



UNIVERSITA' POLITECNICA DELLE MARCHE

DOCTORAL THESIS

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# Sulfur Metabolism in Microalgae

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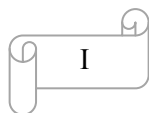
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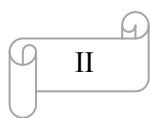
Ancona, 2018



*To my study life.*

*Past present and  
future.....*







# Abstract

Sulfur is a fundamental element for all living organisms. It is acquired as sulfate, which is also the most abundant S form in the ocean, and is assimilated as sulfide, which is fixed in the S-amino acid cysteine. Sulfate assimilation thus requires its reduction to sulfide. For sulfate to be reduced, it needs to be activated to Adenosine PhosphoSulfate (APS). This reaction is catalyzed by ATP-Sulfurylase, which in eukaryotic algae and oceanic cyanobacteria, differently from all other organisms are subject to redox regulation. The other steps of the sulfate assimilation pathways are believed not to differ in algae, as compared to embryophytes. The only other step on which the lack of information leaves crucial open questions is the synthesis of cysteine. Cysteine synthesis is catalyzed by two enzymes, Serine Acetyl Transferase (SAT) and O-Acetyl Serine (Thiol) Lyase (OAS-TL), which in embryophytes form a complex, the Cysteine Synthase Complex (CSC). My thesis will focus on these two steps, with special attention to cysteine synthesis.

With respect to ATP-sulfurylase, I have tried to assess if redox

regulation is mediated by the redox state of the plastoquinon pool of thylakoids. My results suggest that this is not the case. The in vivo blockage of PQ reduction through the use of DCMU, a specific inhibitor of electron transfer from QA to QB, did not affect ATP-S activity.

As for the enzymes of cysteine synthesis, my bioinformatic analysis showed that the phylogeny of SAT and OAS-TL are probably difficult to reconstruct due to the shuffling of these genes across groups with the possible contribution of horizontal gene transfer. By analyzing the protein sequences, I determined that the C-terminal domain of algal SAT, which is believed to be responsible for the interaction with OAS-TL, is very similar to that of embryophytes. This suggests that the interaction of SAT and OAS-TL occurs in algae as in embryophytes. However, the N-terminus of algal SAT, which is believed to be involved in the SAT/SAT interaction, is not equally conserved; therefore, differences in the assemblage of the CSC in algae are possible. In order to clarify this point, I purified OAS-TL from the freshwater cyanobacterium *Synechocystis* sp. PCC 6803, the green marine algae *Tetraselmis suecica* and *Dunaliella tertiolecta*, the green freshwater algae *Chlamydomonas reinhardtii*, the marine diatoms *Thalassiosira pseudonana* and

*Phaeodactylum tricornutum*, and from the marine dinoflagellate *Amphidinium klebsii*. These purified proteins showed some differences in mass, which was however always within the 35-44 kDa range. All the purified proteins were active, although specific activity differed among species. Interestingly, the activity, in most cases, was higher when the enzyme was more diluted; the enzyme is more active, as it also happens in embryophytes. For embryophytes, this has been interpreted as an indication that OAS-TL activity is modulated through protein-protein interaction. To verify the hypothesis that algae have CSC like embryophytes, I studied the ability of algal OAS-TL to form a complex with *Arabidopsis thaliana* SAT (overexpressed in *E. coli*). In all cases, a complex was formed, although the strength of the interaction between SAT and OAS-TL appeared to be different for different algal species. The presence of OAS-TL and SAT in the purified native complexes was confirmed by immunodetection of both proteins. The cysteine synthase complexes that were formed in these experiments were appreciably larger (approximately 600 kDa vs 320 kDa) than those of embryophytes. Further experiments will be required to ascertain the actual stoichiometry and structure of the algal CSC. However, based on the above observation, I

propose that algal CSC is composed by two SAT trimers, with an OAS-TL dimer bound to each SAT monomer. This configuration would give a mass of about 600 kDa, compatible with the results obtained in our CSCS purification experiments. It is also noteworthy that the strength of the binding of algal OAS-TL to AtSAT5 was greater in green algae than in algae of the red lineage. This may be an indication of the fact that a greater proportion of OAS-TL is in the free form, in red-lineage algae, leading to a higher flux of S into cysteines. At this stage, this is only a hypothesis that requires further confirmation.

## **Keywords:**

**Sulfate, Cysteine, O-Acetyl Serine (Thiol) Lyase (OAS-TL),  
Cysteine Synthase Complex (CSC)**



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# 1. Introduction

## 1.1. Impact of sulfur availability on algae ecology, evolution and radiation

Photosynthetic organisms acquire the macronutrient sulfur (S) as sulfate ( $\text{SO}_4^{2-}$ ), with the highest oxidation number of +6. Sulfur, however, is assimilated into organic matter (in cysteine) as sulfide ( $\text{S}^{-2}$ ). Consequently, a conspicuous amount of reducing power is needed to support S assimilation. In embryophytes, this reducing power can derive from linear electron transport, from the pentose phosphate shunt or from the mitochondrial electron transfer chain (Schmidt, 1979). Sulfur assimilation in algae is believed to be more strictly dependent on photosynthesis (Schmidt, 1979).

On average, S is typically below 1 mM (sometimes, much below this value) in freshwater, but it is very abundant in the ocean (about  $29 \text{ mmol L}^{-1}$ ). This makes S never limited in the ocean, although in oligotrophic freshwaters, there are indications that S-limitation may be possible (Giordano, 2005).

The concentration of S in the oceans has not always been so high (Ratti et al., 2011). Sulfate concentration was probably around  $200 \text{ } \mu\text{mol L}^{-1}$  when the first cyanobacteria appeared (Habicht et al., 2002), and raised to less than 5 mM in the Proterozoic Era and to 10-15 mM in the late Palaeozoic Era (Shen et al., 2002; Canfield, 2004; Kah et al., 2004). At the beginning of the Mesozoic, sulfate concentration increased stepwise to a concentration of 13-27 mM (Giordano & Prioretti, 2016.) In concomitance with the Mesozoic increase of oceanic S, phytoplankton composition changed and a domination shift from green algae to the red-lineage algae occurred (Giordano, 2005; Ratti et al., 2011; Knoll et al., 2017). It has been proposed that this shift in dominance was favored, although not fully determined, by the sulfate increase (Sulfate

Facilitation Hypothesis, Ratti et al., 2011; Prioretti et al., 2014).

It is still not clear if the influence of S on phytoplankton radiation was associated with differences in the S metabolism of red-lineage algae compared to green algae or if the increased S availability exerted an indirect effect on the success of some clades (Giordano & Prioretti, 2016); the indirect effect of S may have been associated to the higher S quotas of red lineage algae, which allocate a large portion of S to dimethylsulfoniopropionate (DMSP) (Ratti et al., 2011; Norici et al., 2005). The synthesis of DMSP may have been a response to ocean oxygenation, which led to a decrease of N availability: DMSP, under these circumstances, would have been a cheaper osmolyte than the N-containing glycine betaine (Andreae, 1986; Dacey et al., 1987; Turner & Beidel, 1988; Gröne & Kirst, 1992; Andreae & Meinrat, 1990; Liss et al., 1997; McNeil et al., 1999). DMSP, when a cell is lysed due to predation or other causes, is degraded by bacterial lyases (de Souza & Yoch, 1995a, b; Yoch et al., 1997) and marine algae DMSP lyases (Steinke et al., 1996; Steinke & Kirst, 1996; Stefels, 2000); the products of such degradation are DMS and acrylate, both capable of anti-grazing activity (Steinke et al., 2002a; Steinke et al., 2002b).

Thus, the increased grazing pressures exerted by some micrograzers (especially copepods) at the beginning of the Mesozoic era (see Giordano et al., 2018 for a discussion on this event) may have exerted a selective pressure in favor of those phytoplankton clades more capable of responding to the challenge, among these those groups with a higher S cell quota and thus a higher DMSP content (Prioretti & Giordano, 2016).

## **1.2. Sulfur metabolism**

### **1.2.1. Sulfate acquisition**



Three main types of sulfate transporters have been identified in algae. As described in Fig. 1-1. In the plasmalemma of eukaryotic algae,  $H^+/SO_4^{2-}$  (SULTR) and  $Na^+/SO_4^{2-}$  (SLT) co-transporters have been identified; the expression of both types of transporters is induced by low sulfate availability. The proton gradients across the plasmalemma possibly energize, more or less directly, these transporters. In Cyanobacteria, sulfate transport is carried out by an ATP-binding-cassette (ABC) transporter, with a transmembrane channel constituted by the SulP and SulP2 proteins (SULP stands for sulfate permeases). A sulfate binding protein (Sbp) is linked to the SulP and SulP2 proteins, on the side of the protein emerging on the extracellular face of plasmalemma; ATP-binding proteins (Sabc) are instead present on the cytosolic portion of SulP and SulP2; the Sabc catalyzes the hydrolysis of ATP, which energizes the transport of sulfate (Melis & Chen, 2005). Interesting, a similar type of transporter is present on the inner chloroplast membrane of *Chlamydomonas reinhardtii* (Lindberg & Melis, 2008).

### 1.2.2. Sulfate reduction

In photosynthetic organisms, sulfate reduction occurs in the chloroplast. The only known exception is *Euglena gracilis*, which reduces sulfate in the mitochondrion (Takahashi et al., 2011). Sulfate reduction is a rather complicated process, due to the fact that the redox potential of the sulfate/sulfite pair is too negative ( $E_0' = -0.454$ ) for the cell electron donors to provide the energy for this chemical transformation. For sulfate to be reduced to sulfite, it needs to be activated to adenosine 5'-phosphosulfate (APS.  $E_0' = -0.060$ ) (Schmidt & Jager, 1992). This sulfate adenylation was catalyzed by the enzyme ATP sulfurylase (ATPS; EC 2.7.7.4). Algal ATPS differ from those of vascular plants (Prioretti et al., 2014). Among the most typical features of algal ATPS is the high number of cysteine residues. It has been shown that, in oceanic cyanobacteria and in eukaryotic algae, some of these cysteine residues are involved in

the redox regulation of ATPS (Giordano & Prioretti, 2016; Prioretti et al., 2016). The ATPS of dinoflagellates and freshwater and coastal cyanobacteria, instead, appears not to be subject to redox regulation (Giordano & Prioretti, 2016; Prioretti et al., 2016).

The APS generated in the reaction of ATPS is then reduced to sulfite with the intervention of APS reductase (APR; EC 1.8.4.9). In vascular plants, glutathione is used as the donor of reducing power in this reaction; no direct evidence on whether this is the case in algae exists. The sulfite generated in the reaction catalyzed by APR is further reduced to sulfide by a ferredoxin-dependent sulfite reductase (EC 1.8.7.1); 6 electrons are required for this reduction.

Instead of being used for the production of reduced S, APS can also initiate the sulfation pathway. In this case, APS is probably produced by different isoforms of ATPS from those used in the plastid for sulfate reduction, mostly located in the cytosol; unfortunately, not much is known on sulfation in algae and thus I can only provide limited and anecdotal information on this pathway. Sulfation, in vascular plants, requires that APS is further phosphorylated by APS kinase (APK; EC 2.7.1.25); 3'-phosphoadenosine 5'-phosphosulfate is thus produced PAPS; (Takahashi et al., 2011). PAPS is used by sulfotransferases (EC 2.8.2.24) to donate sulfate groups to all sorts of different molecules (Giordano & Prioretti, 2016). This is mostly done to increase the polar character of molecules and make them more soluble (Dahl et al., 2008).

### **1.2.3. Cysteine synthesis**

Once sulfide is produced, it is assimilated into organic matter by the production of the S-amino acid cysteine. Cysteine synthesis requires two reactions. The first reaction is catalyzed by the enzyme serine acetyltransferase (SAT; EC 2.3.1.30), which produces

O-acetylserine (OAS) from serine and acetyl-CoA (Fig. 1-1,). O-acetylserine, together with sulfide, then acts as the substrate of OAS-(thiol) lyase (OAS-TL; EC 4.2.99.8), which catalyzes the synthesis of cysteine (Leustek et al., 2000; Kopriva, 2006). SAT and OAS-TL are regulated through the assemblage and disassemblage of a complex, the so called Cysteine Synthase Complex (CSC) (Bogdanova & Hell, 1997). The complex does not facilitate the reaction by substrate channeling, because the OAS-TL is not functional when complexed with SAT; the synthesis of cysteine is only accomplished by free OAS-TL (Droux et al., 1998; Wirtz et al., 2001).

In vascular plants, SAT is a hexamer of 29 kDa subunits. It is a member of the hexapeptide acyltransferase enzyme family (Olsen et al., 2004). The functional analysis of SAT domains, using the yeast two-hybrid system, showed that the N-terminal  $\alpha$ -helical domain of SAT is involved in SAT/SAT interaction; the C-terminal domain, instead appears to be responsible for both enzymatic activity and SAT/OAS-TL interaction (Bogdanova & Hell, 1997). The idea of a bifunctional C-terminal SAT domain was further strengthened by the modelling of the C-terminus of plant SAT using bacterial acyltransferase structures as templates (Hell & Wirtz, 2008). The location of SAT is already known in different higher plants: *Arabidopsis* SAT isoform located in mitochondria (Roberts & Wray, 1996; Noji et al., 1998; Wirtz et al., 2001; Krueger et al., 2009), *Arabidopsis* SAT located in the plastid (Murillo et al., 1997; Noji et al., 1998; Krueger et al., 2009), *Arabidopsis* SAT located in the cytosol (Howarth et al., 1997; Noji et al., 1998; Howarth et al., 2003), *Citrullus vulgaris* (watermelon) SAT2 (Saito et al., 1995), spinach SATase *Allium tuberosum* ASAT5 (Urano et al., 2000), *Glycine max* cytosolic isoform SATase1 (Chronis & Krishnan, 2004), through the domain analysed of *O. parapolymorpha* SAT (OpSat1p) and other fungal SATs the new research data revealed a mitochondrial targeting sequence (MTS) at the N-terminus and an  $\alpha/\beta$  hydrolase one domain at the C-terminal region possessed in the fungal (Yeon et al., 2018).

O-acetylserine (thiol) lyase (EC 4.2.99.8) (OAS-TL) is also known as O-acetylserine sulfhydrylase or cysteine synthase (CSase) belongs to the family of  $\beta$ -replacement enzymes (Kopriva et al., 2008). OAS-TL, in embryophytes, is a homodimer with 35

kDa subunits (León et al., 1987). In higher plants and algae, isoforms of OAS-TL are located in the cytosol (Álvarez et al., 2010), mitochondria (Álvarez et al., 2012), and plastids (Heeg et al., 2008).

The structure of the first plant OAS-TL was recently resolved, demonstrating a high degree of conformational similarities between plant and enterobacterial OAS-TL (Bonner et al., 2005); such similarities allow plant and bacteria OAS-TL to form stable homodimers, with a molecular weight from 68 to 75 kDa. Crystallization of OAS-TL from bacteria showed that each OAS-TL subunit carries a tightly bound pyridoxal 5'-phosphate at the catalytic site (Hatzfeld et al., 2000). The conservation of the active site residues strongly supports the idea that the same prokaryotic and eukaryotic OAS-TLs share the same kinetic mechanism.

SAT and OAS-TL of bacteria and embryophytes form CSC of similar size (Becker et al., 1968; Kredich et al., 1969). By using size-exclusion chromatography, a total molecular weight of 309 kDa for the hetero-oligomeric CSC was determined from *Salmonella typhimurium* (Kredich et al., 1969); 160 kDa were attributed to two SAT trimer and 68 kDa were due to a OAS-TL dimer (Kredich & Becker, 1969). In *Arabidopsis thaliana*, the Cysteine Synthase Complex is formed when an excess of sulfide is present and is disassembled when OAS accumulates in the cell (Droux et al., 1998; Hell & Wirtz, 2011).

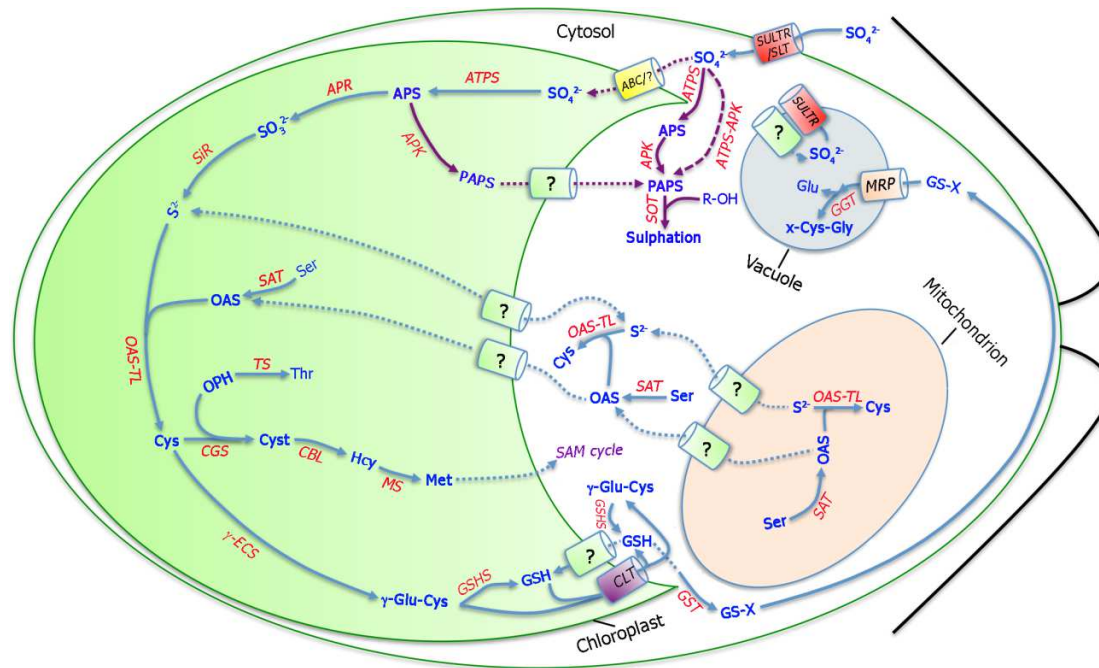


Fig. 1-1. Sulphate metabolism in algal cells.

An overall view of sulphur assimilation and of sulphation is depicted here (Giordano & Prioretti, 2016): the enzymes are indicated in red, metabolites in blue and transporters in black. The light blue lines indicate the reactions of the reductive sulphate assimilation pathway; the dark purple lines indicate reactions of the sulphation pathway; dashed lines indicate putative pathways for metabolite transport that are not been incontrovertibly demonstrated. ABC ATP-binding cassette  $\text{SO}_4^{2-}$  transporter, APK APS kinase, APR APS reductase, APS adenosine 5'-phosphosulphate, ATPS ATP sulphurylase, CBL cystathionine  $\beta$ -lyase, CGS cystathionine  $\gamma$ -synthase, CLT thiol transporter (chloroquine resistance transporter-like transporter, Cys cysteine, Cyst cystathionine,  $\gamma$ -ECS  $\gamma$ -glutamylcysteine synthetase, GGT  $\gamma$ -glutamyltransferase, Glu glutamate,  $\gamma$ -Glu-Cys  $\gamma$ -glutamylcysteine, GSH glutathione, GSHS glutathione synthetase,

GST glutathione-S- transferase, GS-X glutathione conjugate, Hcy homocysteine, Met methionine, MRP multidrug resistance-associated protein, MS methionine synthase, OAS O-acetylserine, OAS-TL OAS (thiol) lyase, OPH O-phosphohomoserine, PAPS 3'-phosphoadenosine 5'-phosphosulphate, R-OH hydroxylated precursor, SAM S-adenosylmethionine, SAT serine acetyltransferase, Ser serine, SiR sulphite reductase, SLT  $\text{Na}^+/\text{SO}_4^{2-}$  transporter, SOT sulphotransferase, SULTR  $\text{H}^+/\text{SO}_4^{2-}$  transporter, Thr threonine, TS threonine synthase, X-Cys-Gly cysteinylglycine conjugate.



## 2. Hypotheses

In this thesis, I focused on the first and on the last step of the S assimilation pathway.

The first step, the one catalyzed by ATPS, still requires to be elucidated with respect to the *in vivo* mechanisms for redox regulation. By similarity with other enzymes (e.g. nitrate reductase; Giordano et al., 2005), I hypothesize that:

**Hp 1: the perception of the availability of redox power for the initiation of sulfate assimilation is constituted by the redox state of the plastoquinone pool of the electron transfer chain in the chloroplast.**

The reduction state of the plastoquinone pool is the indicator of the rate of electron transport and of the level of reduction of ferredoxin, which possibly is the main donor of electrons for sulfate assimilation. It would, therefore, be a good sensor for the overall availability of electrons available for the reduction of sulfate to sulfide. If such a regulation mechanisms exists, this would represent a major difference with respect to embryophytes: in embryophytes, the main control step of sulfate reduction is at APR reduction (Takahashi et al. 2011); if ATPS activity is finely regulated *in vivo* via the redox state of the plastoquinone pool, this would move the first control step of the pathway already at sulfate activation. This is intriguing because it would suggest that a greater control on energy investment exists on S assimilation in algae, which would not commit to the use of ATP for sulfate activation, unless sufficient redox power for the completion of the pathway is available.

