4

Genetics of *Spirulina*

AJAY K.VACHHANI AND AVIGAD VONSHAK

Introduction

Despite the general acceptance that classical genetics and modern genetic engineering techniques are powerful and almost essential tools in studying molecular regulation processes, these techniques have not been applied very efficiently to *Spirulina.* The need for new improved strains to be used for particular purposes is just another reason for the need to develop a gene transfer system and other molecular genetic techniques for *Spirulina.* When reviewing the available relevant literature on *Spirulina,* one is surprised to note how little has been done, even in comparison with the work done on other filamentous blue-green algae.

Indeed, genetic studies on cyanobacteria have been restricted to a few species, especially the unicellular ones which are easy to handle and mainly used for isolation of mutants or cloning of genes involved in photosynthesis. Another group includes the filamentous nitrogen fixers, which have been used in order to study gene regulation involved in the metabolic process of nitrogen fixation, or the developmental process by which vegetative cells are differentiated into heterocysts.

Some of the reasons for the scarcity of genetic studies using *Spirulina* as the experimental organism may be the following.

1 It is essential to use axenic (bacteria free) cultures. Only a few species have been isolated and maintained in this condition.

2 It is much easier to perform classical genetics and isolation of mutants with species that form colonies when plated on agar and have a good plating efficiency. Most of the *Spirulina* isolates plate at a relatively low efficiency and exhibit gliding motility on agar plates. This problem has been overcome recently in our lab and in some others, by reducing the salt concentration in the agar plates and by isolating strains that have lost their gliding ability.

3 It seems that most of the *Spirulina* species have a very high endonuclease and exonuclease activity, which makes the introduction of foreign DNA molecules very difficult.

Table 4.1 Amino acid analog resistant mutants of *Spirulina platensis*

Selected agents	Mutagen	No. of mutants isolated	Frequency	Reference
5-fluorotryptophan	NTG	1	1.1×10^{-7}	Riccardi et al., 1981a
β-2-thienylalanine	NTG	38	1.2×10^{-6}	
Ethionine	NTG	80	to	
p-fluorophenylalanine	NTG	27	7.1×10^{-6}	
Azetidine-2-carboxylic acid	NTG	145	per plated filament of 100 cells	
β-2-thienyl-DL-alanine	UV	–	$\geqslant 10^{-4}$	Lanfaloni et al., 1991
β-2-thienyl-DL-alanine	MNNG	–	$\geqslant 10^{-5}$	
8-azaguanine	MNNG	–	$\simeq 10^{-5}$	
β-2-thienyl-DL-alanine	None (spontaneous)	–	3×10^{-7}	
8-azaguanine	None (spontaneous)	–	6×10^{-7}	

4 Endogenous plasmids, which are used in many microorganisms for construction of shuttle vectors, have not been detected in the *Spirulina* species checked.

So far, the major part of the work on *Spirulina* genetics has been focused on:

1 isolation and characterization of mutants (Table 4.1);

2 cloning and analysis of genes involved in fundamental processes such as protein synthesis, carbon dioxide fixation and nitrogen metabolism (Table 4.2);

3 attempts to construct a gene transfer system for *Spirulina.*

Mutagenesis and Isolation of Mutants

Almost all the work dealing with isolation of mutants was performed by Riccardi's group in Italy. Using various amino acid analogs, Riccardi et al. (1981a) reported the isolation of *Spirulina platensis* mutants resistant to 5-fluorotryptophan, ß-2-thienyl-alanine, ethionine, *p*-fluorophenylalanine or azetidine-2-carboxylic acid. Nitroso-guanidine (NTG) was used as the mutagenic agent, and almost 300 resistant mutants were isolated. Some of the mutants appeared to be very frequent, e.g. more than 100 mutants resistant to azetidine-2-carboxylic acid and 80 resistant to ethionine were isolated.

