, 1342–1346.
- SQUIRES, C. H., DE FELICE, M., DEVEREUX, J. & CALVO, J. M. (1983). Molecular structure of *ilvIH* and its evolutionary relationship to *ilvG* in *Escherichia coli* K12. *Nucleic Acids Research* **11**, 5299–5313.
- TANDEAU DE MARSAC, N. & HOUMARD, J. (1987). Advances in cyanobacterial molecular genetics. In *The Cyanobacteria*, pp. 251–295. Edited by P. Fay & C. Van Baalen. Amsterdam: Elsevier.
- TOMIOKA, N. & SUGIURA, M. (1983). The complete nucleotide sequence of a 16S ribosomal RNA gene from a blue-green alga, *Anacystis nidulans*. *Molecular and General Genetics* **191**, 46–50.
- UMBARGER, H. (1983). The biosynthesis of isoleucine and valine and its regulation. In *Amino Acids: Biosynthesis and Genetic Regulation*, pp. 245–265. Edited by K. M. Hermann & R. L. Somerville. London: Addison Wesley.
- WEK, R. C., HAUSER, C. A. & HATFIELD, G. W. (1985). The nucleotide sequence of the *ilvBN* operon of *Escherichia coli*: sequence homologies of the acetohydroxy acid synthase isozymes. *Nucleic Acids Research* **13**, 3995–4010.
- WIERSMA, P. A., SCHMIEMANN, M. G., CONDIE, J. A., CROSBY, W. L. & MOLONEY, M. M. (1989). Isolation, expression and phylogenetic inheritance of an acetolactate synthase gene from *Brassica napus*. *Molecular and General Genetics* **219**, 413–420.
- YASUI, A., TAKAO, M., OIKAWA, A., KIENER, A., WALSH, C. T. & EKER, A. P. M. (1988). Cloning and characterization of a photolyase gene from the cyanobacterium *Anacystis nidulans*. *Nucleic Acids Research* **16**, 4447–4463.