The last step of S assimilation is that of cysteine synthesis. In the absence of any



data on SAT and OAS-TL from algae, it is not possible to generalize the regulatory mechanism proposed for embryophytes to all photosynthetic organisms. I thus intended to verify whether a CSC is involved in cysteine synthesis in algae and test the following hypothesis:

**HP 2: Cysteine synthesis of algae works through the constitution of a CSC.**



## **3. Materials and methods**

### **3.1. Bioinformatic methods**

#### **3.1.1. Protein sequences and phylogenetic trees**

All protein sequences were obtained from the NCBI Protein Database (<http://www.ncbi.nlm.nih.gov/protein/>), both by looking for the SAT and OAS-TL sequences of specific organisms and by using BLASTP (protein-Basic Local Alignment Search Tool) (Altschul et al., 1990; Goujon et al., 2010; Sievers et al., 2011). The phylogenetic analysis was conducted using the MEGA 5.0 software (Tamura et al., 2011). The Maximum Likelihood method was used for the construction of phylogenetic trees (Saitou & Nei, 1987; Sanderson & Driskell, 2003). The bootstrap values (Fron et al., 1996) shown on the tree branches represented the confidence level of clades, i.e. the number of reiterations (out of 100) of data analysis that gives the same branch (Karlín & Altschul, 1990). The evolutionary distance between branches (corresponding to the branch length) was computed using the Poisson correction method (Zuckermandl & Pauling, 1965) and expressed in terms of the number of amino acid substitutions per site. All positions containing gaps and missing data were eliminated.

#### **3.1.2. Predictions on the location of SAT and OAS-TL proteins**

Prediction of sub-cellular localization of proteins was performed with the Target P 1.1

online software (<http://www.cbs.dtu.dk/services/TargetP/>), as described in Emanuelsson and coauthors, 2007. Also, the on line software identified the sequences signal peptides and prediction of their cleavage sites (Nielsen et al., 1997).

The <http://mobyli.pasteur.fr/cgi-bin/portal.py#jobs::boxshade> online software was used to acquire the bioinformatic about the proteins and then to identify protein homogeneity predicting evolutionary relationship among different species (Koonin & Galperin, 2003).

### **3.1.3. Analysis of SAT and OAS-TL algae sequences**

Fig. 4-4 and Fig. 4-5 showed the degree of SAT and OAS-TL conserved area in different species, respectively. Multiple sequence alignment was done with the T-Coffee on line software (<http://tcoffee.crg.cat/apps/tcoffee/do:regular>) (Notredame et al., 2000; Felix et al., 2005; Moretti et al., 2011; Weimbs & Santa, 2012). Representation of the alignment was done using the Boxshade program ([http://www.ch.embnet.org/software/BOX\\_form.html](http://www.ch.embnet.org/software/BOX_form.html)). In the Boxshade figures, for clarity, only some species were shown for each algal clade.

Pairwise Sequence Alignment through the on line software ([https://www.ebi.ac.uk/Tools/psa/emboss\\_matcher/](https://www.ebi.ac.uk/Tools/psa/emboss_matcher/)) EMBOSS Matcher identifies local similarities between two sequences using a rigorous algorithm based on the LALIGN application (Koonin, & Galperin, 2003; Kleckner et al., 2016).

## 3.2 Experimental organisms

### 3.2.1. *Synechocystis* sp. PCC 6803

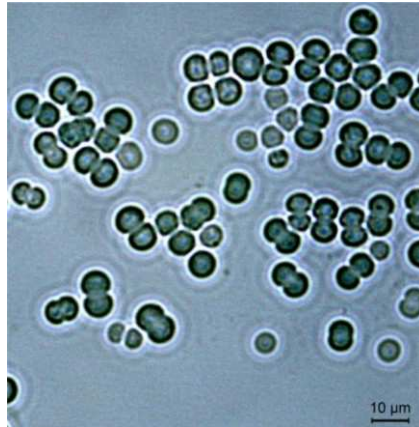


Fig. 3-1. *Synechocystis* sp. PCC 6803.

The cyanobacterium *Synechocystis* (phylum Cyanobacteria, order *Chroococcales*) (Fig. 3-1) (Selim et al., 2018) is a freshwater organism with cells of about 2 μm in diameter, and coccoid in shape. The cells blue-green color is mainly due to phycocyanin, abundant in the antenna complex of photosystem II (PSII). In this thesis I used strain PCC 6803, which is the first photoautotrophic organism whose genome was fully sequenced (Nakamura et al., 1998); the size of *Synechocystis* PCC 6803 genome is 3.57 Mbp. The ease with which this organism can be cultivated and genetically manipulated (Kufryk et al., 2002) made it a model for the study of physiology and genetics of photosynthetic organisms.

### 3.2.2. *Chlamydomonas reinhardtii*

The *Chlamydomonas reinhardtii* (phylum Chlorophyta, class Chlorophyceae, order *Volvocales*) (Fig. 3-2) is a ubiquitous freshwater green algae, with ovoid, biflagellate cells of about 10 μm in diameter (Harris et al., 2009). Its mitochondrial (15.8 Kbps;

Trans & Land, 1988; Rochaix, 1995), plastidial (203.4 Kbps; Maul et al., 2002) and nuclear (121 Mbps; Merchant et al., 2010) genomes have been sequenced and a large collection of mutants is available.

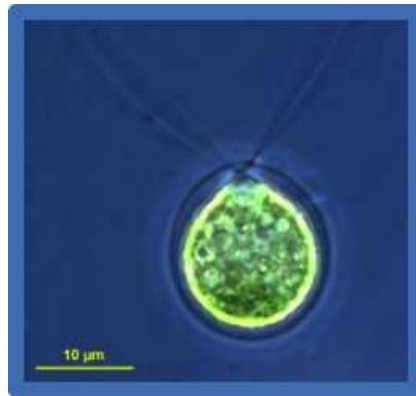


Fig. 3-2. *Chlamydomonas reinhardtii*.

### 3.2.3. *Dunaliella tertiolecta*

Unicellular green algae *Dunaliella* belong to the Chlorophytes (Oren-shamir et al., 1990; Oren, 2005). The algae were first described (Dunal, 1838), but it was not until 1905 that the name *Dunaliella* was given by Teodoresco (1905). *Dunaliella tertiolecta* (phylum Chlorophyta, class Chlorophyceae, order *Volvocales*) (Fig. 3-3) (Mesquita et al., 2013) is a marine green flagellate with a cell size of 10–12 μm. This genus is characterized by the absence of a rigid polysaccharide wall (Gibbs & Duffus, 1976), instead, cells are covered by the amorphous mucilaginous layer of variable thickness called a glycocalyx. *Dunaliella* cells are motile with two equally long flagella. In 2017 (Yao et al., 2017), RNA-Seq technology data were *de novo* assembled and annotated, 17,845 protein-coding transcripts resulted in *Dunaliella tertiolecta* (~95% completeness).

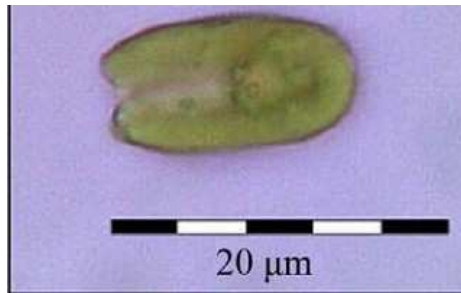


Fig. 3-3. *Dunaliella tertiolecta*.

### 3.2.4. *Tetraselmis suecica*

*Tetraselmis suecica* Strain 1878 (phylum Chlorophyta, class Chlorodendrophyceae, order *Chlorodendrales*) (Fig. 3-4) (Mesquita et al., 2013) is a green marine flagellate alga. It is often classified in the informal group of *Chlorella pyrenoidosa* prasinophytes, some members of which belong to the basal Chlorophyta. A transcriptome was produced by M. Giordano and C. Delwiche (unpublished).



Fig. 3-4. *Tetraselmis suecica*.

(Photo by David Patterson and Bob Andersen via Encyclopedia of Life).

### 3.2.5. *Thalassiosira pseudonana*

*Thalassiosira pseudonana* Cleve 1873 (phylum Heterokonta, class

Coscinodiscusphyceae, order *Thalassiosirales*) (Fig. 3-5). *Thalassiosira pseudonana* is a small centric diatom with an average cell diameter of 2-15  $\mu\text{m}$ . This diatom is found both as a single cell or chain colony of up to 6 cells. It is especially abundant in coastal waters. Its genome has been sequenced (Armbrust et al., 2004).

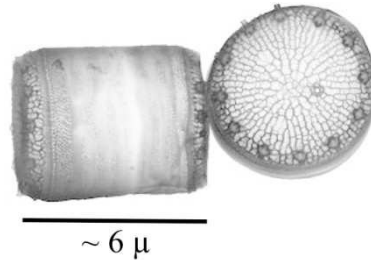


Fig. 3-5. *Thalassiosira pseudonana*.

(SEM Photo by N. Kröger, Alfred Wegener Institute, Germany).

### 3.2.6. *Phaeodactylum tricorutum*

*Phaeodactylum tricorutum* Bohlin 1897 (phylum Heterokonta, class Bacillariophyceae, order Naviculales) (Fig. 3-6) (Miyahara et al., 2014) is a pennate marine diatom, with little silicified cells (Lewin et al., 1958), and four morphotypes: oval, fusiform, triradiate and cruciform, the latter being rarely reported (He et al., 2014). *P. tricorutum* genome has been sequenced (Daboussi et al., 2014).



Fig. 3-6. *Phaeodactylum tricorutum*.



### 3.2.7. *Amphidinium klebsii*

The dinoflagellate group encompasses a variety of morphologically very dissimilar organisms. It includes marine and freshwater species; autotrophic, mixotrophic, and heterotrophic modes of nutrition; pelagic and benthic forms. *Amphidinium klebsii* (phylum Miozoa, Class Dinoflagellata, order *Gymnodiniaceae*) (Fig. 3-7) is a common dinoflagellate in temperate and tropical marine waters. The cell diameter is around 10-15  $\mu\text{m}$ . A transcriptome was produced by M. Giordano and C. Delwiche (unpublished).



Fig. 3-7. *Amphidinium klebsii*.

(photo by Jacob Larsen).

## 3.3. Cultures

### 3.3.1. Culture conditions

Prior to any experiment/measurement, algae were allowed to acclimate to their

culture conditions for at least 4 generations.

### **3.3.1.1. *Synechocystis* sp. PCC 6803**

*Synechocystis* sp. PCC 6803 was cultured in batch, in 250 mL Erlenmeyer flasks containing 150 mL of algal suspension. Only for protein purification, for all species, larger (4 L) vessels were used. The growth temperature was  $20 \pm 0.5$  °C. Cultures were illuminated continuously with PAR (340-700 nm) light at an irradiance of  $50 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . The irradiance was measured with a quantummeter (LI-250 Light Meter; LI-COR, Inc.). Cultures were bubbled with filter-sterilized air. As for all species, experiments were conducted on exponentially growing cells.

### **3.3.1.2. Eukaryotic algae**

All eukaryotic species were cultured in the same conditions used for *Synechocystis*, except for the irradiance, which was  $120 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  (PAR).

## **3.3.2. Growth media.**

### **3.3.2.1. BG-11 medium**

For *Synechocystis* PCC 6803, BG-11 medium (Stanier et al., 1971) was used. BG-11 medium contained:  $\text{NaNO}_3$  ( $17.65 \text{ mmol L}^{-1}$ ),  $\text{K}_2\text{HPO}_4$  ( $0.23 \text{ mmol L}^{-1}$ ),  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  ( $0.304 \text{ mmol L}^{-1}$ ),  $\text{CaCl}_2$  ( $0.245 \text{ mmol L}^{-1}$ ), citric acid ( $0.031 \text{ mmol L}^{-1}$ ), Ammonium Ferric Citrate ( $6 \text{ mg L}^{-1}$ ),  $\text{EDTANa}_2 \cdot 2\text{H}_2\text{O}$  ( $0.0027 \text{ mmol L}^{-1}$ ),  $\text{Na}_2\text{CO}_3$

(0.0189 mmol L<sup>-1</sup>), and trace metals. The trace metal solution had the following composition: H<sub>3</sub>BO<sub>3</sub> (0.0463 mmol L<sup>-1</sup>), MnCl<sub>2</sub>•4H<sub>2</sub>O (0.0091 mmol L<sup>-1</sup>), ZnSO<sub>4</sub>•7H<sub>2</sub>O (0.00077 mmol L<sup>-1</sup>), Na<sub>2</sub>MoO<sub>4</sub>•2H<sub>2</sub>O (0.0016 mmol L<sup>-1</sup>), CuSO<sub>4</sub>•5H<sub>2</sub>O (0.00032 mmol L<sup>-1</sup>), Co(Cl)<sub>2</sub>•6H<sub>2</sub>O (0.000172 mmol L<sup>-1</sup>), Tris-HCl pH 7.6 (10 mmol L<sup>-1</sup>); 0.5 ml of this trace metal solution was used in 1 L of BG-11.

### 3.3.2.2. TAP and TP medium

*Chlamydomonas reinhardtii* was cultured in either Tris-Acetate-Phosphate (TAP) medium or Tris-Phosphate (TP) minimal medium (Gorman & Levine, 1965). TAP medium contained: Trizma base (17.67 mmol L<sup>-1</sup>), NH<sub>4</sub>Cl (7.48 mmol L<sup>-1</sup>), CaCl<sub>2</sub> (0.45 mmol L<sup>-1</sup>), MgSO<sub>4</sub>•7H<sub>2</sub>O (0.41 mmol L<sup>-1</sup>), Na<sub>2</sub>EDTA (0.13 mmol L<sup>-1</sup>), FeSO<sub>4</sub>•7H<sub>2</sub>O (0.018 mmol L<sup>-1</sup>), ZnSO<sub>4</sub>•7H<sub>2</sub>O (0.077 mmol L<sup>-1</sup>), H<sub>3</sub>BO<sub>3</sub> (0.18 mmol L<sup>-1</sup>), MnCl<sub>2</sub>•4H<sub>2</sub>O (0.026 mmol L<sup>-1</sup>), MnCl<sub>2</sub>•4H<sub>2</sub>O (8.04 mmol L<sup>-1</sup>), CuSO<sub>4</sub>•5H<sub>2</sub>O (0.0064 mmol L<sup>-1</sup>), Na<sub>2</sub>MoO<sub>4</sub>•2H<sub>2</sub>O (0.0032 mmol L<sup>-1</sup>), CoCl•6H<sub>2</sub>O (0.012 mmol L<sup>-1</sup>), potassium phosphate buffer (1 mmol L<sup>-1</sup>), pH 7.2; 0.1% (v/v) glacial acetic acid was added to the medium to adjust the pH to 7.2, after the medium was autoclaved. TP medium had the same composition as TAP, with the exception of the acetic acid, which was omitted; the pH of TP medium was adjusted with HCl, after the medium was autoclaved, to obtain a final pH of 7.2.

### 3.3.2.3. Artificial Multipurpose Complement for the Nutrition of Algae (AMCONA)

All other species were cultured in AMCONA medium (Fanesi et al., 2014). The composition of this medium was as follows: NaCl (363 mmol L<sup>-1</sup>), Na<sub>2</sub>SO<sub>4</sub> (8.04

mmol L<sup>-1</sup>), KCl (8.04 mmol L<sup>-1</sup>), NaHCO<sub>3</sub> (2.07 mmol L<sup>-1</sup>), KBr (725 μmol L<sup>-1</sup>), H<sub>3</sub>BO<sub>3</sub> (372 μmol L<sup>-1</sup>), NaF (65.7 μmol L<sup>-1</sup>), MgCl<sub>2</sub>•6H<sub>2</sub>O (41.2 mmol L<sup>-1</sup>), CaCl<sub>2</sub>•2H<sub>2</sub>O (9.14 mmol L<sup>-1</sup>), SrCl<sub>2</sub>•6H<sub>2</sub>O (82 μmol L<sup>-1</sup>), NaNO<sub>3</sub> (549 μmol L<sup>-1</sup>), NaH<sub>2</sub>PO<sub>4</sub>•H<sub>2</sub>O (21 μmol L<sup>-1</sup>), NaSiO<sub>3</sub>•9H<sub>2</sub>O (205 μmol L<sup>-1</sup>), CuSO<sub>4</sub>•5H<sub>2</sub>O (40 nmol L<sup>-1</sup>), Tris-HCl, Ph 8.0 (10 mmol L<sup>-1</sup>), FeCl<sub>3</sub>•6H<sub>2</sub>O (6.56 mmol L<sup>-1</sup>), Na<sub>2</sub>EDTA•2H<sub>2</sub>O (6.56 mmol L<sup>-1</sup>), ZnSO<sub>4</sub>•7H<sub>2</sub>O (254 μmol L<sup>-1</sup>), CoSO<sub>4</sub>•7H<sub>2</sub>O (5.69 μmol L<sup>-1</sup>), MnSO<sub>4</sub>•4H<sub>2</sub>O (6.1 μmol L<sup>-1</sup>), Na<sub>2</sub>MoO<sub>4</sub>•2H<sub>2</sub>O (6.1 μmol L<sup>-1</sup>), Na<sub>2</sub>SeO<sub>3</sub> (1 μmol L<sup>-1</sup>), NiCl<sub>2</sub>•6H<sub>2</sub>O (6.3 μmol L<sup>-1</sup>), Na<sub>2</sub>EDTA•2H<sub>2</sub>O (8.29 mmol L<sup>-1</sup>), Thiamine-HCl (297 μmol L<sup>-1</sup>), Biotin (4.09 μmol L<sup>-1</sup>), B12 (1.47 μmol L<sup>-1</sup>).

### 3.3.3. Determination of cell concentration

Since the cyanobacteria *Synechocystis* sp. PCC 6803 cell size is too small to be easily counted with an optical microscope and also counts with an automatic cell counter are not fully reliable, because cells are undistinguishable from debris and occasional bacteria, cell concentration of *Synechocystis* was routinely measured as optical density at 750 nm with a Beckman DU 640 Spectrophotometer (Beckman Coulter) (Zerulla & Ludt, 2016). At 750 nm, photosynthetic pigments do not significantly absorb light (Rabinowitch & Govindjee, 1969; Govindjee, 2004). In order to convert optical density to cell number, a calibration curve was made with samples containing serially diluted subsamples of a concentrated culture of *Synechocystis* sp. PCC 6803 of known cell concentration. The cell concentration of the reference *Synechocystis* sp PCC 6803 culture was determined by counts under a microscope (Zeiss Axioskop, Germany) applying the following equation:

$$\text{Cell concentration (cell ml}^{-1}\text{)} = [N \times S1 / S2] / V1$$

Where S1 is the slide area (mm<sup>2</sup>); V1 is the sample volume (ml); S2 is 5 times the field area at a 400x magnification (mm<sup>2</sup>); N is the total cell number counted in 5 field areas.

Freshly made, 0.2 µm filtered cell free BG-11 medium was used as the blank.

Slide area (mm<sup>2</sup>) was 24×40, the field area was 400 mm<sup>2</sup>.

To determine cell concentration of all the others experimental algae, cells were counted with an automatic cell counter CASY TT (Roche Innovatis, Mannheim, Germany). An aliquot of 100 µl of culture was diluted in 10 ml of CASYton, an electrolyte solution specifically developed by the counter manufacturer. The sample was sucked into the counter through a measuring capillary (60 µm pore size), at a constant flow rate. Then a pulsed low voltage (1 MHz) was applied between two platinum electrodes; the electrolyte provided the background electrical resistance. When a cell intersected the electric field in the capillary, the cell acted as an insulator and increased resistance between the electrodes. The number of cells was determined from the number of events of current disturbance. The duration of this change in conductivity is proportional to the size of cells (corresponding to the volume of electrolytes displaced by cells). This allowed the determination of a cell volume. Since dead cells are partially empty, they exert a lower resistance to the current and can therefore be discriminated from live cells. Cell concentration was determined twice or thrice per day and used to construct growth curved.

### 3.3.4. Growth rate determination

The specific growth rate,  $\mu$ , was calculated from the equation below (Maqsood, 1974):

$$\mu = \frac{\ln X_1 - \ln X_2}{t(\text{day})}$$

Where  $X_1$  is the cell number at time 1 and  $X_2$  is cell number at time 2. Both time points were chosen to be in the exponential growth phase;  $t$  is the time expressed in days.

### **3.3.5. Biomass harvesting**

For all measurements/experiments, algae acclimated to the growth conditions were collected by centrifugation using a refrigerated centrifuge (Beckman, California, USA) equipped with a JA-10 rotor (Beckman, California, USA). The algal suspension was centrifuged at 5000 g, for 10 min, at 4 °C. The pellet was collected and frozen -80 °C until used.

### **3.4. Overexpression of AtSAT5**

In order to purify OAS-TL, *Arabidopsis thaliana* SAT5 (AtSAT5 or SAT-5) was used as the ligand in the affinity column. This protein was modified by a histidine tag, in view of its utilization as a ligand in affinity chromatography. It was then overexpressed in *E. coli*, after transfection of the bacterium with the plasmid pET28a, into which the AtSAT5 gene had been inserted (Fig. 3-8). The plasmid containing the His-Tagged AtSAT5 was kindly donated by Prof. Ruediger Hell, Heidelberg University.

The amount of plasmid was determined by reading the DNA concentration in MilliQ water using a NANODROP 1000 spectrophotometer V3.8 (ThermoFisher, Massachusetts, USA). The concentration used for the transformation was 20 ng/μl.

#### **3.4.1. Primer design**

In order to verify that the gene of interest was in the plasmid and then in the transformed cells, primers specific for AtSAT5 (Gene ID: 835778, SAT 5: AT5G56760) (Tabata et al., 2000) were designed with the Prim Primer, 5.0 software. Their sequences were shown in Table. 3-1. the gene regions that these primers amplified were located at the beginning, middle and end of the AtSAT5 DNA sequence.