A few of the mutants such as AZ8, PF27 and TA35 appeared to be resistant to more than one analog and to overproduce the corresponding amino acid. These mutants may carry mutations in the mechanisms regulating amino acid biosynthesis.

A second group consisted of mutants that were resistant to one analog only. From this group, one resistant to azetidine-2-carboxylic acid was found to overproduce only proline, while one resistant to fluorotryptophan and one resistant to ethionine did not overproduce any tested amino acid.

Table 4.2 Genes cloned from *Spirulina platensis*

Gene(s) for	Sequenced	Reference
1. Ribulose-1,5-bisphosphate carboxylase (large and small subunits)	No	Tiboni et al., 1984a
2. Glutamine synthetase	No	Riccardi et al., 1985
3. 32 kDa thylakoid membrane protein and α and β subunits of C-phycocyanin	No	De Rossi et al., 1985
4. Translation elongation factor (two genes)	No	Tiboni et al., 1984b
5. Str operon (ribosomal proteins S7 and S12 and translation elongation factors EF-G and EF-Tu)	Yes	Buttarelli et al., 1989
6. β-isopropylmalate dehydrogenase	Yes	Bini et al., 1992
7. Ribosomal protein S2 and part of the gene for elongation factor Ts (EF-Ts)	Yes	Sanangelantoni et al., 1990
8. Acetohydroxy acid synthase (genes for two isoenzymic forms)	Yes	Milano et al., 1992
9. Ribosomal protein S10	Yes	Sanangelantoni and Tiboni, 1993
10. Serine esterase	Yes	Salvi et al., 1994
11. 16S ribosomal RNA; 23S ribosomal RNA; transfer RNA-ile	Yes	Nelissen et. al., 1994
12. delta-12 desaturase (*des* A)	Yes	Deshnium, 1995
13. delta-6 desaturase (*des* D)	Yes	Murata et al., 1996
14. (3R)-hydroxymyristoyl acyl carrier protein dehydrase (*fabZ*) homolog gene	Yes (partial codons)	Los and Murata, 1995
15 ATPase gamma subunit	Yes	Steinemann and Lill, 1995
16 Recombination protein (*rec* A)	Yes	Vachhani and Vonshak, 1995
17. Allophycocyanin genes: *apcA*, *apcB*, *apcC*.	Yes	Anjard, 1996

The mutant overproducing proline is very interesting, because in many studies it has been suggested that proline may be acting as an osmoregulant in salinity-adapted blue-green algae (see Chapter 3). Indeed, when the proline-overproducing strains and control cultures were exposed to 0.3 to 0.9 M NaCl, a marked decrease in growth was observed in the control cultures while the overproducing cells maintained a significant part of the original growth capacity. Studies of this type can be cited to illustrate the potential of simple classical genetic approaches in studying different problems in growth physiology of *Spirulina*.

Riccardi et al. (1981b) further characterized two mutants resistant to ethionine (an analog of methionine) and reported that they had different mode of resistance. One such mutant, ET7, presumably carried a mutation affecting the mechanisms regulating

amino acid biosynthesis. As compared with the wild type strain, ET7 overproduced methionine and other amino acids and did not take up significant quantities of methionine from the medium. In contrast, the mutant ET17 grew at the same rate as the parental strain and did not overproduce methionine but showed a reduction in the amount of amino acids incorporated into the protein.

It was reported by Riccardi et al. (1982) that the mutation responsible for ethionine resistance in strain ET17 most probably involves an altered methionyl-tRNA synthetase, since the methionyl-tRNA present in crude extracts of ET17 showed a reduced affinity for methionine and ethionine.

Spontaneous valine-resistant mutants of *S. platensis* were isolated by Riccardi et al. (1988), and preliminary characterization of three of them indicated that one (strain DR2) was defective in valine uptake and two (strain DR5 and DR9) carried alterations in a valine-mediated mechanism of synthesis of acetohydroxy acid synthase, the first common enzyme of the pathway.

Lanfaloni et al. (1991) standardized the conditions for isolation of 8-azaguanine or *ß*-2-thienyl-DL-alanine resistant mutants of *Spirulina*. Optimal conditions were found to be 1–3 min UV irradiation and 30 min incubation with 50 µg MNNG/ml of trichomes derived from cultures entering stationary phase and sonicated for 10 s and 5 s, respectively. In this respect, from our experience and that of others, it is highly recommended to use fragmented filaments for the mutagenesis step. From other experiments it is also evident that it is important to use slow-growing cultures to obtain a high mutagenesis rate. This is most likely because fast-growing filaments contain more then one complete copy of the genome, thus reducing the chances of isolating the mutants produced, unless enough time is given for segregation to take place.

Cloning and Characterization of *Spirulina* Genes

Glutamine Synthetase

Riccardi et al. (1985) described the isolation of the gene for glutamine synthetase *(glnA)* from *Spirulina platensis*. Their approach was to use a *glnA* gene probe derived from *Anabaena* 7120 and hybridize it to *S. platensis* DNA which had been digested with various restriction endonucleases. The entire *glnA* gene of *S. platensis* was found to be located on an 8 kbp *Hind*III fragment. *Hind*III-cut *S. platensis* genomic DNA and plasmid pAT153 were ligated together and used to transform cells of the *Escherichia coli* strain ET8051, a mutant that carries a deletion of the *glnA* gene and flanking sequences and requires glutamine for growth.

Transformants were selected on a minimal medium and thus the *glnA* gene was isolated by functional complementation of an *E. coli* mutant.

Identity of the cloned *glnA* gene was confirmed by transcription and translation *in vitro* of the purified plasmid DNA. Analysis by SDS-polyacrylamide gel electrophoresis of the [S^{35}] labeled proteins, produced with an *E. coli* cell-free system, demonstrated that a radioactive band of ca. 51 000 daltons, which corresponded to the glutamine synthetase monomer, was evident only when plasmids bearing the appropriate cloned fragments from *Spirulina* or *Anabaena* were utilized.

Acetohydroxy Acid Synthase

Acetohydroxy acid synthase (AHS, EC 4.1.3.18) is the first common enzyme in the biosynthetic pathways leading to the synthesis of valine, isoleucine and leucine and has been demonstrated to be present in *S. platensis* in two isoenzymic forms (Forlani et al. 1991).

An *S. platensis* genomic library was shown to contain a 4.2 kbp *Cla* I fragment and a 3.2 kbp *Cla* I/*Sal* I fragment carrying the presumptive genes encoding the two isoenzymic forms of AHS. The 4.2 kbp *(ilvX)* and the 3.2 kbp *(ilvW)* fragments were subcloned in the plasmid vector pAT153 and it was determined that the *ilvX* gene was able to complement a suitable mutant of *E. coli*, while the *ilvW* gene supported poor growth of the same mutant (Riccardi et al. 1991). Milano et al. (1992) determined the complete nucleotide sequence of *ilvX* and *ilvW* and showed the presence of two 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* PCC7942 AHS enzyme and the large subunits of the *E. coli* AHS I, II and III isoenzymes, revealed a notable degree of similarity. Unlike AHS isoenzymes isolated from *E.coli* and *Salmonella typhimurium* which are tetramers consisting of two large and two small subunits, a small subunit has not been identified for either of the *S. platensis* AHS isoenzymes. Northern blot hybridization analysis demonstrated that the *ilvX* and the *ilvW* genes are transcribed to give mRNA species of approximately 2.15 kbp and 1.95 kbp, respectively.

Genes for the 32 kDa Thylakoid Membrane Protein and Phycocyanin

De Rossi et al. (1985) constructed a cosmid library of *Spirulina platensis* and identified genes involved in photosynthesis (large and small subunits of D-ribulose-1,5-bisphosphate carboxylase, 32 kDa thylakoid protein, α,ß subunits of phycocyanin) and protein synthesis (elongation factors EF-Tu and EF-G).