Table. 3-1. Primer for AtSAT5 amplification

Gene Name	Length	Primer sequence
AtSAT5-1	187 bp	FW: AAACAGCGGTGATTGGGAAC; RV: ATCAGCACAACAGAACCAGC
AtSAT5-2	307 bp	FW: CAGAAGCAGCGTCAGC; RV: TCACGAACACGAGCAG
AtSAT5-3	285 bp	FW: TCCGATGAAGCAGAAG; RV: GGTGGCGTTACGAAGAG
AtSAT5-4	322 bp	FW: ACAATCACGGAAGCCATTAGCAT; RV: GTCAATCAGCACAACAGAACCAG
Vector		FW: (T7) TAATACGACTCACTATAG; RV: (T7 terminator) GCTAGTTATTGCTCAGCGG

FW = forward primer, RV = reverse primer. T7 = T7 RNA Polymerase.

### 3.4.2. AtSAT5 Colony transformation assessment in *E. coli*

The pET28a plasmid (Wirtz, 2004) (Fig. 3-8) was used for the insertion of the AtSAT5 gene into *E. coli*. This plasmid was donated by Prof. Ruediger Hell, Heidelberg University.

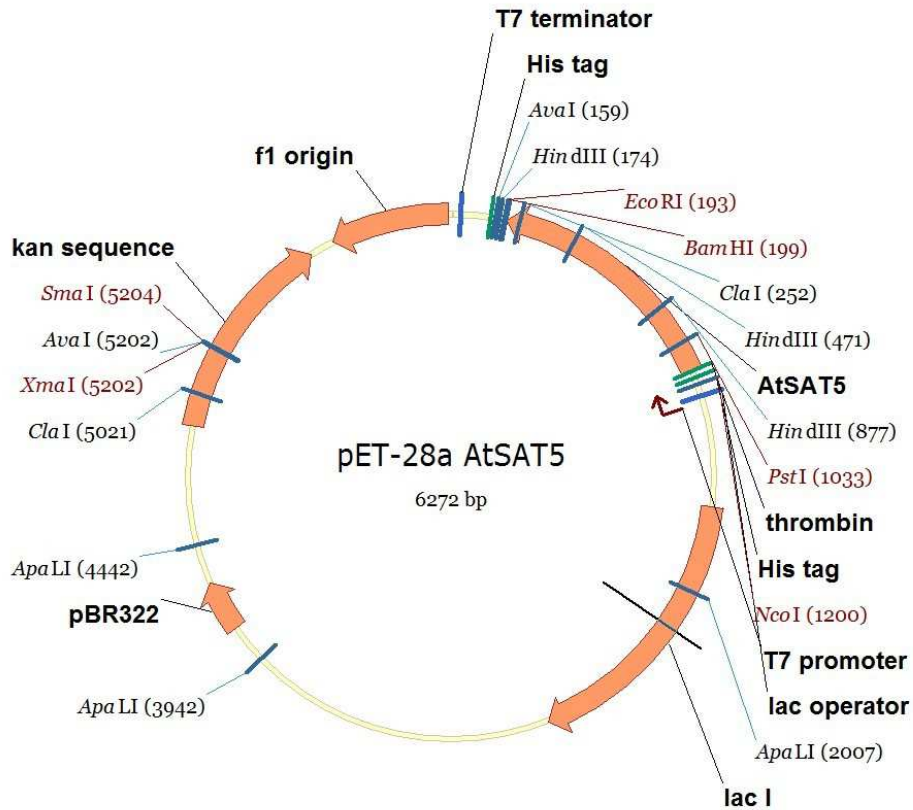
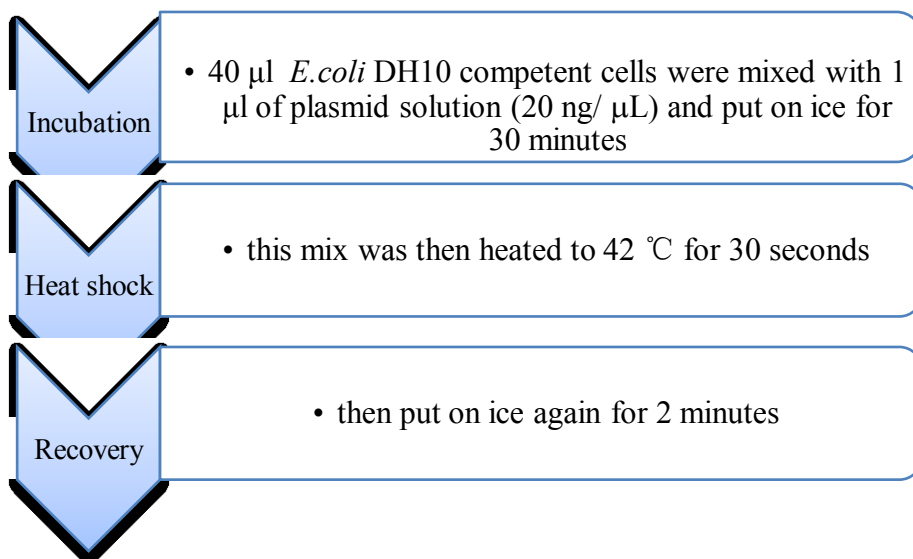


Fig. 3-8. AtSAT5-pET28<sup>a</sup> construct map.

Cloning was conducted using *E.coli* DH10 competent cells, according to the protocol shown below:



Subsequently, 950  $\mu\text{l}$  of Super Optimal Broth with Catabolite (SOC) medium



(Sambrook & Russell, 2001) were added to the *E. coli*/plasmid mix and incubated at 37°C, for 1 hour, in an incubator (SSI5 SHEL LAB Floor Model Shaking Incubator, 5 Cu.Ft.), under continuous shaking at 250 rpm. The SOC medium included the following components: 2% Tryptone, 0.5% Yeast extract, 10 mM NaCl, 2.5 mM KCl, 20 mM MgSO<sub>4</sub>, 10 mM MgCl, 20 mM glucose.

After incubation, 50 µl of transformed *E. coli* were loaded onto a Lysogeny Broth (LB) agar plate, which contained the antibiotic kanamycin. Additional 250 µl of *E. coli* culture were loaded on another LB agar plate with kanamycin. The LB plates contained 1% Tryptone, 0.5% Yeast extract, 1% NaCl, 1.5% agar. The plates were incubated overnight at 37 °C. The following day, if colonies appeared, they were transferred with a sterilized toothpick into a 2 ml sterile Eppendorf tube containing 1ml of 2×TY (1.6% Tryptone, 1% Yeast extract, 0.5% NaCl) medium with Kanamycin. The Eppendorf tube was whirly mixed for 10 minutes; then the toothpick was removed. The Eppendorf tube was put in an incubator (SSI5 SHEL LAB Floor Model Shaking Incubator, 5 Cu.Ft.) at 37 °C, under shaking at 700 rpm, for 3 hours. The *E.coli* DH10 transformed culture was then resuspended 1:1 in a 40% Glycerol solution. This “cloning stock” was stored at -80 °C.

### 3.4.3. Colony PCR

Polymerase Chain Reaction (PCR) was carried out using the Invitrogen™ Platinum™ SuperFi™ PCR Master Mix Kit (Invitrogen, Massachusetts, USA). This kit was designed to work at room temperature. It was a hot-start kit and it was suited for cloning.

The reaction mix had this composition:

3 µl of nuclease-free water

12.5 µl of 2× Platinum™ SuperFi™ PCR Master Mix

1.25 µl of 10 µM forward primer

1.25 µl of 10 µM reverse primer

2 µl of Template DNA

5 µl of 5× SuperFi™ GC Enhancer

The following PCR protocol was applied:

<b>Initial denaturation</b>	98 °C	
<b>Denaturation</b>	98 °C	} ×35 cycles
<b>Annealing</b>	55 °C	
<b>Extension</b>	72 °C	
<b>Final extension</b>	72 °C	
<b>Store</b>	4 °C	

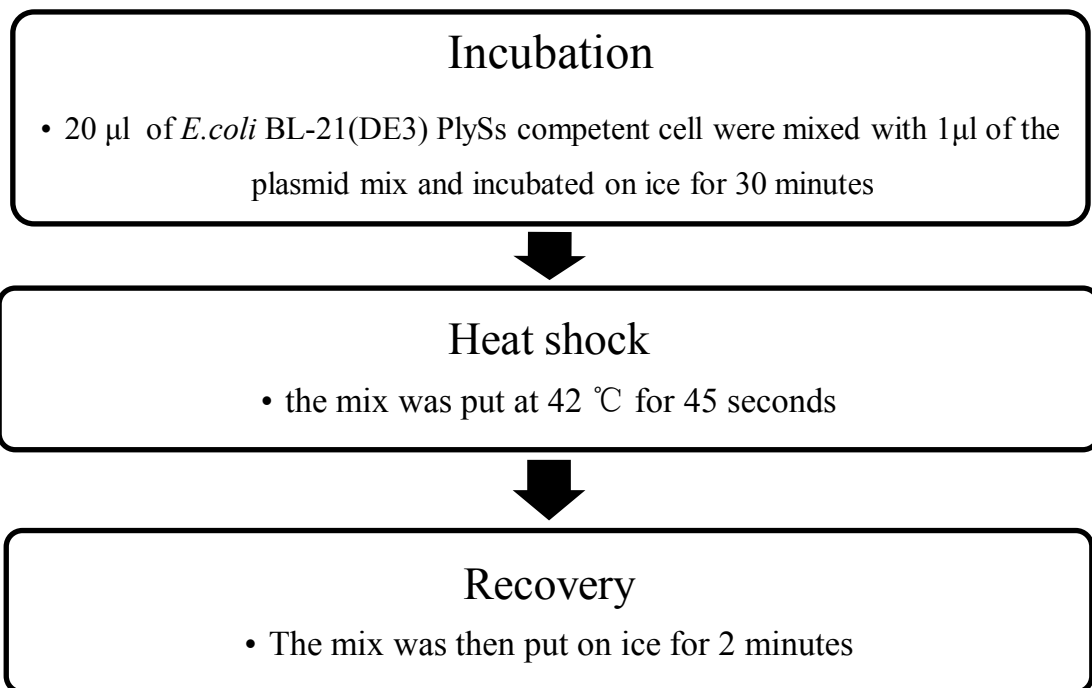
Agarose gels (2%) were used to assess the size of the PCR products. The DNA Ladder with a range 200-300 bp was produced. The gels were run at 100 V for 30

minutes. Then a picture was taken with a Bio-Rad Gel System (Biorad, California, USA).

### 3.4.4. AtSAT5 overexpression

As expression vector, *E.coli* BL-21(DE3) plysS was used. These cells were kindly provided by Dr. Tiziana Cacciamani, MaSByc, DISVA, Università Politecnica delle Marche. BL-21(DE3) pLysS is a chemically competent *E. coli* strain. The BL-21(DE3) pLysS strain allowed inducible protein expression from the gene of interest, under the control of the lac UV5 promoter, via the T7 RNA polymerase. The pLysS plasmid also contained the T7 lysozyme gene (lysozyme had the function of repressing low-level transcription prior to induction), a chloramphenicol resistance gene and a p15A replication origin which was compatible with those found in pBR322 and pUC derived plasmids.

This competent strain could be made permeable to exogenous DNA through the following procedure:



Subsequently, 90  $\mu$ l of SOC medium was added to the mix. This suspension was incubated for 1 hour at 37  $^{\circ}$ C, under continuous shaking at 600 rpm. Then, 600  $\mu$ l of 2 $\times$ TY medium containing kanamycin and chloramphenicol, were added. A further incubation at 37  $^{\circ}$ C and 400 rpm was conducted overnight. The next morning, 40  $\mu$ l of the culture incubated overnight was added to an Eppendorf tube containing 2 $\times$ TY medium and kanamycin and chloramphenicol. These cultures were allowed to grow to an optical density (600 nm) of 1. At that point, 100  $\mu$ l of 100 mM isopropyl-1-thio- $\beta$ -D-galactopyranoside (IPTG) was added to induce the expression of AtSAT5 protein. The incubation at 37  $^{\circ}$ C and 400 rpm was continued for 4 hours. The suspension was then centrifuged at 4500 g, 4  $^{\circ}$ C, for 10 min; the pellet, containing the *E. coli* cells overexpressing AtSAT5, was collected and stored at -80  $^{\circ}$ C.

The production of the AtSAT5 containing *E. coli* was scaled up by New Brunswick™ Innova® 44/44R Shaker, setting the shaking speed at 250 RPM and a temperature of 37 $^{\circ}$ C. When the OD<sub>600</sub> reached 0.6-0.8, 250  $\mu$ l of 1 M IPTG were added into the culture (250 ml) for inducing the protein SAT5 expression. The incubation at 37  $^{\circ}$ C and 400 rpm was continued for 4 hours. The suspension was then centrifuged at 4500 g, 4  $^{\circ}$ C, for 10 min; the pellet, containing the *E. coli* cells overexpressing AtSAT5, was collected and stored at -80  $^{\circ}$ C, these pellets were used for the followed purification experiment.

### **3.4.5. Extraction of AtSAT5 protein from *E. coli***

The *E. coli* cells were resuspended in 10 ml of binding buffer (buffer B: 20 mM Imidazole, 50 mM Tris (pH 8.0) and 250 mM NaCl), then used for the affinity (binding) chromatography. To protect the proteins, buffer B was added containing 10

$\mu\text{l}$  of 500 mM phenylmethylsulfonyl fluoride (PMSF) protease inhibitor (VWR, USA). The PMSF stock solution was prepared in ethanol, filtered through 0.2  $\mu\text{m}$  filter (sterilized, USA) and kept at  $-20\text{ }^{\circ}\text{C}$  till used. Cells in buffer B were sonicated on ice using a SONICS Vibra-Cell™ (SONICS & MATERIALS INC, Newton, CT, USA), with an energy of 60% amplitude for 5 minutes, with 5 seconds bursts and 10 seconds intervals. The slurry was spun down at 12000 g, for 10 min, at  $4\text{ }^{\circ}\text{C}$ , in a J2-MC Beckman centrifuge equipped with a JA-20 rotor (Beckman, California, USA). The supernatant was filtrated through a 0.45  $\mu\text{m}$  membrane filter (VWR, Pennsylvania, USA), and then again through a 0.2  $\mu\text{m}$  filter (VWR, Pennsylvania, USA) (Fig. 3-9). Then, 10  $\mu\text{l}$  of 500 mM PMSF was added to the filtered crude extract and the samples were stored at  $-20\text{ }^{\circ}\text{C}$ , until they were used.

### **3.4.6. OAS-TL purification**

The purification of OAS-TL was carried out by affinity chromatography, using the protein overexpressed in *E. coli* as the ligand. A 1 ml HiTrap™ Chelating Column (GE Healthcare, Buckinghamshire, UK) was used (Fig. 3-9). Prior to its utilization, the column was washed with MilliQ water for 5 min, at a flow rate of 1 ml/min (which was maintained for all the following steps). The column was then loaded with 50 mM  $\text{NiCl}_2$ , for 5 min. Subsequently, the column was equilibrated with the binding buffer (buffer B: 20 mM Imidazole, 50 mM Tris (pH 8.0), and 250 mM NaCl), for 10 min.

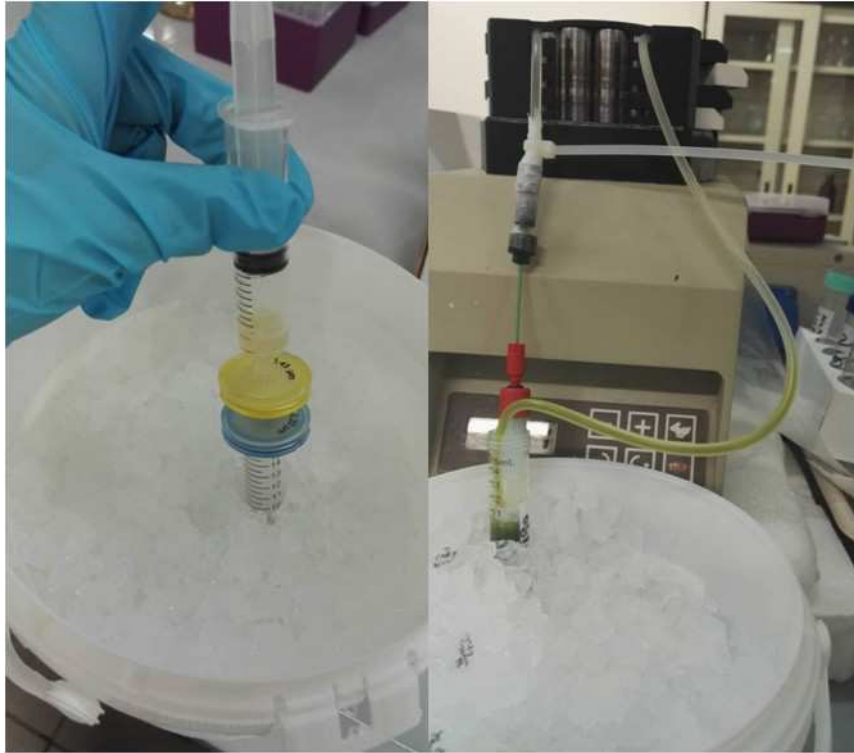


Fig. 3-9. Protein loading onto the affinity chromatography column.

The His-tagged AtSAT5 was then loaded onto the column. The loading lasted 1.5 hour. The column was conditioned with washing buffer (Washing buffer: 80 mM Imidazol, 50 mM Tris (pH 8.0), and 250 mM NaCl). Subsequently, the column was washed for 5 minutes with the same buffer (Washing buffer) used for conditioning. Finally, a volume of 5 ml washing buffer containing 10 mM O-Acetylserine was added; this step was applied to remove any residual bacterial OAS-TL. The column was finally washed for 5 minutes with the same washing buffer and the algal extract was loaded.

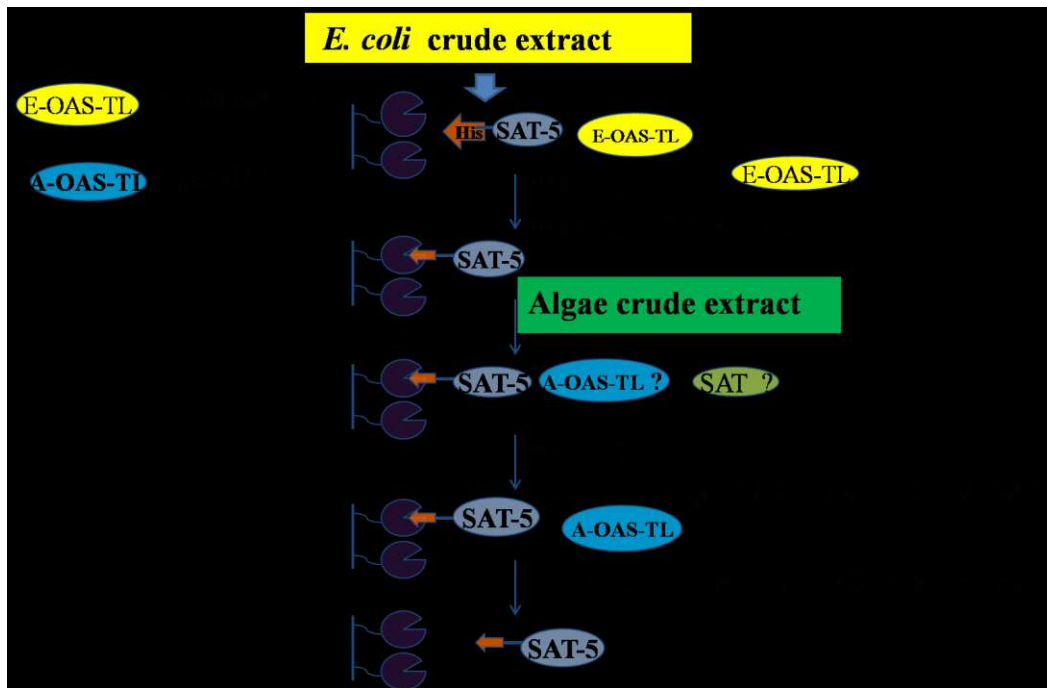


Fig. 3-10. Procedure followed for affinity chromatography.

### 3.4.7. Algal OAS-TL extraction

Algae in mid-exponential growth phase were used for the extraction of OAS-TL. Cells were harvested by centrifugation in a J2-MC Beckman centrifuge with a JA-10 rotor (Beckman, California, USA), at 5000 g, 4 °C, for 10 minutes. The pellet was stored at -80 °C until it could be processed further.

When needed, the pellets were resuspended in 10 ml of buffer B additioned with 50 mM PMSF, 0.001 mM MgCl<sub>2</sub>, 0.1% Triton-100 (v/v), and 1% Glycerol (v/v) and subject to sonication, centrifugation, and filtration as described above for the extraction of AtSAT5 from *E. coli*.

About 10 ml of crude extract were then loaded onto the column, with a flow rate of 1ml/min. The extract was allowed to circulate through the columns for 1.5 hours (Fig. 3-10). The column was then washed for 10 minutes with 10 mL buffer W, which contained 80 mM Imidazole, 50 mM Tris (pH 8.0), and 250 mM NaCl; this was done to wash out unbound proteins. The column was then washed for 5 min with 5 ml of

Buffer W added with 10 mM OAS; this step was necessary to displace the protein OAS-TL bound to the affinity ligand. The eluted solution was collected in 10 fractions of 500  $\mu$ l each. A further 5 min wash with Buffer W followed. Finally, the His-tagged AtSAT5 protein was eluted with 5 ml Buffer E, which contained 400 mM Imidazole, 50 mM Tris (pH 8.0), and 250 mM NaCl.