Southern blotting and hybridization with the *Anabaena* 7120 32 kDa probe gave positive signals on different restriction fragments of two cosmids (pSpR6 and pSpE17), suggesting the presence, in *S. platensis*, of more than one gene for the 32 kDa protein, as has been reported for *Anabaena* 7120.

The entire cluster of genes coding for the α and ß subunits of phycocyanin from *Agmenellum quadruplicatum* along with the gene for the 33 kDa phycobilisome protein was used as a probe, and one cosmid (pSpE31) was isolated by colony hybridization.

Ribulose-1,5-Bisphosphate Carboxylase

Tiboni et al. (1984a) reported the cloning of the genes for the large and small subunits of ribulose-1,5-bisphosphate carboxylase from *S. platensis*. The probe for the large subunit gene was an internal 1 kbp fragment from the *Chlamydomonas reinhardii* gene. The genes for the large and small subunits were found to be very closely located on a 4.6 kbp DNA fragment. Genes for both the subunits appeared to be expressed, albeit to a different extent, in minicells of *E. coli*. The amount of the large subunit produced in the bacterial host represented at least 10 per cent of the total protein.

ß-Isopropylmalate Dehydrogenase

ß-Isopropylmalate dehydrogenase (EC 1.1.1.85) is a key enzyme in the isopropylmalate (IMP) pathway, which is the most common route for leucine biosynthesis in many species. Bini et al. (1992) cloned the gene for *ß*-isopropylmalate dehydrogenase from *S. platensis* by heterologous hybridization using the *Nostoc* UCD7801 *leuB* gene as a probe. The entire *leuB* coding region was sequenced along with 645 bp of the 5' flanking region and 956 bp of the 3' flanking region. An open reading frame of 1065 nucleotides, capable of encoding a polypeptide of 355 amino acids was identified. Comparison of the amino acid sequences published for corresponding proteins from either bacteria or yeast and the amino acid sequence deduced from the nucleotide sequence of the *S. platensis* gene revealed a homology of 45 per cent or more. Northern hybridization analysis revealed that the *S. platensis leuB* gene was transcribed as a single monocistronic RNA, approximately 1200 bases long.

Genes for Ribosomal Proteins and Elongation Factors

Tiboni et al. (1984b) used probes derived from the *tuf A* (elongation factor Tu) gene of *Escherichia coli* to detect homologous sequences on *Spirulina platensis* DNA. They reported the isolation of a 6 kbp fragment of *S. platensis* DNA which appears to contain two sequences homologous to the *E. coli* gene. Thus, *S. platensis* presumably contains two *tuf* genes.

The genes encoding ribosomal proteins S12 and S7, as well as the protein synthesis elongation factors Tu (EF-Tu) and G (EF-G) of *S. platensis*, were identified and cloned by Tiboni and Pasquale (1987). Gene expression for ribosomal protein S12 was determined by genetic complementation. *E. coli* HB101, a streptomycin resistant strain, when transformed with a plasmid bearing the putative S12 gene, showed a streptomycin sensitive phenotype demonstrating that the S12 protein is not only synthesized in the bacterial cell but also integrated in the bacterial ribosomes. Gene expression for the EF-Tu gene was determined by production of the protein in *E. coli* minicells. Buttarelli et al. (1989) reported the nucleotide sequence of a 5.3 kbp DNA fragment carrying the *str* operon (ca. 4.5 kbp) of *S. platensis*. The *str* operon includes the following genes: *rpsL* (ribosomal protein S12), *rpsG* (ribosomal protein S7), *fus* (translation elongation factor EF-G) and *tuf* (translation elongation factor Ef-Tu). Primary structures of the four gene products were derived from the nucleotide sequence of the operon and compared with the available corresponding structures from eubacteria, archaebacteria and chloroplasts. In almost all cases, extensive homology was found and the order was S12>EF-Tu>EF-G>S7. The largest homologies were usually found between the cyanobacterial proteins and the corresponding chloroplast gene products. No codon usage bias was detected in *S. platensis*.