### **3.4.8. Determination of protein content**

Proteins were determined according to the method by Peterson (1977), a modification of Lowry's method (Lowry et al., 1951). The denaturing power of SDS in combination with NaOH allowed the complete disruption of membranes. To 480  $\mu$ l of a solution containing 1% SDS and 0.1 M NaOH, a volume of 20  $\mu$ l of sample was added, followed by 500  $\mu$ l of reagent A (Table. 3-2); the solution was let sit for 10 min. Reagent A was a mixture of copper and Na-K tartrate: the former specifically binds to proteins in an alkaline environment; the tartrate was used to stabilize the Cu ions (Lowry et al., 1951). Subsequently, 250  $\mu$ l of solution B (Table. 3-2) were added. Solution B contained the Folin & Ciocalteu's phenol reagent (Sigma-Aldrich) and carried out the oxidation of the Cu-protein complexes; this gave, almost instantaneously, a blue color to the protein solution (Lowry et al., 1951). The samples were incubated for about 30 minutes at room temperature to allow a complete development of the blue color. Finally, the absorbance was measured at 750 nm in a Beckman DU 640 Spectrophotometer (Beckman Coulter, California, USA).

Protein concentration was calculated by interpolating the samples' absorbances into a calibration curve constructed with known quantities of bovine serum albumin (BSA). The BSA standards were prepared as the samples. Standard curves were made for all the buffers employed in the various purification steps, to take into account possible interferences by all buffer components.



Table. 3-2. Reagents for Peterson's protein assay

Reagents	Stock solution
Solubilizing solution	1% SDS, 0.1 M NaOH a: CTC (CopperTartrate/Carbonate) 0.1% CuSO <sub>4</sub> 5 H <sub>2</sub> O 0.2% NaKTartrate 4 H <sub>2</sub> O
Reagent A (v/v) (a: b: c: d = 1: 1: 1: 1)	10% Na <sub>2</sub> CO <sub>3</sub> b: 10% SDS c: 0.8 M NaOH d: MilliQH <sub>2</sub> O
Reagent B (v/v) (d: e = 5: 1)	e: Folin-Ciocalteu reagent (1 Folin-Ciocalteu :5 MilliQ H <sub>2</sub> O)

### 3.4.9. SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

OAS-TL purity was assessed through SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE). Proteins were denatured by mixing the samples with 4 volumes of 5X Laemmli Sample Buffer containing 10% (w/v) SDS, 20% (v/v) Glycerol, 100 mM Tris pH 7, 0.1% (w/v) Bromophenol blue, 25% (v/v) β-Mercaptoethanol (Laemmli, 1970). The mixture was quickly whirly mixed for 1 min, then incubated for 10 minutes at 95.5 °C, transferred on ice for 2 minutes and finally centrifuged at 12,000 g at room temperature. Samples were then loaded on a 12% polyacrylamide gel. Resolving gel and stacking gel buffers were prepared as described in Table. 3-3 and 3-4. First, an appropriate volume (Table. 3-5) of resolving gel was loaded into the electrophoresis sandwich; a thin layer of isopropanol was deposited on its surface to ensure that the surface was flat and horizontal. After the gel polymerized, isopropanol was removed with the syringe. The stacking gel was then added (Table. 3-6). After polymerization, the gel sandwich was placed in an electrophoresis chamber (Bio-Rad Laboratories Inc., California, USA). The running buffer contained 25 mM Tris-HCl pH 8.3, 192 mM glycine and 0.1% (W/V) SDS. The electrophoresis was performed at 80 V for 30 min (or until samples ran through the stacking gel) and at 120 V for

additional 120 min or until bromophenol blue ran out of the gel. Protein pre-stained molecular weight markers (10-180 kDa, Abcam, Cambridge, UK) were used to identify the mass of the proteins in the samples.

Protein bands were visualized by Coomassie Staining (Table. 3-7). The Coomassie Staining was stopped when the first protein bands became visible, by washing the gel three times with tap water. The Coomassie destaining solution was then added to the stained gel (Table. 3-8).

Table. 3-3. Recipe for the SDS-PAGE resolving gel buffer

1 x Resolving Gel Buffer	Final concentration	For 100 ml
Tris pH 8.8	1.5 M	18.18 g
SDS	0.4% (w/v)	0.4 g

Table. 3-4. Recipe for the SDS-PAGE Stacking gel buffer

1 x Stacking Gel Buffer	Final concentration	Add to 100 ml
Tris pH 6.8	0.5 M	6.06 g
SDS	0.4 % (w/v)	0.4 g
Add few grains of bromphenol blue		

Table. 3-5. Resolving gel (for 2 gels in the Protean minigel system)

Resolving Gel	H <sub>2</sub> O	Resolving Gel Buffer	Acrylamide solution, 30% (w/v)	10% APS	TEMED
12%	4.73 ml	3.3 ml	5.37 ml	90 µl	16 µl

Table. 3-6. Stacking gel (for 2 gels in the Protean minigel system)

Stacking Gel	H <sub>2</sub> O	Stacking gel buffer	Acrylamide solution, 30% (w/v)	10% APS	TEMED
4%	2.61 ml	0.72 ml	1.13 ml	45 µl	9 µl

Table. 3-7. Coomassie Staining solution

	Final concentration	Add to 1 L
Methanol	50% (v/v)	500 ml
Acetic acid	1% (v/v)	10 ml
Coomassie Brilliant Bleu R-250 (more sensitive)	0.1% (w/v)	0.1 g

Table. 3-8. Coomassie Destaining solution

Coomassie Destaining Solution recipe	Final concentration	Add to 1 L
Ethanol	20% (v/v)	200 ml
Acetic acid	10% (v/v)	100 ml

### 3.4.10. Western Blots

The transfer of protein from the gel to a 0.45  $\mu\text{m}$  PVDF membrane (Amersham, GE Healthcare, Buckinghamshire, UK) for immune detection was carried out in a wet system (Bio-RAD, California, USA). The transfer sandwich was assembled as shown in Fig. 3-11. The PVDF membrane was activated by soaking in methanol before the blotting sandwich was assembled. Subsequently, the blotting sandwich was put in the transfer chamber (Biorad, California, USA). The transfer was conducted at 350 mA for 1 hour, at 4  $^{\circ}\text{C}$ .

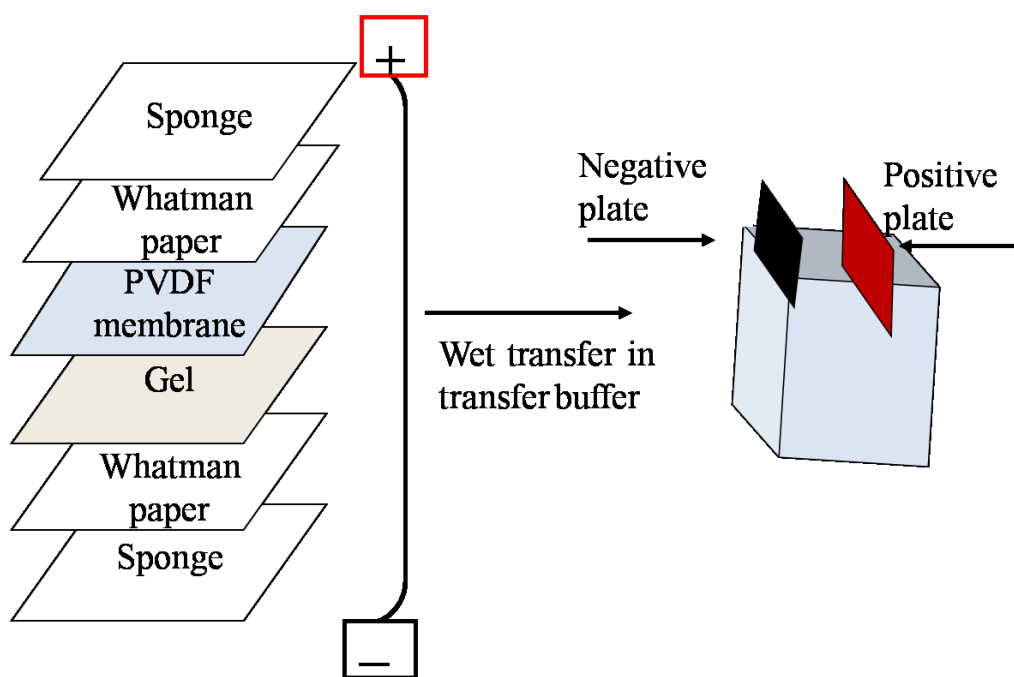


Fig. 3-11. Assembly of a western blot sandwich.

This scheme was modified from Mahmood, 2012.

The membrane was blocked by soaking for 1 hour in a blocking solution containing 5% (w/v) BSA, 0.5% (v/v) Tween 20 in TBS (20 mM Tris, pH7.6, 0.137 M NaCl) while slowly shaking at room temperature. After blocking, the membrane was washed with

0.5% TBS-T three times, for 5 min each time. As primary antibody, OAS-TL-A antibodies (from rabbit) were used. The membrane was incubated overnight in TBS-T buffer, containing 0.05% (v/v) Tween-20, 0.5% (w/v) BSA and the primary antibody (OAS-TL A as 1:5000 dilution), at 4 °C. It was then washed 3 times, for 5 min each time, with 1x TBS-T. Then the secondary antibody (goat anti-rabbit Horse Radish Peroxidase (HRP), HRP-conjugate (Pierce), Abcam, Cambridge, UK) was added in a 1:10000 dilution. The membrane was incubated for 1 hour in TBS-T buffer containing 0.05% (v/v) Tween-20, 0.5% (w/v) BSA and the secondary antibody. It was then washed 3 times for 5 min with TBS-T buffer (TBS-T contained 0.05% (v/v) Tween-20).

An identical procedure was used for the immunodetection of SAT5. The primary antibody in this case had a 1:5000 dilution.

Immunodetection was carried out with Amersham™ ECL™ start Western blotting detection reagent (Amersham, GE Healthcare, Buckinghamshire, UK). The basic principle of operation of this detection kit was shown in Fig. 3-12.

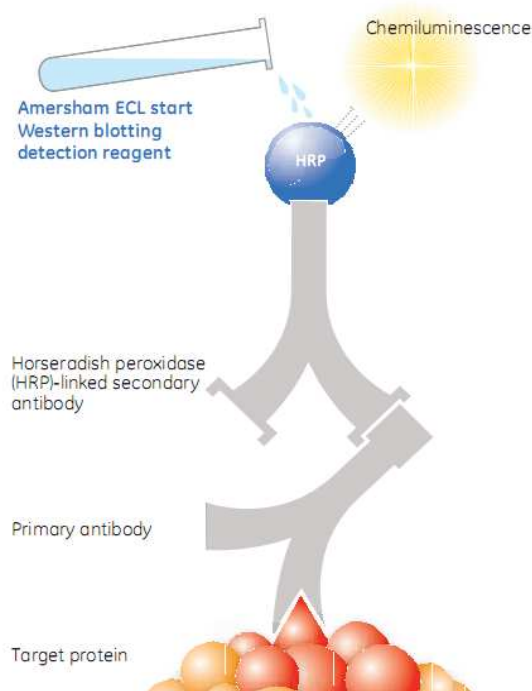


Fig. 3-12. Principle of operation of the Amersham ECL Western blotting detection reagent.

After the addition of 1 ml luminol peroxide detection reagent on the membrane, the HRP enzyme catalyzes the oxidation of luminol, which generates the emission of photons at a wave number of 428 nm.

After the membrane was wetted with 1 ml of ECL reagent, the membrane was wrapped in the transparent plastic film and incubated in the dark for 5 min. The excess of working solution was then removed with a pipette, and the membrane was transferred in a light-tight photographic cassette and fixed into position with tape. A photographic film was located on the membranes and the cassette was closed. All these operations were conducted in a dark room. The film was exposed to the membranes for several seconds to minutes, depending on signal strength. The film was then developed with ChemiDoc™ XRS+ imaging system (BIO-RAD, California, USA).

The Molecular weight of proteins was determined by comparing the position of protein bands of unknown mass with prestained marker proteins of known molecular weight (Garfin, 1990). A standard curve was prepared by measuring the Retention Factor ( $R_f$ ) of prestained marker protein bands. The sample  $R_f$  was interpolated Log( $M_r$ ) vs  $R_f$  standard curve to estimate the molecular weight of the protein of interest (Garfin, 1990; Lord, 2003).

### **3.5. OAS-TL activity measurements**

OAS-TL uses sulfide and O-acetylserine (OAS) as substrates (Kredich, 1966). OAS is generated from the reaction between serine and acetyl coenzyme A catalyzed by SAT (Kredich, 1966; Hell et al., 2002; Droux, 2004). The measurement of OAS-TL activity was based on the spectrophotometric determination of cysteine production. In fact, what was measured was the change of absorbance consequent to the reaction

of the cysteine produced by OAS-TL with ninhydrin (cat. 485-47-2, Sigma-Aldrich, MO, USA). Ninhydrin reacted with the primary amino group of cysteine. As a product, Ruhemann's purple was formed, which absorbed at 560 nm (Gaitonde, 1967). O-acetylserine (thiol) lyase activity was measured as described in (Rolland et al., 1996; Droux et al., 1998) the reaction mix contained 50 mM HEPES-KOH, pH 7.5, 5 mM Na<sub>2</sub>S, 10 mM O-Acetyl serine (OAS), 5 mM DTT, 10 µl of crude extract or purified OAS-TL protein, in a total volume of 100 µl. The reaction mixture was incubated, in the absence of sulfide, for 5 min, at 25 °C. The reaction was initiated by the addition of Na<sub>2</sub>S; the reaction was allowed to run for 10 min at 25 °C and was stopped by the addition of 50 µl of 20% trichloroacetic acid (TCA). In the blank, TCA was added before Na<sub>2</sub>S. Samples and blank, after completion of the reaction, were centrifuged (Microfuge\* 20, Beckman Coulter, California, USA) at room temperature, at 13,000 rpm, for 5 min. The supernatant was then added to a 0.07 M Ninhydrin solution, which was composed of 12 M HCl and 100% acetic acid in a 1:3 (v/v) ratio, incubated at 95.5 °C for 10 min and cooled down for 1-2 min on ice. A volume of 550 µl of 100% ethanol was added to the mixture and the absorbance at 560 nm was measured in a glass cuvette. OAS-TL activity was expressed as g cysteine · g<sup>-1</sup> protein · min<sup>-1</sup>; the amount of cysteine produced was derived from the equivalence: 1 unit of Absorbance at 560 nm = 36.6 nmol cysteine (Wirtz & Droux, 2004).

### **3.6. Cysteine Synthase Complex purification**

Purification of CSCs from all the experimental species was conducted by Fast Protein Liquid Chromatography (FPLC) (ÄKTA pure system GE Healthcare, Buckinghamshire, UK), using a size exclusion column. The basic principle of size exclusion chromatography is depicted in Fig. 3-13. Gel filtration does not rely on the chemical interaction of the protein with the mobile phase; rather it is based on the

effective molecular radius of the protein, which, for most typical globular proteins, relates to mass.

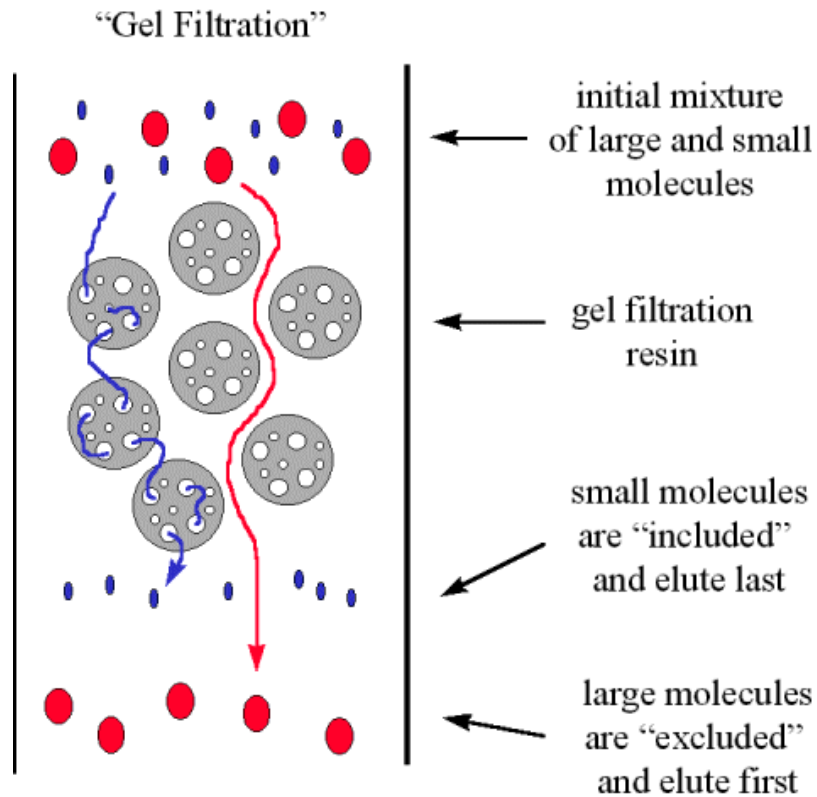


Fig. 3-13. Basic principles of size exclusion chromatography.

(<http://www.mikeblaber.org/oldwine/bch5425/lect31/lect31.htm>) (BCH5425 Molecular Biology and Biotechnology Spring 1998 Dr. Michael Blaber)

The running buffer contained 20 mM Tris-HCl and 150 mM NaCl; the pH was adjusted to 7.5. The column used for this analysis was a Superdex 200 5/150 GL (GE Healthcare, Buckinghamshire, UK), with 3 ml of bed volume, with a protein separation size range from 10 to 600 kDa. The choice was based on what known for *A. thaliana* CSC having a mass of about 320 kDa (Wirtz et al., 2010). The flow rate of the mobile phase was set at 0.1 ml/ min, and fraction collection was such that each fraction contained 0.1 ml of elution buffer.

The previous algal OAS-TL purified samples (section 3.4.7) were centrifuged for 10 min at 10000 g, at room temperature. The supernatant containing OAS-TL (100  $\mu$ l)



was mixed with AtSAT 5 according to the concentration ratio 2 dimer (OAS-TL) to 1 hexamer (AtSAT 5) (Wirtz et al., 2010) and then it was loaded onto the FPLC column with a syringe, as shown in Fig. 3-14. The fractions were collected with Fraction collector F9-R (GE Healthcare, Buckinghamshire, UK).



Fig. 3-14. Fast Protein Liquid Chromatography (FPLC).

(ÄKTA pure system, GE Healthcare, Buckinghamshire, UK).

A LMW Gel Filtration Calibration Kit (Cat. No. 17-0442-01, Amersham, Buckinghamshire, UK) was used to calibrate the column (Fig. 3-15 and Fig. 3-16); a regression curve was generated, which was used to calculate the molecular weight of the proteins in the eluate. The following protein standards were contained in the LMW Kit: 1. Ferritin (440 kDa) 0.4 mg/ml; 2. Aldolase (158 kDa) 4 mg/ml; 3. Ovalbumin (43 kDa) 4 mg/ml; 4. Ribonuclease A (13.7 kDa) 3 mg/mL. The average elution volume was corrected by the total bed column volume and the void volume ( $K_{av}$ ).  $K_{av}$  was calculated with the following equation:

$$K_{av \text{ protein}} = \frac{\text{Elution volume protein} - \text{Void volume}}{\text{Column Bed volume} - \text{Void volume}}$$

The Column Void Volume was measured with a blue dextran solution (2000 kDa). Proteins were detected at 280 nm, using the UV detector of the ÄKTA pure system (GE Healthcare, Buckinghamshire, UK).

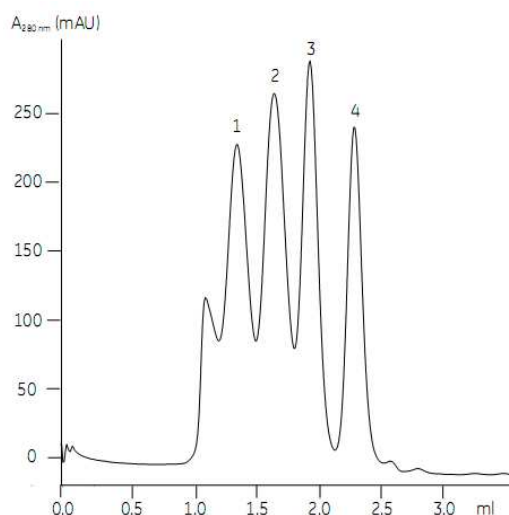


Fig. 3-15. FPLC Protein standards for column calibration.

1, Ferritin (440 kDa) 0.4 mg/ml; 2, Aldolase (158 kDa) 4 mg/ml; 3, Ovalbumin (43 kDa) 4 mg/ml; 4, Ribonuclease A (13.7 kDa) 3 mg/ml; See text for details.

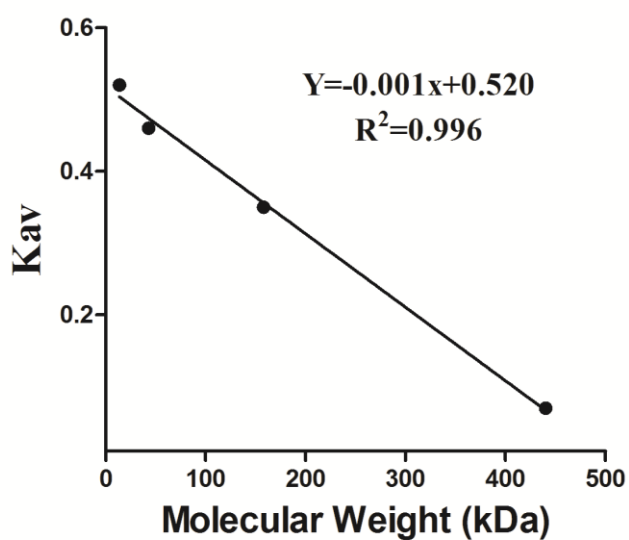


Fig. 3-16. FPLC column calibration curve.