The *E. coli* gene for ribosomal protein S2 was used by Sanangelantoni et al. (1990) as a probe to clone a 6.5 kbp region of the *S. platensis* genome. Sequence analysis showed that the fragment contained the gene for ribosomal protein S2 and a part of the gene for the elongation factor Ts (EF-TS). The arrangement of *rpsB* and *tsf* with a spacer region in between resembles the arrangement of these genes in *E. coli*.

Deduced amino acid sequence of the *rpsB* gene showed higher similarity with the *E. coli* (68.5 per cent) than with the tobacco chloroplast (39.3 per cent) S2 ribosomal

protein. The authors point out that this finding is rather unexpected, since the endosymbiont hypothesis for the origin of plastids proposes that they arose from an ancestral photosynthetic prokaryote related to cyanobacteria. The deduced amino acid sequence of the second incomplete ORF which was located 51 bp downstream of the *rpsB* gene was found to be 50.6 per cent identical to the *E. coli* elongation factor Ts. No sequence similarity was observed in the spacer region present between the two genes in *S. platensis* and *E. coli.*

Sanangelantoni and Tiboni (1993) reported the cloning of the structural gene *(rps10)* encoding ribosomal protein S10 and also determined the location of the *rps10* gene relative to the *tuf* gene in *S. platensis.* Alignment of the predicted S10 sequence of *S. platensis* with the homologous sequences from cyanelles, bacteria, archaebacteria and eukaryotes revealed a high degree of sequence homology (74 per cent amino acid identity) with the cyanellar protein. The *rps10* gene of *S. platensis* is adjacent to the *str* operon genes, unlike the situation in *E. coli* where it is located in a different operon—the S10 operon.

Serine Esterase

The gene for serine esterase from *Spirulina platensis* was cloned, identified and expressed in *E. coli* (Salvi et al. 1994). The approach used was of shotgun cloning. Chromosomal DNA of *Spirulina* was isolated and digested with *Bgl*II. Three- to five-kbp fragments of genomic DNA were cloned into plasmid pPLc2833. *E. coli* HB101 cells carrying pc1875 were transformed and selection was made on the basis of utilization of tributyrin with and without induction of the lambda promoter. The primary structure of the esterase deduced from the DNA sequence displayed a 32 per cent identity with the sequence of carboxyl esterase of *Pseudomonas fluorescens.* The findings reported suggest that the esterase of *S. platensis* is a serine enzyme.

Attempts to Develop Gene Transfer Systems for *Spirulina*

The absence of plasmids in *Spirulina* (there have been only two preliminary reports so far) has been a major obstacle in the development of gene transfer systems. Any attempt made to transfer genes into *Spirulina* would require the following points to be tackled:

1 entry of DNA into *Spirulina*;

2 evasion of restriction digestion by the cyanobacterial nucleases;

3 stable maintenance of the DNA (either by an independent *ori* or by integration into the host genome);

4 expression of the selection marker.

Of the points listed above, the presence of restriction endonucleases may, perhaps, be the most formidable. These enzymes have been found in many cyanobacterial species, and Kawamura et al. (1986) reported the presence of three restriction endonucleases

in *Spirulina platensis* subspecies *siamese.* Recently Tragut et al. (1995) have reported the identification of four restriction enzymes in the soluble protein fraction of *Spirulina platensis* strain pacifica. Specificities of these enzymes are listed in Table 4.3.

As mentioned earlier there have been two reports on the presence of plasmids in *Spirulina.* Qin et al. (1993) have reported isolation of CCC DNA of size 2.40 kbp and 1.78 kbp from *Spirulina* strains S_6 and F_3 respectively. Prof. Hiroyuki Kojima (Government Industrial Research Institute, Osaka, Japan) has reported (personal communication) the presence of megaplasmids in *Spirulina*. His group is presently working to confirm these results.