Each dot represents a standard; from left to right: Ribonuclease A (13.7 kDa); Ovalbumin (43 kDa); Aldolase (158 kDa); Ferritin (440 kDa). The average elution volume corrected by the

total bed column volume and the void volume ( $K_{av}$ ) is shown on the Y-axis, as a function of the protein mass in kDaltons (on the X-axis).

The Protein fractions were then subject to SDS-PAGE, according to the protocol described in (3.4.9.) paragraph 1. The proteins were transferred onto a PVDF membrane and identified by immunodetection (see section 3.4.10).

Before loading the sample, the column was equilibrated with running buffer; 5 column volumes (CV) of running buffer were used.

### **3.7. Determination of the impact of the plastoquinone redox state on ATPS activity**

In order to assess whether ATPS redox regulation (Prioretti et al., 2016) is mediated by the redox state of the plastoquinone pool, *Thalassiosira pseudonana* cultures were treated with 3-(3' 4'-dichlorophenyl)-1, 1-dimethylurea (DCMU), which specifically and effectively inhibits  $Q_B$  reduction at PSII (Fig. 3-17). DCMU was dissolved in 100% DMSO to obtain a stock concentration of 20 mM. An amount of DCMU stock equivalent to 0.5‰ of the total culture volume was added, so that the final DCMU concentration in the culture was 10  $\mu$ M.

*T. pseudonana* was chosen as the experimental organism because its ATPS is likely to be redox-regulated (Prioretti et al., 2016). To control cultures, 0.5‰ (v/v) DMSO was added.

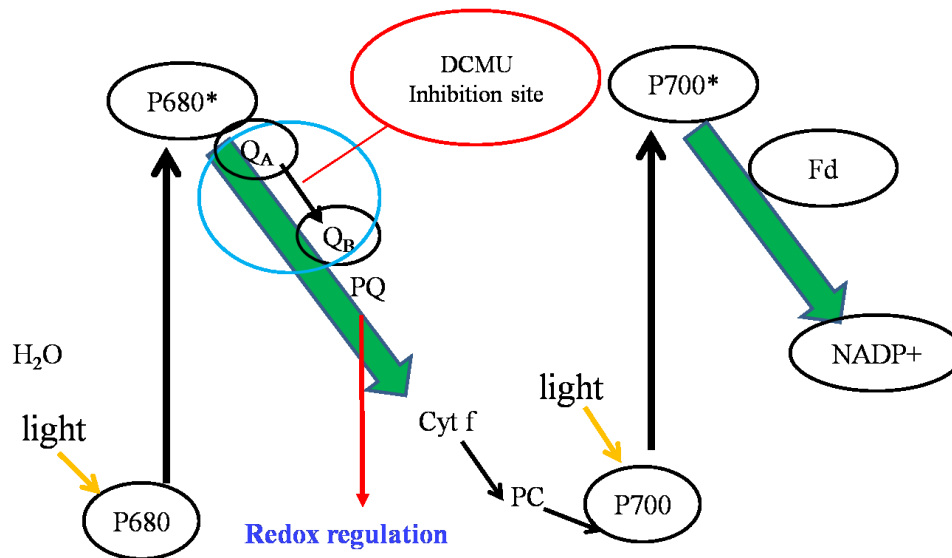


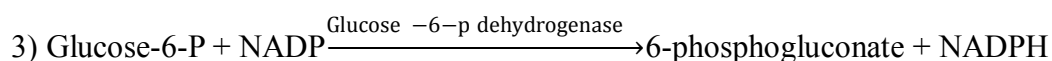
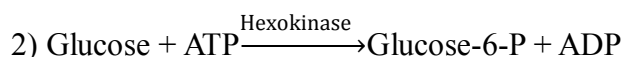
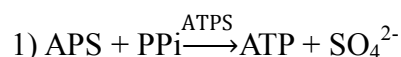
Fig. 3-17. DCMU blocks the electron transfer from  $Q_A$  to  $Q_B$ .

The ATPS enzyme activity was measured after 0, 1, 3 and 6 hours from the addition of the inhibitor (or just the solvent for the controls). The concentration of DCMU that consistently stopped PSII electron transfer rate, as determined with a Dual-PAM-100 fluorimeter (Heinz Walz GmbH, Effeltrich Germany), was used in the experiment. Cells were collected by centrifugation at 3500 g for 10 minutes. The pellet was washed three times with a 0.5 mM NaCl solution and then resuspended in 800  $\mu$ l of an extraction medium containing: 50 mM Tris-HCl (pH 8), 1 mM EDTA, 10 mM  $MgCl_2$  (Giordano et al., 2000). The resuspended pellet was frozen by the addition of liquid  $N_2$  and, while frozen, it was homogenized with a ceramic mortar and pestle. The slurry was transferred into a 1.5 ml plastic tube to which Triton X-100 (0.1% v/v) and glycerol (10% v/v) were added to a final volume of 1 ml. Samples were then kept on ice for 30 minutes to allow the Triton to completely solubilize the proteins. Subsequently, the slurry was centrifuged for 15 minutes at 12000 g, at 4  $^{\circ}C$ . The supernatant was collected and stored at -20  $^{\circ}C$  until used. The protein concentration in the crude extract was determined by the Peterson's method (Peterson, 1977).

ATP sulfurylase (ATPS) activity was measured spectrophotometrically at 25  $^{\circ}C$ , according to Burnell (1984). The reaction mixture contained: APS (1 mM), Sodium

diphosphate tetrabasic (PPi, 1 mM), MgCl<sub>2</sub> (5 mM), glucose (5 mM), Nicotinamide adenine dinucleotide phosphate (NADP, 300 μM), hexokinase and glucose-6-P dehydrogenase from baker's yeast (5 units, H8629 Sigma-Aldrich), Tris-HCl pH 8 (50 mM). The volume of crude extract added to the assay mixture was 40 μl for a final volume in the cuvette of 0.5 ml.

Since it is difficult to determine ATPS activity in the forward direction, the reverse reaction was used. The ATPS reaction was coupled with the reactions catalyzed by hexokinase and glucose-6-P dehydrogenase; the adenosine 5-phosphosulfate (APS)-depending reduction of NADP to NADPH was measured as the change in absorbance at 340 nm.



The rate of NADPH production was recorded for 10 minutes at 340 nm, using a Beckman DU 640 Spectrophotometer (Beckman Coulter). The values of absorbance were converted to enzyme activity using the Lambert-Beer law:

$$\text{Enzyme activity } (\mu\text{mol min}^{-1} \text{ ml}^{-1}) = \left[ \frac{\Delta A_{340 \text{ nm}}}{\epsilon_{\text{NADH}} \cdot d} \right] \cdot \left[ \frac{V_{\text{tot}}}{V_{\text{sample}}} \right]$$

Where:  $\Delta A_{340 \text{ nm}}$  is the rate of NADPH production per minute measured at 340 nm;  $\epsilon_{\text{NADH}}$  (6.22 mM<sup>-1</sup> cm<sup>-1</sup>) is the extinction coefficient of NADPH at 340 nm;  $d$  (1 cm) is the optical path length;  $V_{\text{tot}}$  (500 μl) is the total volume in the reaction vessel;  $V_{\text{sample}}$  (40 μl) is the volume of crude extract used for the assay. The activity of the enzyme per unit of protein was calculated by dividing the results of the above equation (with units of nmol min<sup>-1</sup> ml<sup>-1</sup>) by the concentration of protein in the sample (mg ml<sup>-1</sup>); the final results thus had units of μmol NADH min<sup>-1</sup> (mg protein)<sup>-1</sup>.

Three different types of blanks were tested: 1) using boiled crude extract, 2) assaying

the crude extract without APS, 3) assaying the complete mixture without enzyme extract. All three types of blanks gave very similar results; for the experiments, blanks were prepared without APS in the assay mixture.



## 4. Results

### 4.1. Bioinformatics

#### 4.1.1. Phylogeny of Serine Acetyltransferase

The phylogenetic tree of SAT showed two main branches (Fig. 4-1).

1) The first branch showed two main sub-branches. The first one (from the top) comprise sequences from early-diverging chlorophytes, such as the prasinophyte *Tetraselmis suecica*, core-Chlorophyceae belonging to Trebuxiophyceae and Chlorophyceae (Leliaert et al., 2003) and embryophytes (i.e. *Arabidopsis thaliana* and *Glycine max*). The first sub-branch could be divided into two further groups, only the first one of which included embryophyte sequences.

The second sub-branch was constituted by red algae and algae of the red lineage (diatoms and brown algae), but it also includes sequences from the marine cyanobacterium *Lyngbya confervoides*.

2) The second branch of the SAT tree was more heterogeneous; it could be separated into two sub-branches. The first sub-branch from the top included sequences from terrestrial and oceanic cyanobacteria (*Scytonema millei* VB511283, *Prochlorococcus* sp. Scb245), green algae (*Ostreococcus tauri* and *Micromonrias* sp.) cryptophytes (*Guillardia theta*), prymnesiophytes (*Emiliania huxleyi* and *Chrysochromulina* sp.), brown algae such as *Ectocarpus siliculosus* (also present in the first branch), and the diatom *Thalassiosira pseudonana* (also in the first branch).

The second sub-branch contained sequences from mamiellophyceae green algae (*Micromonas* sp., *Bathycoccus prasinos* and *Ostreococcus tauri*), which constituted



a compact group somewhat distant from the heterokonts *Aureococcus anophagefferens* (Pekagophyceae) and *Ectocarpus siliculosus* (Phaeophyceae) and even more distant from the diatom *Thalassiosira oceanica*.

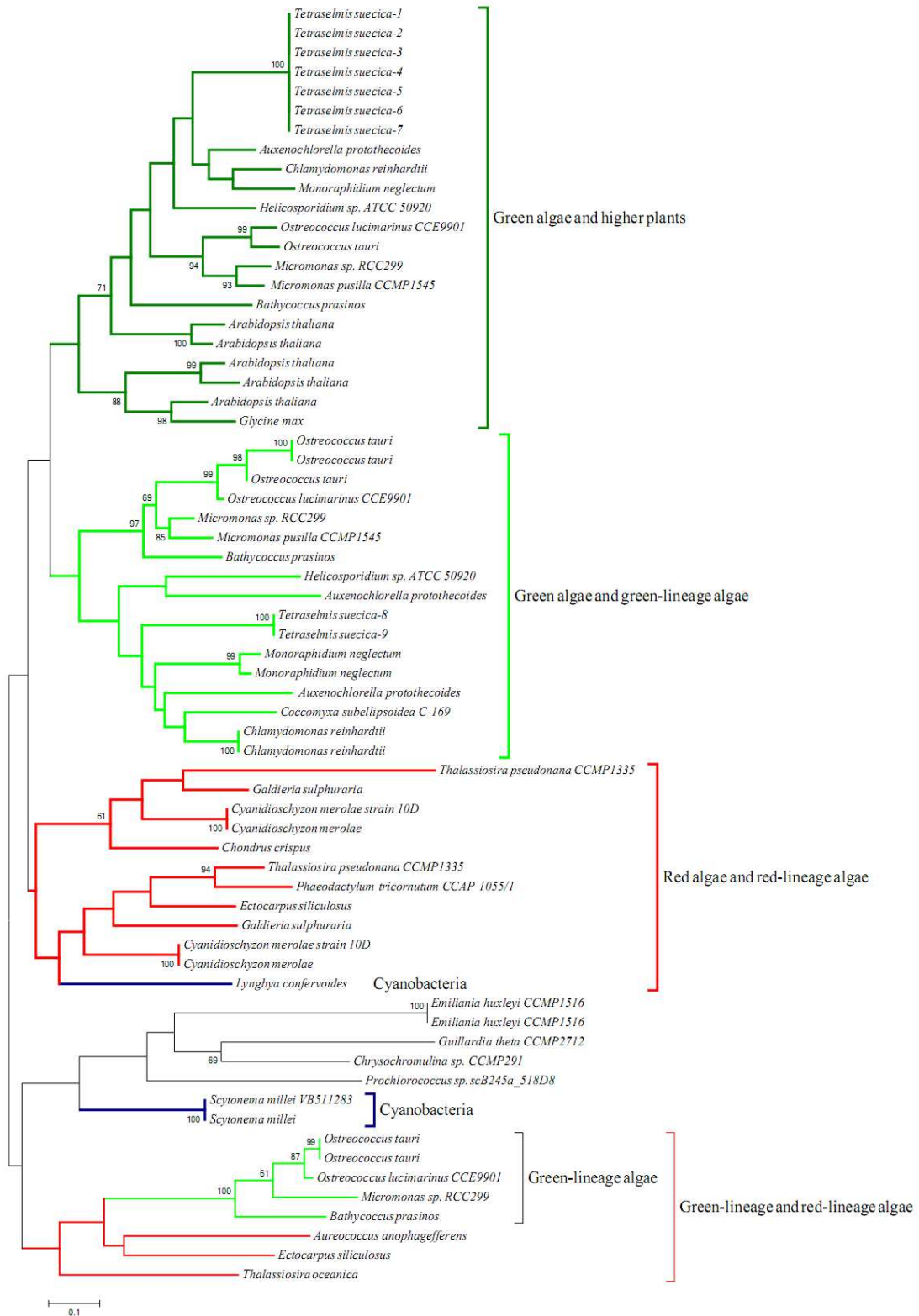


Fig. 4-1. Serine Acetyltransferase phylogenetic tree.

Dark green lines indicated green algae and higher plant; light green lines indicated green algae; red lines indicated lineage algae; blue lines indicated Cyanobacteria.

### 4.1.2. Phylogeny of O-Acetylserine (thiol) lyase

O-Acetylserine (thiol) lyases phylogenetic tree showed two main branches (Fig. 4-2).

1) In the first branch, *Bacillus cereus* sequence constituted a sister clade of a more composite group that included most of the species used to build the tree (Fig. 4-2).

In this group, two sub-branches could be identified: the branch in the lower position included dinoflagellates, diatoms, and prymnesiophytes; the green freshwater algae *Monoraphidium neglectum* constituted a sister group to these species.

In the other sub-branch, several groups could be distinguished: all cyanobacteria sequences grouped together. Another group was represented by sequences from green algae (the freshwater species *Chlamydomonas reinhardtii*, the marine species *Coccomyxa subellipsoidea* C-169, *Ostreococcus tauri*, *Ostreococcus lucimarinus* CCE9901, *Tetraselmis* sp. GSL018, *Coccomyxa subellipsoidea* C-169 and *Tetraselmis suecica*) and several sequences from the embryophyte *Arabidopsis thaliana* (due to the presence of different isoforms, in this organism). A third group was given by sequences from red algae and algae of the red lineage (*Porphyrapurpurea*, *Chondrus crispus*, *Galdieria sulphuraria*, *Cyanidioschyzon merolae* strain 10D), with the green mixotrophic algae *Auxenochlorella protothecoides* as the only intruder.

2) The second branch was formed by species belonging to heterogeneous taxa: the freshwater green algae *Auxenochlorella protothecoides*, *Chlamydomonas reinhardtii*, *Monoraphidium neglectum*, the marine green algae *Tetraselmis suecica*, *Bathycoccus prasinos* and *Nannochloropsis gaditana*, the dinoflagellate *Amphidinium klebsii*, the diatom *Phaeodactylum tricorutum*, the red algae *Cyanidioschyzon merolae*, the brown algae *Ectocarpus siliculosus*, the amoeba *Dictyostelium discoideum*, the protozoan *Thecamonas trahens*, the ascomycete *Aspergillus fumigatus*. No obvious

connection could be found between the position of the OAS-TL sequences in this branch and the known phylogeny of the organisms, suggesting a shuffling of genes through horizontal gene transfer.

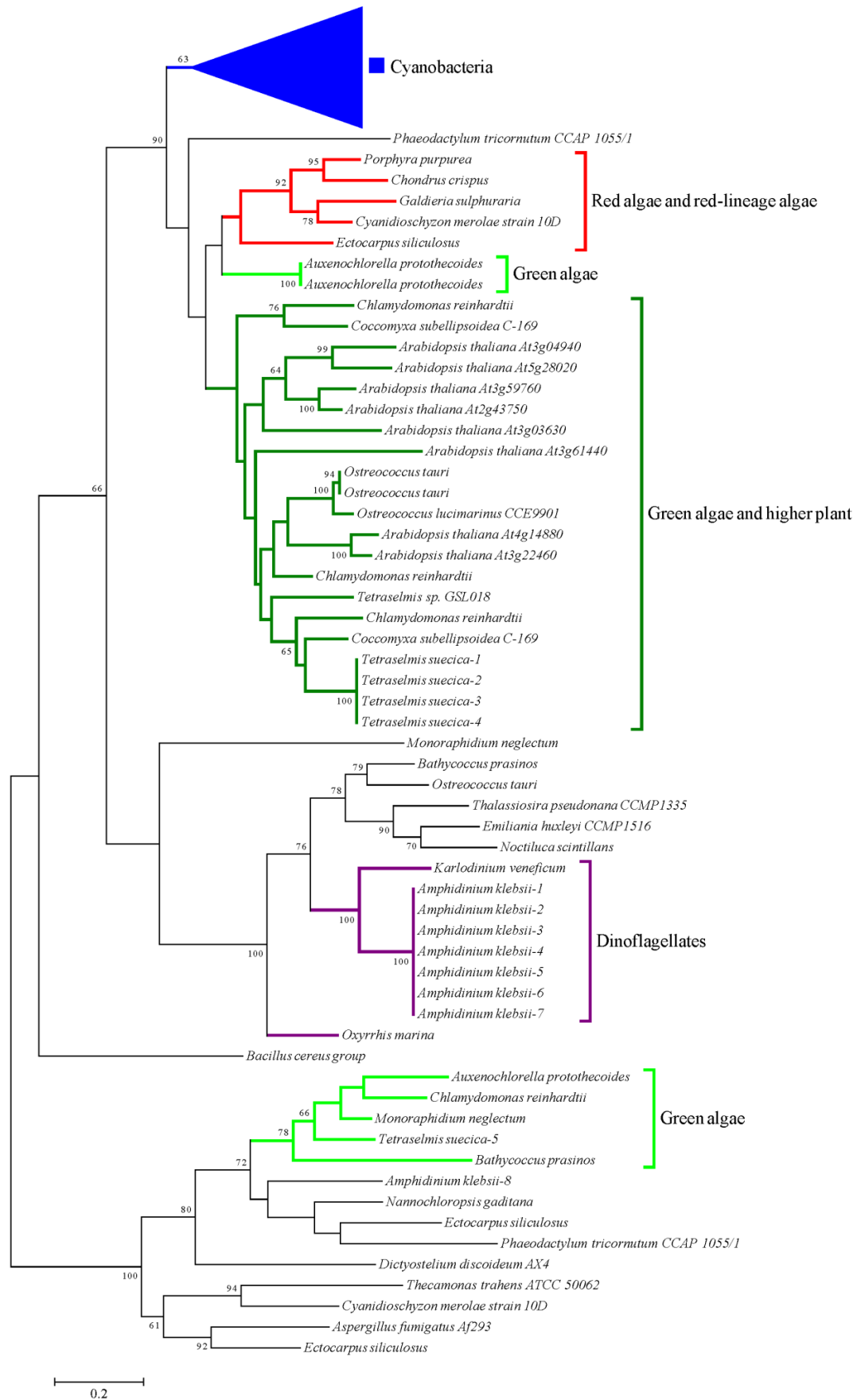


Fig. 4-2. The O-Acetylserine (thiol) lyase phylogenetic tree.

The dark green lines indicated green algae and higher plant; light green lines indicated green algae; red lines indicated red lineage algae; purple lines indicated dinoflagellates; the blue triangle indicated Cyanobacteria. The species of cyanobacteria included in this analysis were shown in the Table. 4-2.

### **4.1.3. Observations on SAT and OAS-TL protein sequences**

#### **4.1.3.1. Number of cysteine residues in algae SAT sequences**

The number of cysteines represents an indication of the presence of potential disulfide bonds in the protein and of possible sites for redox regulation. For this reason, I verified whether a trend could be identified with respect to the number of cysteine residues (Table. 4-1). In Cyanobacteria, terrestrial, marine and freshwater species contained more or less similar numbers of Cys residues. In Chlorophyta, the SAT sequences from freshwater species had more Cys residues (typically 8 or 9) than marine species (5 to 7 residues). Somewhat interestingly, species able to grow both in freshwaters and marine environments (e.g. *Monoraphidium neglectum*) had the lowest number of cysteine residues (2 or 3) in their SAT sequences. In the red algae and in their descendants, the number of Cys residues was in the most case between 4 and 6. Only sequences from *Emiliania* (7 and 11), *Cyanidioschizon* (9) and some sequence from *Thalassiosira* (7 and 8) contained higher numbers of Cys.

#### **4.1.3.2. Predicted location of SAT proteins**

As shown in Table. 4-1, Cyanobacteria, being prokaryotes, did not have organellar localization of proteins (Table. 4-1). The SAT sequences of Cyanobacteria were between 238 and 272 amino acids (aa)-long. In Chlorophyta, SAT protein contained

229-480 aa and its location was in the chloroplasts or in mitochondria, depending on the species. In the case of *Tetraselmis suecica*, it was not possible to obtain a reliable location for SAT. The haptophyte *Emiliana huxley* SAT protein sequence was 344 to 656 aa-long; all two *Emiliana huxleyi* CCMP 1516 sequences appeared to be located in the mitochondrion. In Rhodophyta, SAT protein was from 323 to 406 aa. In Heterokontophyta, the amino acid number varied greatly, from 183 to 603 aa. Prediction software was unable to identify the location of heterokonts SAT.

Table. 4-1. Number of Cysteines residues, sequence length and location of algae serine acetyltransferase (SAT)

Phylum	Name	Environment	Total CYS number	Len	cTP	mTP	SP	other	Loc	RC	TPlen
Cyanobacteria	<i>Lyngbia sp.</i>	M	5	272	-	-	-	-	-	-	-
	<i>Scytonema millei</i>	T	4	269	-	-	-	-	-	-	-
	<i>Scytonema millei VB511283</i>	T	4	264	-	-	-	-	-	-	-
	<i>Synechocystis sp. PCC 6803</i>	F	3	249	-	-	-	-	-	-	-
	<i>Synechococcus sp. PCC 7803</i>	M	3	247	-	-	-	-	-	-	-
	<i>Prochlorococcus sp. scB245a_518D8</i>	M	3	238	-	-	-	-	-	-	-
Chlorophyta	<i>Auxenochlorella protothecoides</i>	F	7	271	0.077	0.522	0.076	0.039	M	3	38
	<i>Auxenochlorella protothecoides</i>	F	3	355	0.243	0.199	0.018	0.064	_	4	38
	<i>Bathycoccus prasinus</i>	M	5	370	0.803	0.097	0.028	0.207	C	3	9
	<i>Bathycoccus prasinus</i>	M	7	286	0.098	0.191	0.032	0.886	_	2	9
	<i>Chlamydomonas reinhardtii</i>	F	8	392	0.915	0.202	0.002	0.044	C	2	26
	<i>Chlamydomonas reinhardtii</i>	F	8	480	0.810	0.503	0.006	0.009	C	4	26
	<i>Chrysochromulina sp. CCMP 291</i>	M/F	3	416	0.240	0.213	0.061	0.087	C	5	26
	<i>Coccomyxa subellipsoidea C169</i>	F	8	296	0.023	0.419	0.022	0.772	_	4	-
	<i>Helicosporidium sp. ATCC 50920</i>	T	5	309	0.643	0.136	0.017	0.210	C	3	17
	<i>Helicosporidium sp. ATCC 50920</i>	T	6	307	0.061	0.076	0.019	0.877	_	1	17
	<i>Micromona spusilla CCMP 1545</i>	M	4	350	0.341	0.480	0.012	0.098	M	5	117
	<i>Micromona spusilla CCMP 1545</i>	M	2	306	0.103	0.144	0.046	0.860	_	2	117
	<i>Micromonas sp. RCC 299</i>	M	1	258	0.069	0.054	0.438	0.721	_	4	117
	<i>Micromonas sp. RCC 299</i>	M	3	363	0.846	0.236	0.006	0.039	C	2	117
	<i>Micromonas sp. RCC 299</i>	M	5	276	0.107	0.109	0.027	0.858	_	2	117
	<i>Monoraphidium neglectum</i>	F	2	318	0.025	0.456	0.120	0.327	M	5	11
	<i>Monoraphidium neglectum</i>	F	3	471	0.261	0.082	0.163	0.676	_	3	11
	<i>Ostreococcus lucimarinus CCE 9901</i>	M	2	374	0.842	0.257	0.005	0.033	C	3	18
	<i>Ostreococcus lucimarinus CCE 9901</i>	M	3	270	0.112	0.315	0.066	0.668	_	4	18
	<i>Ostreococcus lucimarinus CCE 9901</i>	M	5	265	0.154	0.120	0.032	0.827	_	2	18
<i>Ostreococcus tauri</i>	M	5	284	0.045	0.473	0.014	0.105	M	5	18	
<i>Ostreococcus tauri</i>	M	6	436	0.702	0.235	0.014	0.105	C	3	18	
<i>Tetraselmis suecica-1</i>	M	6	308	0.121	0.225	0.083	0.597	_	4	-	



	<i>Tetraselmis suecica-2</i>	M	5	303	0.097	0.148	0.060	0.798	_	2	-
	<i>Tetraselmis suecica-3</i>	M	5	251	0.069	0.161	0.133	0.614	_	3	-
	<i>Tetraselmis suecica-4</i>	M	5	234	0.058	0.276	0.093	0.739	_	3	-
	<i>Tetraselmis suecica-5</i>	M	5	250	0.058	0.276	0.093	0.739	_	3	-
	<i>Tetraselmis suecica-6</i>	M	5	229	0.012	0.090	0.680	0.700	_	5	-
	<i>Tetraselmis suecica-7</i>	M	4	258	0.058	0.276	0.093	0.739	_	3	-
	<i>Tetraselmis suecica-8</i>	M	7	283	0.106	0.440	0.012	0.582	_	5	-
Rhodophyta	<i>Chondrus crispus</i>	M	5	380	0.492	0.177	0.016	0.019	C	4	26
	<i>Cyanidioschyzon meroiae 10D</i>	M	4	402	0.565	0.881	0.001	0.022	M	4	38
	<i>Cyanidios chyzon meroiae</i>	M	9	406	0.751	0.616	0.003	0.030	C	5	38
	<i>Galdieria sulphuraria</i>	M	3	323	0.104	0.082	0.148	0.909	_	2	62
	<i>Galdieria sulphuraria</i>	M	6	344	0.667	0.327	0.019	0.089	C	4	62
Haptophyta	<i>Emiliania huxleyi CCMP 1516</i>	M	11	656	0.030	0.843	0.014	0.218	M	2	117
	<i>Emiliania huxleyi CCMP 1516</i>	M	7	344	0.291	0.863	0.008	0.010	M	3	117
Heterokontophyta	<i>Ectocarpus siliculosus</i>	M	5	479	0.539	0.083	0.049	0.549	_	5	-
	<i>Ectocarpus siliculosus</i>	M	6	333	0.317	0.130	0.036	0.715	_	4	-
	<i>Thalassiosira oceanic THAOC 04795</i>	M	7	554	0.017	0.673	0.011	0.737	_	5	26
	<i>Thalassiosira pseudonana CCMP 1335</i>	M	4	234	0.034	0.113	0.170	0.887	_	2	26
	<i>Thalassiosira pseudonana CCMP 1335</i>	M	8	603	0.020	0.025	0.821	0.079	S	2	26
	<i>Phaeodactylum tricornutum CCAP 1055/1</i>	M	4	539	0.042	0.325	0.353	0.075	S	5	18

The column “Environment” indicates whether the species is marine (M), freshwater (F) or terrestrial (T). The column “Number of Cys” refers to the number of cysteine residues in the sequence; Len, sequence length; cTP, chloroplast transit peptide; mTP, mitochondrial targeting peptide; SP, secretory pathway signal peptide; C, Chloroplast; M, Mitochondrial; RC, reliability class ranging area, from (1 to 5); Tplen, predicted presequence length. If the sequence contained a chloroplast or mitochondrion transit peptides, cTP or mTP score was assigned; if the sequence contained a secretory pathway signal peptide, a SP score was assigned; for any other location an “other” score was assigned. The reliability class RC ranged from (1 to 5), with the higher number indicating a more robust prediction. RC was measured calculating the difference between the highest and the second highest output scores: reliability was 1 when the difference was > 0.800; reliability was 2 when the difference was between 0.600 and 0.800; a reliability score of 3 indicates that the difference was between 0.400 and 0.600; a reliability of 4 was attributed when the difference was between 0.200 and 0.400; a reliability of 5 corresponds to a difference between the two highest scores < 0.200. The cleavage site was predicted and the presequence length (TPlen) reported.

#### 4.1.3.3. Number of cysteine residues in algal OAS-TL sequences

As shown in Table. 4-2. all Cyanobacteria, both marine and freshwater species, showed less than 3 cysteine residues in their OAS-TL sequences; some sequences contained no Cys residues at all. The cysteine number in the OAS-TL sequences of Chlorophyta was extremely variable: with no obvious difference between marine and freshwater species. Rhodophyta and their descendants of the red lineage contained an extremely variable number of cysteines. This heterogeneity may reflect the presence of different isoforms. A finer phylogenetic analysis and possibly more sequences (at least for certain groups) were required to ascertain whether the different number of cysteine is associated with different origins of the proteins.

#### 4.1.3.4. Predicted OAS-TL location

As shown in Table. 4-2, Cyanobacteria, being prokaryotes, did not have organellar localization of proteins (Table. 4-2). Cyanobacteria OAS-TL sequence length was between 307 and 336 aa. In Chlorophyta the protein length was between 307 and 491 aa; the predicted location was more often in mitochondria than in chloroplasts, some sequences could not be located in any particular compartment. In Dinophyta the sequences ranged from 331 to 388 aa; the predicted location was mostly in chloroplasts. In *Emiliana huxleyi* CCMP 1516 the sequence length was 382 aa and it was predicted to be located in mitochondria; Rhodophyta showed the OAS-TL length ranging from 326 to 390 aa, located both in chloroplasts and mitochondria. In Heterokontophyta, the sequences were 342-428 aa long.

Table. 4-2. O-acetylserine (thiol) lyase (OAS-TL) of algae with different taxonomy, ecology, and the location prediction

Phylum	Name	Environment	Total CYS number	Len	cTP	mTP	SP	other	Loc	RC	TPlen
Cyanobacteria	<i>Acaryochloris marina</i>	M	3	336							
	<i>Anabaena 90</i>	F	2	313							
	<i>Anabaena 90</i>	F	0	320							
	<i>Anabaena variabilis</i> ATCC 29413	M	2	320							
	<i>Anabaena</i> sp. Wa102	F	2	320							
	<i>Bacilluscereus</i> group	M	1	307							
	<i>Calothrix</i> sp. 336/3	F	2	313							
	<i>Calothrix</i> sp. PCC 7507	F	1	320							
	<i>Chlorogloeopsis frütschii</i>	M	2	320							
	<i>Cyanobacterium</i> PCC 7702	M	2	320							
	<i>Cylindrospermopsis raciborskii</i>	F	2	314							
	<i>Cylindrospermum stagnale</i>	F	2	322							
	<i>Thermosynechococcus</i> sp. NK55a	M	2	321							
	<i>Dactylococcopsis salina</i>	F	3	317							
	<i>Dolichospermum circinale</i>	F	1	313							
	<i>Fischerella</i>	F	2	311							
	<i>Fischerella muscicola</i>	F	0	320							
	<i>Fischerella muscicola</i>	F	2	319							
	<i>Fischerella</i> sp. JSC-11	F	3	344							
	<i>Fischerella</i> sp. PCC 9605	F	2	320							
	<i>Fischerella</i> sp. PCC 9431	F	0	320							
	<i>Fischerella</i> sp. PCC 9339	F	0	313							
	<i>Halotheca</i> sp. PCC 7418	M	3	322							
	<i>Hapalosiphon</i> sp. MRB220	F	0	320							
	<i>Hassalliabyssosidea</i> VB512170	M	0	319							
	<i>Mastigocladopsis repens</i>	F	2	320							
	<i>Mastigocladus laminosus</i>	F	0	320							
	<i>Microchaete</i> sp. PCC 7126	F	1	320							
	<i>Microcoleus vaginatus</i>	F	1	326							
	<i>Microcystis panniformis</i>	F	2	319							
	<i>Myxosarcina</i> sp. G11	F	1	321							
	<i>Nodulariaspumigena</i>	F	0	320							
	<i>Nostocpunctiforme</i>	F/M	0	320							
<i>Nostoc</i> sp. PCC 7107	F/M	0	315								
<i>Nostoc</i> sp. PCC 7120	F/M	1	320								
<i>Nostoc</i> sp. PCC 7120	F/M	2	319								
<i>Nostoc</i> sp. PCC 7524	F/M	1	320								
<i>Oscillatorianigroviridis</i> sp. PCC 7112	F	1	326								
<i>Planktothrix agardhii</i>	F	2	311								

<i>Planktothrix agardhii</i> NIVA-CYA 126/8	F	2	311								
<i>Planktothrix</i>	F	2	319								
<i>Pleurocapsa</i> sp. PCC 7319	F	1	332								
<i>Pseudanabaena</i> sp. PCC 6802	M/F	1	320								
<i>Raphidiopsis brookii</i>	F	2	314								
<i>Rivularia</i> sp. PCC 7116	F	3	320								
<i>Scytonema hofmanni</i>	F	0	320								
<i>Scytonema hofmanni</i> UTEX B 1581	F	1	319								
<i>Scytonema millei</i>	M	0	320								
<i>Scytonema millei</i>	M	1	320								
<i>Scytonema tolypothrichoides</i>	F	1	317								
<i>Stigonematales</i>	F	2	319								
<i>Synechococcus</i> sp. CC9605	M	4	322								
<i>Synechococcus</i> JA-3-3Ab	F	1	326								
<i>Synechococcus</i> sp. PCC 6312	F	2	320								
<i>Synechococcus</i> sp. PCC 7335	F	3	346								
<i>Synechococcus</i> sp. PCC 7336	F	1	320								
<i>Synechococcus</i> sp. WH 8109	M	4	322								
<i>Synechocystis</i> sp. PCC 6714	F	2	312								
<i>Synechocystis</i> sp. PCC 6803	F	0	331								
<i>Thecamonastrahens</i> ATCC_50062	M	5	385								
<i>Thermosynechococcus</i> sp. NK55a	M	2	321								
<i>Tolypothrix bouteillei</i>	M	2	320								
<i>Tolypothrix campylonemoides</i> VB511288	M	2	320								
<i>Tolypothrix</i> sp. PCC 7601	F	2	320								
<i>Trichormus azollae</i>	F	2	320								
<i>Xenococcus</i> sp. PCC7305	M	2	321								
<i>Auxenochlorella protothecoides</i>	M	1	352	0.034	0.037	0.760	0.057	S	2	26	
<i>Auxenochlorella protothecoides</i>	M	5	361	0.308	0.067	0.038	0.644	—	4	26	
<i>Bathycoccus prasinus</i>	F	10	384	0.495	0.175	0.034	0.236	C	4	13	
<i>Bathycoccus prasinus</i>	F	8	491	0.067	0.064	0.235	0.444	—	4	-	
<i>Chlamydomonas reinhardtii</i>	F	7	359	0.052	0.863	0.029	0.064	M	2	11	
<i>Chlamydomonas reinhardtii</i>	F	6	414	0.885	0.100	0.003	0.070	C	2	53	
<b>Chlorophyta</b> <i>Chlamydomonas reinhardtii</i>	F	8	382	0.016	0.293	0.612	0.018	S	4	26	
<i>Chlamydomonas reinhardtii</i>	F	6	387	0.136	0.890	0.036	0.072	M	2	33	
<i>Chlorella variabilis</i>	F	0	328	0.085	0.115	0.035	0.860	—	2	-	
<i>Coccomyxa subellipsoidea</i> C-169	F	4	405	0.534	0.335	0.006	0.102	C	5	60	
<i>Coccomyxa subellipsoidea</i> C-169	F	1	378	0.081	0.167	0.111	0.587	—	3	60	
<i>Monoraphidium neglectum</i>	F	5	406	0.077	0.171	0.283	0.069	S	5	23	
<i>Monoraphidium neglectum</i>	M	7	440	0.414	0.794	0.003	0.015	M	4	7	

	<i>Nannochloropsis gaditana</i>	M	9	420	0.081	0.210	0.058	0.209	M	5	43
	<i>Ostreococcus lucimarinus CCE9901</i>	F	3	305	0.028	0.141	0.222	0.818	-	3	-
	<i>Ostreococcus tauri</i>	F	4	440	0.156	0.281	0.011	0.510	-	4	-
	<i>Ostreococcus tauri</i>	F	5	345	0.150	0.675	0.039	0.033	M	3	9
	<i>Tetraselmis</i> sp. GSL0	M	11	428	0.319	0.393	0.002	0.044	M	5	17
	<i>Tetraselmis suecica</i> -1	M	3	314	0.121	0.064	0.180	0.715	-	3	-
	<i>Tetraselmis suecica</i> -2	M	3	315	0.137	0.069	0.147	0.762	-	2	-
	<i>Tetraselmis suecica</i> -3	M	2	297	0.036	0.415	0.145	0.391	M	5	21
	<i>Tetraselmis suecica</i> -4	M	6	345	0.239	0.517	0.023	0.119	M	4	26
	<i>Tetraselmis suecica</i> -5	M	3	333	0.121	0.064	0.180	0.715	-	3	-
<b>Dinophyta</b>	<i>Amphidinium klebsii</i> -1	M	3	347	0.019	0.196	0.053	0.950	-	2	-
	<i>Amphidinium klebsii</i> -2	M	3	331	0.018	0.058	0.125	0.948	-	1	-
	<i>Amphidinium klebsii</i> -3	M	3	342	0.018	0.058	0.125	0.948	-	1	-
	<i>Amphidinium klebsii</i> -4	M	3	329	0.184	0.207	0.051	0.266	-	5	-
	<i>Amphidinium klebsii</i> -5	M	3	334	0.018	0.058	0.125	0.948	-	1	-
	<i>Amphidinium klebsii</i> -6	M	3	356	0.018	0.356	0.045	0.878	-	3	-
	<i>Amphidinium klebsii</i> -7	M	3	355	0.016	0.403	0.043	0.893	-	3	-
	<i>Amphidinium klebsii</i> -8	M	7	343	0.025	0.037	0.129	0.936	-	1	-
	<i>Karlodinium veneficum</i>	M	4	357	0.838	0.198	0.036	0.058	C	2	46
	<i>Karlodinium veneficum</i>	M	4	388	0.432	0.305	0.074	0.346	C	5	34
	<i>Noctiluca scintillan</i>	M	8	356	0.022	0.716	0.022	0.531	M	5	12
<i>Oxyrrhis marina</i>	M	4	385	0.860	0.279	0.037	0.037	C	3	5	
<b>Rhodophyta</b>	<i>Chondrus crispus</i>	M	6	326	0.238	0.094	0.111	0.486	-	4	-
	<i>Cyanidioschyzon meroiaie</i> 10D	M	7	390	0.541	0.432	0.031	0.139	C	5	20
	<i>Cyanidioschyzon meroiaie</i>	M	4	389	0.837	0.037	0.023	0.025	C	2	20
	<i>Galdieria sulphuraria</i>	M	9	385	0.211	0.402	0.042	0.186	M	5	43
<b>Haptophyta</b>	<i>Emiliana huxleyi</i> CCMP 1516	M	9	382	0.035	0.806	0.014	0.069	M	2	17
<b>Heterokontophyta</b>	<i>Ectocarpus siliculosus</i>	M	6	345	0.196	0.035	0.116	0.751	-	3	-
	<i>Ectocarpus siliculosus</i>	M	13	422	0.014	0.064	0.576	0.010	S	3	26
	<i>Ectocarpus siliculosus</i>	M	8	354	0.646	0.087	0.285	0.156	C	4	10
	<i>Phaeodactylum tricorutum</i> CCAP 1055/1	M	3	342	0.052	0.427	0.014	0.575	-	5	-
	<i>Phaeodactylum tricorutum</i> CCAP 1055/1	M	6	356	0.265	0.060	0.107	0.450	-	5	-
	<i>Thalassiosira pseudonana</i> CCMP 1335	M	6	355	0.160	0.211	0.052	0.589	-	4	-

The column “Environment” indicates whether the species is marine (M), freshwater (F) or terrestrial (T). The column “Number of Cys” refers to the number of cysteine residues in the sequence; Len, sequence length; cTP, chloroplast transit peptide; mTP, mitochondrial targeting peptide; SP, secretory pathway signal peptide; C, Chloroplast; M, Mitochondrial; RC, reliability class ranging area, from (1 to 5); Tplen, predictedpresequence length. If the sequence contained a chloroplast or mitochondrion transit peptides, cTP or mTP score was assigned; if the sequence contained a secretory pathway signal peptide, a SP score was assigned; for any other location an “other” score was assigned. The reliability class RC ranged from (1 to 5), with the higher number indicating a

more robust prediction. RC was measured calculating the difference between the highest and the second highest output scores: reliability was 1 when the difference was  $> 0.800$ ; reliability was 2 when the difference was between 0.600 and 0.800; a reliability score of 3 indicates that the difference was between 0.400 and 0.600; a reliability of 4 was attributed when the difference was between 0.200 and 0.400; a reliability of 5 corresponds to a difference between the two highest scores  $< 0.200$ . The cleavage site was predicted and the presequence length (TPlen) reported.

#### **4.1.4. Comparison of SAT and OAS-TL sequences among algae and with *A. thaliana* sequences**

##### **4.1.4.1. Alignment of the SAT sequences of Cyanobacteria with *A. thaliana* SAT isoforms**

The alignment of the SAT sequence of freshwater cyanobacterium *Synechocystis* sp. PCC 6803 and the marine cyanobacterium *Synechococcus* sp. WH 7803 with the SAT sequences of the higher plant model organism *A. thaliana* (Mayer et al., 1999; Lin et al., 1999; Rhee et al., 2000; Theologis et al., 2000) was performed in order to observe the homology. Table 4-3 showed the details of this analysis. *A. thaliana* SAT sequences carried an amino-terminal extension (112-192 aa) that was not present in the cyanobacterial sequence. *Synechocystis* sp. PCC 6803 SAT contained a C-terminus extension that ranged 1-27 aa. The alignment of *Synechocystis* sp. PCC 6803 SAT protein with one of the *A. thaliana* SAT protein is shown in Fig. 4-3. When hypothetical deletions are omitted, a 168 amino acid-long region with the identity of 42.2% (70 identical amino acids) could be found. If the 32 additional equivalent amino acids were included in the identity analysis (i.e. equivalent amino acids are considered equal), the homology was 61.4%. The amino acid alignment with the other four *A. thaliana* SAT isoforms showed 41.3%, 42.4%, 44.6%, 46.7% identity, respectively (Table 4-4). If the additional equivalent amino acids were included, the homology was (Table 4-4) 61%, 60.3%, 63.6%, 63.5%. *A. thaliana* SAT sequences had an amino-terminal extension (112-192 aa) which was not present in the *Synechococcus* sp. WH 7803 sequence. *Synechococcus* sp. WH 7803 had a C-terminus extension of 2-29 amino acid residues. The identity of *Synechococcus* sp. WH 7803 SAT amino acid sequence to the five SAT isoforms of *A. thaliana*, ranged between 40.1% and 47.9%; if the equivalent amino acids were included, the

homology range was 56.3-64.2% (See supplementary data for details). The alignments of all other algae species were shown in Table. 4-4.