Experiments describing successful transformation of *Spirulina platensis* by electroporation have been reported by Cheevadhanarak et al. (1993) and Kawata et al. (1993).

Cheevadhanarak et al. (1993) used the plasmid pKK232–8 which bears a promoterless chloramphenicol acetyltransferase (CAT) gene. Shotgun cloning of *Spirulina* genomic DNA upstream of this gene resulted in expression of CAT activity due to the *Spirulina* promoter sequences, and such constructs were selected in *E. coli.* Plasmids with a functional CAT gene were electroporated into *Spirulina* and transformants selected on appropriate selective medium. Transformants were stably maintained under selection pressure for several generations. PCR analysis of transformant DNA with CAT based primer sequences revealed the presence of the CAT gene, and Southern hybridization experiments indicated that pKK232–8 derivatives had integrated into the *Spirulina* genome.

Kawata et al. (1993) reported transformation of *S. platensis,* with heterologous DNA by electroporation. Vector DNA of three forms was used: The *E. coli* plasmid vector pHSG397, its *Eco* RI linearized DNA and linearized pHSG379 flanked with random fragments of host DNA at both ends. Integrative transformation was evaluated by chromosomal integration of the chloramphenicol acetyltransferase (CAT) gene and was confirmed by PCR amplification using appropriate primers. A second marker used was *lacZ,* and the activity of ß-galactosidase was assayed by MUG (4-methylumbelliferyl-*ß*-galactoside) method with HPLC.

In both the reports discussed above, stability of the transformants has been a major problem and hence more work remains to be done in these systems. It is clear that any transformation system developed for *Spirulina* must be an efficient one that will allow not only a good degree of expression of the gene introduced but also be able to maintain itself in a fast-growing culture.

Table 4.3 Specificities of restriction enzymes of Spirulina platensis subspecies siamese and strain pacifica

Strain	Enzyme description		Specificity	Reference
S. platensis subspecies *siamese*	*Spl*I	New enzyme	C/GTACG	Kawamura et al., 1986
	*Spl*II	Isoschizomer of *Tth*111I	GACNNNGTC	
	*Spl*III	Isoschizomer of H*ae*III	GGCC	
S. platensis strain pacifica	*Spa*I	Isoschizomer of *Tth*111I	GACN/NNGTC	Tragut et al., 1995
	*Spa*II	Isoschizomer of *Pvu*I	CGAT/CG	
	*Spa*III	Isoschizomer of *Pvu*II	CAG/CTG	
	*Spa*IV	Isoschizomer of *Hin*dIII	AAGCTT	

Concluding Remarks

The fairly poor level of information on the genetics and molecular biology of *Spirulina* is eventually going to limit the rate at which new information on its cell-biology, physiology and biochemistry can be accumulated. Development of a reliable genetic engineering methodology will be a definite breakthrough in this field. It will open the way, not only for better basic research, but will enable a better selection and screening program for development of new and better strains to be used by the industry. Although it may still take some time before a reliable gene transfer system is developed, studies on *Spirulina* genetics must continue. Even without the existence of a gene transfer methodology, basic work can be done along two main lines.

1 By using classical methodology more mutants can be isolated. Beside the fact that they can be used for biochemical studies, they will be very useful in the future once the transformation system is available.

2 Isolation of genes involved in specific regulation processes or associated with specific requirements of the biotechnology industry may be performed. This can be done by:

   (a) using easily selected markers like genes encoding resistance to inhibitors or genes coding for production of easily detected products;

   (b) using sequence homology of already characterized genes from other prokaryotes;

   (c) using *Spirulina* DNA for complementation of mutants in *E. coli,* or single cell cyanobacteria that have a reliable transformation system.

Today, the molecular genetics of *Spirulina* seems a neglected field of research and any piece of information accumulated at this stage is undoubtedly of high value. It is our hope that this situation will improve, probably as a result of an increased level of funding by the biotechnology industry which is going to benefit from these developments.

References

ANJARD, C. (1996) Direct submission of DNA sequence, *GenBank accession #X95898.*

BINI, F., DE ROSSI, E., BARBIERATO, L. and RICCARDI, G. (1992) Molecular cloning and sequencing of the ß-isopropylmalate dehydrogenase gene from the cyanobacterium *Spirulina platensis, J. Gen. Microbiol.,* **138,** 493.

BUTTARELLI, F.R., CALOGERO, R.A., TIBONI, O., GUALERZI, C.O. and PON, C.L. (1989) Characterization of the *str* operon genes from *Spirulina platensis* and their evolutionary relationship to those of other prokaryotes, *Mol. Gen. Genet.,* **217,** 97.

CHEEVADHANARAK, S., KANOKSLIP, S., CHAISAWADI, S., RACHDAWONG, S. and TANTICHAROEN, M. (1993) Transformation system for *Spirulina platensis.* In MASOJIDEK, J. and SETLIK, I. (Eds) *Book of Abstracts of the 6th International Conference on Applied Algology,* p. 109, Czech Republic, September.

DE ROSSI, E., RICCARDI, G., SANANGELANTONI, A.M. and CIFERRI, O. (1985) Construction of a cosmid library of *Spirulina platensis* as an approach to DNA physical mapping, *FEMS Microbiol. Letters,* **30,** 239.

DESHNIUM P. (1995) Direct submission of gene sequence, *GenBank accession # X86736.*

FORLANI, G., RICCARDI, G., DE ROSSI, E. and DE FELICE, M. (1991) Biochemical evidence for multiple forms of acetohydroxy acid synthase. In *Spirulina platensis, Arch. Microbiol.,* **155,** 298.

KAWAMURA, M., SAKAKIBARA, M., WATANABE, T., KITA, K., HIRAOKA, N., OBAYASHI, A., TAKAGI, M. and YANO, K. (1986) A new restriction endonuclease from *Spirulina platensis, Nucleic Acids Res.,* **14,** 1985.

KAWATA, Y., YANO, S. and KOJIMA, H. (1993) Transformation of *Spirulina platensis* by chromosomal integration. In MASOJTDEK, J. and SETLIK, I. (Eds) *Book of Abstracts of the 6th International Conference on Applied Algology,* p. 72, Czech Republic, September.

LANFALONI, L., TRINEI, M., RUSSO, M. and GUALERZI, C.O. (1991) Mutagenesis of the cyanobacterium *Spirulina platensis* by UV and nitrosoguanidine treatment, *FEMS Microbiol. Lett.,* **83,** 85.

LOS, D.A., and MURATA, N. (1995) Direct submission of DNA sequence, *GenBank accession #U41821.*

MILANO, A., DE ROSSI, E., ZANARIA, E., BARBIERATO, L., CIFERRI, O. and RICCARDI, G. (1992) Molecular characterization of the genes encoding acetohydroxy acid synthase in the cyanobacterium *Spirulina platensis, J. Gen. Microbiol.,* **138,** 1339.

MURATA, N., DESHNIUM, P. and TASAKA, Y. (1996) Biosynthesis of gamma-linolenic acid in the cyanobacterium *Spirulina platensis.* In HUANG, Y. and MILLES, D.E. (Eds) *Gamma-linolenic acid, metabolism and its role in nutrition and medicine,* p. 22, Champaign, Illinois: AOC Press.

NELISSEN, B., WILMOTTE, A., NEEFS, J.M. and DE WACHTER, R. (1994) Phylogenetic relationships among filamentous helical cyanobacteria investigated on the basis of 16S ribosomal RNA gene sequence analysis, *Syst. App. Microbiol.,* **17,** 206.