SAT1-A.thalia	143	SSIRLDVQAFKDRDPACLSYS SAILHLKGYLALQAYRVAHKLWKQGRKLL	192
Synechocystis	3	NSLIADFRIIFERDPAARNWLEVLFCYPGLQALLIHRFHRLYTLGLPFF	52
SAT1-A.thalia	193	ALALQSRVSEVFGIDIHFAARIGK GILLDHGTGVVIGETAVIGDRVSILH	242
Synechocystis	53	PRLMSHLARFFTGIEIHPGAQIQGVFIDHGMGVVIGETAIVGDYSLIYQ	102
SAT1-A.thalia	243	GVTLLGGTGKETGDRHPNIGDGALLGACVTILGNIKIGAGAMVAAGSLVLK	292
Synechocystis	103	GVTLLGGTGKESGKRHPTLGENVVVGAGAKVLGNIAIGDNVRIGAGSVVLR	152
SAT1-A.thalia	293	DVPSHSMVAGNPAKLI	308
Synechocystis	153	DVPADFTVVGVPGRMV	168

Fig. 4-3. Alignment of the SAT protein of the freshwater cyanobacterium *Synechocystis* sp. PCC 6803 and the SAT protein of *A. thaliana*.

Identical amino acids are marked with a vertical line; equivalent amino acids are marked with a semicolon; positions with lower identity are identified by a point.

#### 4.1.4.2. Alignment of the SAT sequences of green algae with *A. thaliana* SAT isoforms

Two of the *A. thaliana* SAT sequences (SAT-1, SAT-3) had an amino-terminal extension which was not present in *C. reinhardtii* SAT1. The alignment data indicated that *C. reinhardtii* SAT1 and SAT2 had an N-terminal extension (2-95 aa) that was not present in the other three *A. Thaliana* sequences. *C. reinhardtii* SAT sequences also showed a C-terminus extension of 4-117 amino acid residues. The identity of the two *C. reinhardtii* SAT sequences to the five SAT isoforms of *A. thaliana*, ranged between 50.2% and 59.1%; if the equivalent amino acids were included, the homology increased to 71.7-78.7%. Only one of the three SAT sequences of *T. suecica* carried an amino-terminal extension (around 20 aa), which was not present in one of *A. thaliana* SAT sequences. Also, the *T. suecica* SAT sequence showed a shorter C-terminus than *A. thaliana* (Table. 4-3). The identity of the three *T. suecica* SATs to the five SAT



isoforms of *A. thaliana*, ranged between 48.6% and 62.7%; it increased to 67.9-76.3% if the equivalent aa were considered identical (Table. 4-4), (See supplementary data for details).

#### **4.1.4.3. Alignment of the SAT sequences of red algae and red-lineage algae with *A. thaliana* SAT isoforms**

*A. thaliana* SAT sequences carried an N-terminus extension (45-83 aa) (Table. 4-3) that was not present in the *C. merolae* strain 10D. *C. merolae* strain 10D showed a 4-30 aa C-terminus extension. The identity of *C. merolae* strain 10D SAT sequences to the five SAT isoforms of *A. thaliana* ranged between 45.3% and 57.0% (65.8%-74.3% with equivalent amino acids). *T. pseudonana* CCMP 1335 SAT had an N-terminal extension (199-295 aa), which was not present in three out of five *A. thaliana* SAT sequences. *T. pseudonana* CCMP 1335 also had a C-terminal extension.

The identity of *T. pseudonana* CCMP 1335 SAT with *A. thaliana* isoforms ranged between 46.7% and 52.1% (70.9%-74.2% with equivalent amino acids). The SAT sequence of the diatom *P. tricornutum* CCAP 1055/1 carried N-terminus (164-231 aa) and C-terminus (16-127 aa) extension compared to *A. thaliana* sequences (Table. 4-3). The identity of the *P. tricornutum* CCAP 1055/1 SAT with the five *A. thaliana* isoforms ranged between 26.3% and 50.8% (41.9%-72.6% with equivalent amino acids) (Table. 4-4). *A. thaliana* SAT sequences carried N-terminus (13-18 aa) and C-terminus (21-26 aa) extensions that were not present in the *E. huxleyi* CCMP 1516 sequence (Table. 4-3). The identity of *E. huxleyi* CCMP 1516 SAT to the five *A. thaliana* sequences ranged between 44.1% and 47.7% (58.8%-61.1% with the equivalent amino acids) (Table. 4-4), (See supplementary data for details).

Table. 4-3. Comparison of N'-terminus and C'-terminus sequences of algal SAT the corresponding regions as *A. thaliana* SAT isoforms

	SAT1- <i>A. thaliana</i>		SAT2- <i>A. thaliana</i>		SAT3- <i>A. thaliana</i>		SAT4- <i>A. thaliana</i>		SAT5- <i>A. thaliana</i>	
	N'-terminal	C'-terminal	N'-terminal	C'-terminal	N'-terminal	C'-terminal	N'-terminal	C'-terminal	N'-terminal	C'-terminal
<i>Synechocystis</i> sp. PCC 6803	-140	+6	-103	+1	-192	+27	-115	+27	-112	+27
<i>Synechococcus</i> sp. WH 7803	-140	+17	-103	+12	-192	+29	-116	+29	-112	+29
1- <i>C. reinhardtii</i>	-6	-17	+86	-22	-34	+4	+20	+4	+42	+4
2- <i>C. reinhardtii</i>	+2	+95	+64	+90	-11	+117	+22	+117	+41	+117
1- <i>T. suecica</i>	-14	-21	+17	-26	-75	0	-15	0	-6	0
2- <i>T. suecica</i>	-17	-32	+20	-37	-74	-11	-15	-11	-5	-11
3- <i>T. suecica</i>	-68	-35	-31	-40	-120	-14	-14	-14	-40	-14
1- <i>C. merolae</i> strain 10D	+46	+4	+83	-3	0	+30	+45	+30	+70	+30
2- <i>C. merolae</i> strain 10D	+40	+16	+77	+11	0	+22	+39	+22	+69	+22
<i>T. pseudonana</i> CCMP 1335	+263	-17	+295	-26	+199	+5	+263	+5	+278	+5
1- <i>P. tricornutum</i> CCAP 1055/1	+208	-16	+240	-21	+164	+8	+228	+8	+228	+8
2- <i>P. tricornutum</i> CCAP 1055/1	+205	-122	+243	-127	+168	-107	+231	-106	+231	-107
<i>E. huxleyi</i> CCMP 1516	+14	-21	+18	-26	-45	0	+13	0	+18	0

In this table, the positive numbers indicate the numbers of additional residues in algae sequences when aligned with their counterparts, the negative numbers indicate the number of residues missing in algae sequences when aligned with *A. thaliana* sequences.

Table. 4-4. Identity and Similarity of algal SAT protein sequence with *A. Thaliana* isoforms

	SAT1- <i>A. thaliana</i>		SAT2- <i>A. thaliana</i>		SAT3- <i>A. thaliana</i>		SAT4- <i>A. thaliana</i>		SAT5- <i>A. thaliana</i>	
	Identity	Similarity	Identity	Similarity	Identity	Similarity	Identity	Similarity	Identity	Similarity
<i>Synechocystis</i> sp. PCC 6803	42.2 %	61.4 %	41.3 %	61.0 %	42.4 %	60.3 %	44.6 %	63.6 %	46.7 %	63.5 %
<i>Synechococcus</i> sp. WH 7803	42.8 %	62.0 %	42.8 %	62.0 %	40.1 %	56.3 %	46.4 %	60.8 %	47.9 %	64.2 %
1- <i>C. reinhardtii</i>	55.1 %	76.7 %	55.3 %	75.1 %	50.2 %	71.7 %	51.9 %	72.2 %	54.4 %	73.0 %
2- <i>C. reinhardtii</i>	62.2 %	78.7 %	59.8 %	75.0 %	55.7 %	73.4 %	55.3 %	74.7 %	59.1 %	78.5 %
1- <i>T. suecica</i>	48.6 %	72.1 %	49.8 %	70.1 %	49.8 %	69.0 %	48.3 %	68.5 %	54.6 %	75.0 %
2- <i>T. suecica</i>	62.7 %	76.3 %	60.7 %	73.8 %	53.6 %	67.9 %	55.0 %	70.8 %	55.4 %	73.8 %
3- <i>T. suecica</i>	62.7 %	76.3 %	60.7 %	73.8 %	53.6 %	67.9 %	55.0 %	70.8 %	55.4 %	73.8 %
1- <i>C. merolae</i> strain 10D	51.8 %	68.2 %	50.6 %	67.5 %	49.4 %	72.0 %	45.3 %	65.8 %	54.0 %	70.5 %
2- <i>C. merolae</i> strain 10D	54.8 %	74.3 %	57.0 %	72.6 %	52.2 %	69.8 %	47.7 %	67.0 %	53.7 %	68.2 %
<i>Trpseudonana</i> CCMP 1335	52.1 %	71.8 %	52.1 %	71.4 %	46.7 %	74.2 %	47.7 %	73.2 %	50.6 %	70.9 %
1- <i>P. tricomutum</i> CCAP 1055/1	50.4 %	70.5 %	50.8 %	70.5 %	45.7 %	70.2 %	47.7 %	72.6 %	50.8 %	68.4 %
2- <i>P. tricomutum</i> CCAP 1055/1	26.3 %	52.6 %	29.7 %	45.9 %	28.0 %	48.0 %	36.0 %	44.0 %	29.0 %	41.9 %
<i>E. huxleyi</i> CCMP 1516	47.1 %	59.3 %	47.7 %	59.9 %	44.1 %	58.8 %	45.6 %	61.1 %	45.9 %	59.7 %

#### **4.1.4.4. Alignment of the OAS-TL sequence of Cyanobacteria with OAS-TL isoforms of *A. thaliana***

*A. thaliana* contains four major OAS-TL isoforms (A, B, C, C1), (García et al., 2014). The sequences of these enzymes carried N-terminus (2-110 aa) and C-terminus (1-13 aa) extensions (Table. 4-5) that were not present in the cyanobacterial sequences. The identity of the two OAS-TL sequences of *Synechosystis* sp. PCC 6803 to the four OAS-TL isoforms of *A. thaliana* ranged between 34.0% and 61.1% (52.3%-78.5%, with equivalent amino acids) (Table. 4-6). Also the OAS-TL sequence of the marine cyanobacterium *Synechococcus* sp. WH 7803 differed at the N-terminus, in positions 1-110, relative to *A. thaliana* OAS-TL sequences (Table. 4-5). In contrast, *Arabidopsis* and *Synechococcus* had C-terminus domains of similar length. The identity of the two OAS-TL sequences of *Synechococcus* sp. WH 7803 to the four OAS-TL isoforms of *A. thaliana* ranged between 48.5% and 57.6% (66.3%-74.8%, if the equivalent amino acids were considered) (Table. 4-6), (See supplementary data for details).

#### **4.1.4.5. Alignment of the OAS-TL sequence of green algae with OAS-TL isoforms of *A. thaliana***

When the three OAS-TL sequences of the green algae *C. reinhardtii* were compared to *A. thaliana* isoforms, it was observed that one of the *C. reinhardtii* OAS-TL sequences contained an N-terminal extension (36-72 aa) that was not present in *A. thaliana*-A. On the other hand, *A. thaliana* OAS-TL (B, C, C1) sequences carried an N-terminal extension (2-80 aa) that was mostly not present in *C. reinhardtii* OAS-TL sequences. *C. reinhardtii* had a C-terminus extension (2-20 aa) that was not observed

in *Arabidopsis* (Table. 4-5). The identity between *C. reinhardtii* and *A. thaliana* OAS-TL was between 29.7% and 74.1% (Table. 4-6) (50.0%-83.5% including the equivalent amino acids) (Table. 4-6), (See supplementary data for details).

*A. thaliana* OAS-TL sequences carried an N-terminal extension (5-113 aa) (Table. 4-5), which was not present in *Tetraselmis suecica* sequence. *A. thaliana* OAS-TL sequences carried a C-terminal extension (4-22 aa) that was partially 3 aa present in only one of the *Tetraselmis suecica* OAS-TL isoforms (Table. 4-5). The amino acid identity between *Tetraselmis suecica* and *A. thaliana* OAS-TL ranged between 28.9% and 66.6% (48.2%-80.4% with the equivalent amino acids) (Table. 4-6), (See supplementary data for details).

#### **4.1.4.6. Alignment of the OAS-TL sequence of red algae and red-lineage algae with OAS-TL isoforms of *A. thaliana***

The *C. merolae* strain 10D OAS-TL sequences, when compared to OAS-TL-A of *A. thaliana* had an additional sequence (44-62 aa) at the N-terminus (Table. 4-5). When *C. merolae* OAS-TL sequences were aligned with the other *A. thaliana* OAS-TL sequences, the embryophytes sequences had an extension at the N-terminus (1-42 aa). *C. merolae* sequences also contained a C-terminal extension (3-28 aa) as compared to *A. thaliana* sequences (Table. 4-5). The identity between *C. merolae* OAS-TL and those of *A. thaliana* ranged between 34.7% and 64.2% (54.5%-80.4% with the equivalent aminoacids) (Table. 4-6), (See supplementary data for details).

*T. pseudonana* CCMP 1335 alignment with AtOAS-TL-A showed additional amino acids (10 aa and 42 aa) in the diatom at the N-terminus. In all other cases, *A. thaliana* OAS-TL had an amino-terminal extension (5-113 aa) (Table. 4-5). One of *T. pseudonana* OAS-TL contained additional aa at the C-terminus (8-11 aa) (Table. 4-5), while the other sequences showed a 1-18 aa C-terminal extension in *A. thaliana*

OAS-TL. The amino acid identity of *T. pseudonana* CCMP 1335 to *A. thaliana* isoforms varied between 33.7% and 63.0% (53.5%-77.4% with the equivalent amino acids) (Table. 4-6), (See supplementary data for details).

*P. tricornutum* 1055/1 sequences missed a section at the N-terminus that was present in *A. thaliana* (1-106 aa), while the C-terminus between these two species more or less similar (Table. 4-5). The identity of *P. tricornutum* and *A. thaliana* OAS-TL was between 36.2% and 50.8% (52.4%-79.7% if equivalent amino acids were considered identical) (Table. 4-6), (See supplementary data for details).

In *E. huxleyi* CCMP 1516 OAS-TL there was a 22 aa extension at the N-terminus compared to OAS-TL-A. While, the other three OAS-TL sequences of *A. thaliana*, which, in turn had amino acids at the N-terminus (27-79 aa) that were mostly not present in *E. huxleyi* enzyme (Table. 4-5). At the C-terminus, *E. huxleyi* contained a 25 aa amino acid extension, as compared to *A. thaliana* (Table. 4-5). The OAS-TL amino acid identity between *E. huxleyi* CCMP 1516 and *A. thaliana* ranged between 38.1% and 43.0% (53.5%-57.3% if equivalent amino acids were counted) (Table. 4-6), (See supplementary data for details).

When the OAS-TL sequences of *A. thaliana* were compared with those of the dinoflagellate *Amphidinium klebsii*, the embryophyte was found to have an N-terminal extension (4-112 aa) (Table. 4-5). In contrast, the C-terminus showed additional amino acids (1-3 aa; 1-16 aa, depending on isoforms) in *A. klebsii* sequence (Table. 4-5). The OAS-TL amino acid identity between *A. klebsii* and *A. thaliana* was between 32.3% and 39.5% (50.5%-56.8 considering equivalent aa as identical) (Table. 4-6), (See supplementary data for details).

Table. 4-5. Alignment of N'-terminus and C'-terminus sequences of algal OAS-TL corresponding regions in *A. thaliana* OAS-TL isoforms

	<i>A-A. thaliana</i>		<i>B-A. thaliana</i>		<i>C-A. thaliana</i>		<i>C1-A. thaliana</i>	
	N'-terminal	C'-terminal	N'-terminal	C'-terminal	N'-terminal	C'-terminal	N'-terminal	C'-terminal
1- <i>Synechocystis</i> sp. PCC 6803	+17	-10	-53	-10	-91	-13	-38	-12
2- <i>Synechocystis</i> sp. PCC 6803	-2	-1	-72	-1	-110	-4	-46	-3
1- <i>Synechococcus</i> sp. WH 7803	-2	0	-72	0	-110	-3	-46	-2
2- <i>Synechococcus</i> sp. WH 7803	-1	+1	-71	+1	-101	-2	-45	-1
1- <i>C. reinhardtii</i>	+72	+2	-8	+20	-41	+17	+2	+18
2- <i>C. reinhardtii</i>	+36	+9	-17	+9	-50	+6	-7	+7
3- <i>C. reinhardtii</i>	+50	+15	-10	+15	-43	+12	0	-7
4- <i>C. reinhardtii</i>	+35	+2	-51	+2	-89	-1	-19	0
1- <i>T. suecica</i>	-5	-8	-75	-8	-113	-11	-49	-10
2- <i>T. suecica</i>	+9	-4	-61	-4	-101	-7	-37	-6
3- <i>T. suecica</i>	-2	-4	-72	-22	-110	-21	-46	-20
4- <i>T. suecica</i>	-10	+3	-80	+3	-118	0	-54	1
1- <i>C. merolae strain 10D</i>	+62	+3	-1	+3	-34	0	-11	+1
2- <i>C. merolae strain 10D</i>	+44	+21	-9	+21	-42	+11	-4	+28
1- <i>T. pseudonana</i> CCMP 1335	+10	+11	-63	+11	-101	+8	-39	+9
2- <i>T. pseudonana</i> CCMP 1335	+42	-1	-6	-1	-39	-4	-2	-3
3- <i>T. pseudonana</i> CCMP 1335	-5	-15	-72	-15	-113	-18	-49	-7
1- <i>P. tricornutum</i> CCAP 1055/1	+2	-1	-68	-1	-106	-4	-43	-3
2- <i>P. tricornutum</i> CCAP 1055/1	+10	+3	-60	+3	-98	0	-37	+1
<i>E. huxleyi</i> CCMP 1516	+22	+24	-42	+26	-79	+26	-27	+23
1- <i>A. klebsii</i>	-4	+3	-72	+3	-112	0	-48	+1
2- <i>A. klebsii</i>	-7	-14	-75	-14	-115	-17	-51	-16
3- <i>A. klebsii</i>	+5	+3	-115	+3	-103	0	-39	+1
4- <i>A. klebsii</i>	0	+1	-51	+1	-108	-2	-44	-1

In this table, the positive numbers indicate the numbers of additional residues in algae sequences when aligned with their counterparts, the negative numbers indicate the number of residues missing in algae sequences when aligned with *A. thaliana* sequences.

Table. 4-6. Identity and Similarity of algae OAS-TL with reference species of *A. thaliana* isoforms

	<i>A-A. thaliana</i>		<i>B-A. thaliana</i>		<i>C-A. thaliana</i>		<i>C1-A. thaliana</i>	
	Identity	Similarity	Identity	Similarity	Identity	Similarity	Identity	Similarity
1- <i>Synechocystis</i> sp. PCC 6803	60.6 %	76.5 %	61.1 %	78.5 %	60.6 %	76.6 %	53.6 %	71.2 %
2- <i>Synechocystis</i> sp. PCC 6803	37.7 %	53.7 %	36.8 %	52.3 %	38.0 %	53.0 %	34.0 %	52.3 %
1- <i>Synechococcus</i> sp. WH 7803	55.2 %	71.9 %	55.2 %	73.7 %	56.5 %	74.4 %	48.5 %	68.3 %
2- <i>Synechococcus</i> sp. WH 7803	57.3 %	72.9 %	57.6 %	74.8 %	55.7 %	74.5 %	50.6 %	66.3 %
1- <i>C. reinhardtii</i>	64.6 %	80.0 %	64.4 %	82.8 %	65.0 %	83.2 %	57.9 %	76.5 %
2- <i>C. reinhardtii</i>	34.2 %	51.7 %	29.7 %	50.0 %	33.2 %	51.2 %	33.0 %	50.3 %
3- <i>C. reinhardtii</i>	66.7 %	83.5 %	65.6 %	79.8 %	68.4 %	82.2 %	56.9 %	74.5 %
4- <i>C. reinhardtii</i>	74.1 %	86.1 %	69.5 %	84.9 %	73.4 %	86.7 %	61.6 %	77.1 %
1- <i>T. suecica</i>	35.7 %	57.2 %	33.0 %	55.9 %	34.3 %	56.9 %	34.9 %	53.7 %
2- <i>T. suecica</i>	66.2 %	79.0 %	66.6 %	80.4%	64.2 %	78.6 %	59.0 %	74.3 %
3- <i>T. suecica</i>	34.1 %	54.0 %	32.7 %	54.0%	31.6 %	48.4 %	28.9 %	48.2 %
4- <i>T. suecica</i>	46.0 %	61.5 %	44.3 %	60.6%	45.4 %	61.4 %	42.8 %	55.9 %
1- <i>C. merolae</i> strain 10D	64.2 %	80.4 %	59.5 %	77.2 %	61.9 %	78.3 %	54.8 %	70.1 %
2- <i>C. merolae</i> strain 10D	35.8 %	52.8 %	35.2 %	52.8 %	38.1 %	55.0 %	34.7 %	54.5 %
3- <i>C. merolae</i> strain 10D	43.9 %	57.5 %	41.4 %	57.2 %	41.1 %	58.9 %	38.5 %	54.7 %
1- <i>T. pseudonana</i> CCMP 1335	40.6 %	56.6 %	39.7 %	57.6 %	40.4 %	58.7 %	37.7 %	55.6 %
2- <i>T. pseudonana</i> CCMP 1335	61.9 %	75.0 %	63.0 %	77.4 %	62.4 %	77.4 %	54.5 %	71.3 %
3- <i>T. pseudonana</i> CCMP 1335	37.6 %	54.0 %	35.5 %	57.0 %	36.6 %	57.0 %	33.7 %	53.5 %
1- <i>P. tricornutum</i> CCAP 1055/1	51.0 %	67.5 %	47.5 %	65.6 %	49.3 %	65.9 %	42.0 %	61.6 %
2- <i>P. tricornutum</i> CCAP 1055/1	43.5 %	58.7 %	40.4 %	57.4 %	42.0 %	57.1 %	36.2 %	52.4 %
3- <i>P. tricornutum</i> CCAP 1055/1	61.7 %	75.7 %	63.2 %	78.9 %	64.7 %	79.7 %	54.6 %	72.2 %
<i>E. huxleyi</i> CCMP 1516	43.0 %	57.3 %	40.2 %	57.1 %	39.8 %	56.0 %	38.1 %	53.5 %
1- <i>A. klebsii</i>	39.5 %	56.8 %	38.5 %	57.4 %	38.2 %	58.0 %	36.1 %	53.4 %
2- <i>A. klebsii</i>	37.4 %	56.3 %	37.0 %	53.8 %	35.9 %	54.9 %	35.7 %	55.1 %
3- <i>A. klebsii</i>	38.8 %	54.7 %	37.3 %	53.7 %	36.3 %	53.1 %	32.4 %	48.3 %
4- <i>A. klebsii</i>	36.6 %	53.9 %	36.5 %	54.0 %	36.4 %	53.5 %	32.3 %	50.5 %



## 4.1.5. Main motives of SAT and OAS-TL algal sequences

### 4.1.5.1. SAT sequences main motives in algae

The SAT protein, apparently, share with the embryophyte enzyme a C-terminal hexapeptide-repeat domain. This domain is common in a number of acyltransferases and is believed to be involved both in catalysis and hetero-oligomerization (i.e. formation of the Cysteine Synthase Complex) (Yeon et al., 2018). Our sequences alignment revealed a partial but long consensus sequence, with the following interesting features: i) a peculiar six-residue periodicity can be found in the consensus region; ii) each of the six hexapeptide units starts with isoleucine (I), leucine (L) or valine (V); iii) in four of the units, the second residue is glycine (G) and the fifth residue isalanine (A) or valine (V).