QIN, S., TONG., S., ZHANG, P. and TSENG, C.K. (1993) Isolation of plasmid from the blue-green alga *Spirulina platensis, Chin. J. Oceanol.Limnol.,* **11,** 285.

RICCARDI, G., SORA, S., and CIFERRI, O. (1981a) Production of amino acids by analog-resistant mutants of the cyanobacterium *Spirulina platensis, J. Bact.,* **147,** 1002.

RICCARDI, G., SANANGELANTONI, A.M., CARBONERA, D., SAVI, A. and CIFERRI, O. (1981b) Characterization of mutants of *Spirulina platensis* resistant to amino acid analogues, *FEMS Microbiol. Lett.,* **12,** 333.

RICCARDI, G., SANANGELANTONI, A.M., SARASINI, A. and CIFERRI, O. (1982) Altered methionyl-tRNA synthetase in a *Spirulina platensis* mutant resistant to ethionine, *J. Bact.,* **151,** 1053.

RICCARDI, G., DE ROSSI, E., VALLE G.D. and CIFERRI O. (1985) Cloning of the glutamine synthetase gene from *Spirulina platensis, Plant Mol. Biol.,* **4,** 133.

RICCARDI, G., DE ROSSI, E., MILANO, A. and DE FELICE, M. (1988) Mutants of *Spirulina platensis* resistant to valine inhibition, *FEMS Microbiol. Lett.,* **49,** 19.

RICCARDI, G., DE ROSSI, E., MILANO, A., FORLANI, G. and DE FELICE, M. (1991) Molecular cloning and expression of *Spirulina platensis* acetohydroxy acid synthase genes in *Escherichia coli, Arch. Microbiol.,* **155,** 360.

SALVI, S., TRINEI, M., LANFALONI, L. and PON, C.L. (1994) Cloning and characterization of the gene encoding an esterase from *Spirulina platensis, Mol. Gen. Genet.,* **243,** 124.

SANANGELANTONI, A.M. and TIBONI, O. (1993) The chromosomal location of genes for elongation factor Tu and ribosomal protein S10 in the cyanobacterium *Spirulina platensis* provides clues to the ancestral organization of *str* and S10 operons in prokaryotes, *J. Gen. Microbiol.,* **139,** 2579.

SANANGELANTONI, A.M., CALOGERO, R.C., BUTTARELLI, F.R., GUALERZI, C.O. and TIBONI, O. (1990) Organization and nucleotide sequence of the genes for ribosomal protein S2 and elongation factor Ts in *Spirulina platensis, FEMS Microbiol. Lett.,* **66,** 141.

STEINEMANN, D. and LILL, H. (1995) Sequence of the gamma-subunit of *Spirulina platensis:* a new principle of thiol modulation of F0F1 ATP synthase? *Biochim. Biophys. Acta.,* **86,** 1230.

TIBONI, O. and DI PASQUALE, G. (1987) Organization of genes for ribosomal proteins S7 and S12, elongation factors EF-Tu and EF-G in the cyanobacterium *Spirulina platensis, Biochim. Biophys. Acta,* **908,** 113.

TIBONI, O., DI PASQUALE, G. and CIFERRI, O. (1984a) Cloning and expression of the genes for ribulose-1,5-bisphosphate carboxylase from *Spirulina platensis, Biochim. Biophys. Acta,* **783,** 258.

TIBONI, O., DI PASQUALE, G. and CIFERRI, O. (1984b) Two *tuf* genes in the cyanobacterium *Spirulina platensis, J. Bact.,* **159,** 407.

TRAGUT, V., XIAO, J., BYLINA, E.J. and BORTHAKUR, D. (1995) Characterization of DNA restriction-modification systems in *Spirulina platensis* strain pacifica. *J. Appl. Phycol.,* **7,** 561.

VACHHANI, A.K. and VONSHAK, A. (1995) Direct submission of DNA sequence, *GenBank accession # U33924.*