Fig. 4-4 showed the alignment of algal SATs and the five *Arabidopsis thaliana* SAT isoforms. The alignment revealed the similarity in the structures of algal and embryophyte enzymes. The six-residue periodical amino acid sequence of algae SAT was (-I/V/L)-G-XXXX-(I/V/L)-, thus very similar in structure to that of *Arabidopsis thaliana* (Vuorio et al., 1991; Vuorio & Taina, 1994; Bogdanova & Hell, 1997; Pye, et al., 2004). In 1997, Bogdanova and Hell proved through truncated proteins experiments that the C-terminus of SAT was necessary for the interaction with OAS-TL. Also, this domain appeared to be associated with the putative transferase activity (Bogdanova & Hell, 1997). The hexapeptide domain was also found to be responsible for the formation of  $\beta$ -turns (Vuorio, 1991). In agreement with Bogdanva and Hell (1997) and based on the structural similarity suggested by the sequence alignments, it seems logical to assume that the catalytically functional domain of algae SATs is the central part of the sequence, where the region with the highest similarity to the *Arabidopsis thaliana* proteins was.

SAT1-A.thaliana 1 -----  
 SAT2-A.thaliana 1 -----  
 SAT3-A.thaliana 1 -----  
 SAT4-A.thaliana 1 -----  
 SAT5-A.thaliana 1 -----  
 Synechocystis 1 -----  
 Synechococcus 1 -----  
 1-C.reinhardtii 1 -----  
 2-C.reinhardtii 1 -----M-----  
 1-T.suecica 1 -----  
 2-T.suecica 1 -----  
 3-T.suecica 1 -----  
 1-C.merolae 1 -----  
 2-C.merolae 1 -----  
 T.pseudonana 1 MDNTKKYLQAVLLTAPMLMALAFTPSISTVMSVHQYSSNQCDGFLPPSRARIRHYSSPRQ  
 1-P.tricornutum 1 -----MVAVLVLTLSSLELVNGWIHQCTVMSLYIRR-----PTVSASLAFS-----  
 2-P.tricornutum 1 -----  
 E.huxleyi 1 -----  
 consensus 1 -----

SAT1-A.thaliana 1 -----MA  
 SAT2-A.thaliana 1 -----  
 SAT3-A.thaliana 1 -----ML  
 SAT4-A.thaliana 1 -----  
 SAT5-A.thaliana 1 -----M  
 Synechocystis 1 -----  
 Synechococcus 1 -----  
 1-C.reinhardtii 1 -----M  
 2-C.reinhardtii 2 -----SF  
 1-T.suecica 1 -----  
 2-T.suecica 1 -----  
 3-T.suecica 1 -----  
 1-C.merolae 1 -----MF  
 2-C.merolae 1 -----MF  
 T.pseudonana 61 RCCYQPTLLHNYKSSDDSHKNSTTPSTWQSSLKDLVSQIDSKPSTDDDEQPQSRMDLVPQI  
 1-P.tricornutum 43 -----HVTTHLDEHDM-----EF  
 2-P.tricornutum 1 -----MD  
 E.huxleyi 1 -----  
 consensus 61 -----

SAT1-A.thaliana 3 CING-----ENRDFSSSS--SLSSLP-----MIVS-----  
 SAT2-A.thaliana 1 -----  
 SAT3-A.thaliana 3 PVTS-----RRHFTMSLYMLRSSSPHINHH-----S-----  
 SAT4-A.thaliana 1 -----  
 SAT5-A.thaliana 2 PP-----AGELRHQSPS--KEK-----  
 Synechocystis 1 -----  
 Synechococcus 1 -----  
 1-C.reinhardtii 2 RSVFS----D-----TCSAAPSTSRP---HAVSVIPSRPRRYA-GGRT-----  
 2-C.reinhardtii 4 PCLG-----SKHALSGAS--SRAQQS-----VVA-----  
 1-T.suecica 1 --ES-----LEWVKDAIP-----  
 2-T.suecica 1 -----  
 3-T.suecica 1 TWASP----D-----KAGNLPAYSNG---RPVTVLPR-----  
 1-C.merolae 3 SCLTS--V-----AQNGRLQRTS---KQVPLVARERRRHRLAVRT-----  
 2-C.merolae 3 ATTG-----RLYQTLRVHPSHRALQSVRKRVR---SR-----  
 T.pseudonana 121 DAKSSQEDQEHRRPKYQLGLGKNRPLKSS---NASMSTADVVDVQSKQPDSSPSENKDES  
 1-P.tricornutum 56 GANRTLETSTG-TTRYDLGIGKNAPLGS---SNTTIPAMLAGPSPDPNVPTPVATESTQ  
 2-P.tricornutum 3 DSGQ-----TYTVLNYPLENGQVLAEAQLRYQTYG-Q--L-----  
 E.huxleyi 1 --ML-----RFSRR--TPLP---AARRACS-----  
 consensus 121 -----

SAT1-A.thaliana 26 -----RNFSA-----R-DDGETGDEFPPERIFP-----  
 SAT2-A.thaliana 1 -----MNGDELPFESGFE-----  
 SAT3-A.thaliana 29 -----FLLPSFVSSKFKHHTL-SP-----PPSPPP-----PPPMAA-----  
 SAT4-A.thaliana 1 -----  
 SAT5-A.thaliana 17 -----LSSV-----T-QS-----DEAE---A--A--SA-----  
 Synechocystis 1 -----  
 Synechococcus 1 -----  
 1-C.reinhardtii 37 -----LGGHTLLSS-----R-NV-----GSVAVNTMARDRLSAQ-----  
 2-C.reinhardtii 26 -----RVWF-----RGGSGRRCSFVLASNTEPASSKPSKA-----  
 1-T.suecica 12 -----AYLTS-----R-SECNSN-----FD  
 2-T.suecica 1 -----  
 3-T.suecica 26 -----GNDNMFSS-----  
 1-C.merolae 39 -----LRPALESS-----L-SR-----FTEQ---E--QAFPT-----  
 2-C.merolae 32 -----GQCLRSARLQHVVRGH-GR-----SSNDGV-----SEARDS-----  
 T.pseudonana 177 DV--ASAPFQYWNAPQPVAKPITPQH-----L-NK-----TSRQRYDMGMGKHAPL-----  
 1-P.tricornutum 111 SVPFSAASRGENWVALDPVHKPSTPAS-----R-TR-----HSPHPVSEQTAKPVNG-----  
 2-P.tricornutum 35 -----NETRDNVMV-----V-C-----HALT-----GNASLH-----  
 E.huxleyi 19 -----LRLRR-----CI--SSTSSLQTTI-----  
 consensus 181 -----



SAT1-A.thaliana 48 ---VYA-----RG--T-----L-----  
 SAT2-A.thaliana 14 ---VYA-----KG--T-----H-----  
 SAT3-A.thaliana 59 CIDTCRTGKPKQISP--RDSSKHHDDDESGFR---YMNIFRY-----  
 SAT4-A.thaliana 4 CIDTCRTG-----NTQDDDSRFc-----cIKNFFR-----  
 SAT5-A.thaliana 32 -----AIS--AA-----  
 Synechocystis 1 -----  
 Synechococcus 1 -----  
 1-C.reinhardtii 65 EREELEAVRARLNQQL--VT---GVSPSASSDL--SVDERPTLL-----AKA-----  
 2-C.reinhardtii 57 SNNKMSP-----ED--SI---DWTRAGVPAY-----L-----  
 1-T.suecica 25 ---QWL-----RD--AS---TVSTGGE-----  
 2-T.suecica 1 -----  
 3-T.suecica 34 -----S-----  
 1-C.merolae 60 LKADAKPK-----T--AS---NSRTAGVFNRR--LKEE-----EGAcFVEFL-----  
 2-C.merolae 62 AIEHITD---T-----RSETSRSAAVGARVVWPLGS-----  
 T.pseudonana 220 GETTNRRTGNTVTKQHLLT--TG---DEESARLTKA--VWDKGFHFHKDGASSLENG-----  
 1-P.tricornutum 157 LSSTQKPAPTTSTR--MV--AR---DNRSAKLRAA--LWDEGHYQKHEPPLLSPHPHDPT-----  
 2-P.tricornutum 56 AWWGDMLG-----P--GK---VFDTDK-----  
 E.huxleyi 36 -----  
 consensus 241 -----

SAT1-A.thaliana 55 -----NPV--ADPV-----LLDF-----T-----  
 SAT2-A.thaliana 21 -----KSE--FDSN-----L-----L-----  
 SAT3-A.thaliana 94 -----PDRSSFNGTQTKLHTRPILLED-----LD-----  
 SAT4-A.thaliana 29 -----PG-FSVN---RKIHHTQIED-----  
 SAT5-A.thaliana 37 -----A--A-----  
 Synechocystis 1 -----  
 Synechococcus 1 -----  
 1-C.reinhardtii 107 -N-----NCP--cEDEVHPSNGW--RPMP-----H-----  
 2-C.reinhardtii 79 -----QSN--VDFNI--cGEESQALT-----L-----  
 1-T.suecica 39 -----QMSQAIS-----E-----  
 2-T.suecica 1 -----  
 3-T.suecica 35 -----GDAY--QPQE-----H-----  
 1-C.merolae 94 -R-----RAV--EDcRRNVQAGG-----  
 2-C.merolae 90 -----PPQ--IGGGFGPVISVDDMVRT-----LT-----  
 T.pseudonana 268 -SVVVQKSSLPIAS--PDPI---ANSAEDVHIGDSSQSTGVTNYQQIDLSIPPSVYSLRcN  
 1-P.tricornutum 208 TSTAVADTTKQSSSTVFNH--EIVQRSTTITADGSPPTAFYFDIDLSIPDSVY-----  
 2-P.tricornutum 73 -----  
 E.huxleyi 36 -----  
 consensus 301 -----

SAT1-A.thaliana 67 ----NSSYDFIWDIIRREAKLEAE----EPPVLSSEFLYASILSHDCLEQALSFVLANRL  
 SAT2-A.thaliana 30 ----DPRSDFIWDIIRREAKLEAE----KEPILSSFLYAGILAHDCLEQALGFVLANRL  
 SAT3-A.thaliana 118 ----RDAEVDVWAKIIRREAKSDIA----KEPIVSAVYHSAIVSQRSLERLANTLSVKL  
 SAT4-A.thaliana 45 ----DDVWIKMLLEAKSDVK----CEPILSNMYASITSHRSLESALAHILSVKL  
 SAT5-A.thaliana 39 ----DAEAAGIWTQIKAEARRDAE----KEPILASMYLSTIILSHSSLERSITSHFLGNKL  
 Synechocystis 1 -----  
 Synechococcus 1 -----  
 1-C.reinhardtii 127 ----HLSKPELWERIRCEAQMDAS----SEPLASNLFSITLHPSLEKSMFAFLANKL  
 2-C.reinhardtii 97 ----GRAGEMMKQIRTEAQADAN----SEPLSSFLYASIIAHDTFEQALAFVLANRL  
 1-T.suecica 48 ----MMKKSFWLIRSEAGRDAE----KEPILSSFLWGSILSHDFERLAFILANRL  
 2-T.suecica 1 ----FWWLIRSEAGRDAE----KEPILSSFLWGSILSHDFERLAFILANRL  
 3-T.suecica 44 ----HKDTPALWERIRSEARSDSD----TERSLASLHSTILVHHSLAKTMAFVLANKL  
 1-C.merolae 109 ----HGTEDEWVERVRLAEAAAR----EECLLASFLYATVLNHDLEACLAFHLANKL  
 2-C.merolae 112 ----YSSDFVWELVRREAEIGAA----NEPLASFLYATVLNHRCLDLELAFHLANKL  
 T.pseudonana 323 NETASSQFVDFVWDLMRHEAQEAQ----REPILVSELYSTILNHPLELAFHLANKL  
 1-P.tricornutum 261 ----ADDGSVDLWDLIRWDAKCEAQ----REPILVSELYSTILNHPLELAFHLANKL  
 2-P.tricornutum 73 ----YLVVcNcILGScYGSTSc----PVSIRPGTDQPYGLDFPVSVKDITVRLcQcM  
 E.huxleyi 36 ----DTQSTWSKLQSEAREAIASSVVKRGGYGLRDLIQRVLSHGSLADGLSATIcAKL  
 consensus 361 -----

SAT1-A.thaliana 118 QN-----PTLLATQLMDIFcNVMVHDRGIQSSIRLDVcAGKDRDP  
 SAT2-A.thaliana 81 QN-----PTLLATQLLDIFcYGVMMHDKGIQSSIRHDVcAGKDRDP  
 SAT3-A.thaliana 170 SN-----LNLPSNTLEDFEGVLOGNPFIVESVKLDLL-AVKERDP  
 SAT4-A.thaliana 93 SN-----LNLPSNTLEDFELFSVLEESPEIIESTKQDLI-AVKERDP  
 SAT5-A.thaliana 90 cS-----STLLSTLMDLFLNFTSSDESLRNATVADIR-AAVVRDP  
 Synechocystis 1 -----MLNSLIADFR-IIEBRDP  
 Synechococcus 1 -----MFNHIRADIA-IIRERDP  
 1-C.reinhardtii 178 AN-----PTMLGMQLMRLISEAYEDDAGLIEAcMADLcAVVDRDP  
 2-C.reinhardtii 148 AN-----STMLSTQLFEIFHNFLSKEFDVRCALSLDLA-ACRERDP  
 1-T.suecica 99 AD-----ATMLPTELEDFDYDTLKTSPETVFBASMQcCQ-AAERDP  
 2-T.suecica 47 AD-----ATMLPTELEDFDYDTLKTSPETVFBASMQcCQ-AAERDP  
 3-T.suecica 95 QS-----HTLPATHLLHLFQEAFNDDPDIMAAVVADMN-AVDRDP  
 1-C.merolae 160 AS-----TTLPSMTLNEIIRALEKAFEARVAIRLDL-AVADRDP  
 2-C.merolae 162 AS-----PFFQNTQYVKLFRDALYQDKSYREAIRADLL-AVVRDP  
 T.pseudonana 379 ES-----SAMLSTQVMBIVREARDGDEEFQRNLRADIM-AVDRDP  
 1-P.tricornutum 314 QS-----PAMMISTQLQSLIVASLQRcEIFRRLRADLM-AVDRDP  
 2-P.tricornutum 121 LR-----DEPKVASVHNAVVGSGFGMQAVFPAVcAGST-RAAFTDA  
 E.huxleyi 89 EDGATGLDYAAMcSAAYAADA-SIVDRAAADLERASVHALATcVSSAVVGEALTVVcEDA  
 consensus 421 -----



SAT1-A. thaliana 158 A ALSVSSAIIHLKGYEALQAYRVAHKLWQ---G-R-KLLALALQSRVSEVFGIDIHP--  
 SAT2-A. thaliana 121 A ALSVSSAIIHLKGYEALQAYRVAHKLWNE---G-R-KLLALALQSRVSEVFGIDIHP--  
 SAT3-A. thaliana 210 A CISVWHCELFKGFELACQAHRIAHLELWQ---D-R-KLLALALQSRVSEVFAVDIHP--  
 SAT4-A. thaliana 133 A CISVWHCELFKGFELACQAHRIAHLELWQ---N-R-KIVALLQSRVSEVFAVDIHP--  
 SAT5-A. thaliana 130 A CISVSHCLLNKGFELACQAHRIASHKLLWQ---S-R-KLLALALQSRVSDVFAVDIHP--  
 Synechocystis 18 AARNWLEVLFCYEGCALIHRFSHELTYL---G-L-PPFPRLMSHLARFFFTGIEIHP--  
 Synechococcus 18 AARGPLETILCYEGCALSLHRLSHELWHS---RLPKLKLARLLSOLGRNLTGVEIHP--  
 1-C. reinhardtii 218 ACDSESQAMLYFKGFALOCORVAHMLWQK---G-R-KLLALALQSRMSEVFEVDIHP--  
 2-C. reinhardtii 188 AASSSHALLFKGYEALQTRLAHALWNR---K-Q-KVMALALQSRVSEVFAVDVHP--  
 1-T. suecica 139 AARGMSDALLYKGFHVAQACRAHVWLWR---G-R-TVLALALQSKVSEVLAIDIHP--  
 2-T. suecica 87 AARGMSDALLYKGFHVAQACRAHVWLWR---G-R-TVLALALQSKVSEVLAIDIHP--  
 3-T. suecica 135 AAEKMSHCMLNFKGFALQSYRISHWLFK---N-R-RALASALQSRVSEVFEVDIHP--  
 1-C. merolae 200 AATRVLDALLFKGFHALQTRVAHMLWQ---N-R-QALAMLYSVCVCKLQIDIHP--  
 2-C. merolae 202 AMKHCVAVLMYKGYEALQAYRLAHLWQ---D-R-KVLALFLOSEISKVFAVDIHP--  
 T. pseudonana 419 AATCLPDVFLFKGFHALQSYRISNYLWRS---G-R-RVLAQYLOSQVSOVFCIDIHP--  
 1-P. tricornutum 355 AVQSLPDVFLFKGFHALSHRVAHMLWKK---QN-K-RVLAQYLOSQVSOVFCIDIHP--  
 2-P. tricornutum 161 HGQPLCKHVPEI---ACG-AQHSAMQCAISEVCRQRAIYQDP  
 E. huxleyi 148 AFAGLRIYLFKGFHSVQCARVAHFVWNPNGS-G-R-MIALALQSEMSDFVGVDIHP--  
 consensus 481 ..\*.....\*

SAT1-A. thaliana 211 -----AARIGKGI-----LDHGTGVVIGETAVIGDR-----  
 SAT2-A. thaliana 174 -----AARIGKGI-----LDHGTGVVIGETAVIGNG-----  
 SAT3-A. thaliana 263 -----GAKIGKGI-----LDHATAVIGETAVVGN-----  
 SAT4-A. thaliana 186 -----GAKIGKGI-----LDHATGVVIGETAVVGN-----  
 SAT5-A. thaliana 183 -----AAKIGKGI-----LDHATGVVIGETAVIGNN-----  
 Synechocystis 71 -----GAGIGGVF-----IDHGMGVVIGETAVIGDY-----  
 Synechococcus 73 -----GARIGHVF-----IDHGMGVVIGETAEVGDR-----  
 1-C. reinhardtii 271 -----AARLGRGL-----LDHATGVVIGETAVVGN-----  
 2-C. reinhardtii 241 -----AARIGKVL-----LDHGTGVVIGETAVIGNN-----  
 1-T. suecica 192 -----AARLGRGL-----LDHGTGVVIGETAVVGN-----  
 2-T. suecica 140 -----AARLGRGL-----LDHGTGVVIGETAVVGN-----  
 3-T. suecica 188 -----GAKLGRGI-----IDHATGVVIGETAVGN-----  
 1-C. merolae 253 -----AARIGGVF-----IDHGTGVVIGETAVGN-----  
 2-C. merolae 255 -----AARIGGVM-----IDHATGVVIGETAVGN-----  
 T. pseudonana 472 -----NAEIGGVM-----LDHGTGVVIGETAHIGNN-----  
 1-P. tricornutum 409 -----NAEIGGIM-----LDHGTGVVIGETAVGN-----  
 2-P. tricornutum 198 AWPTDPERATHGLRVARQLGMISYRTPQGLGSKFGRERQRCRGDDDDTGGPAYGSHARWQV  
 E. huxleyi 204 -----AARIGGIT-----LDHGTGVVIGETAVIGNN-----  
 consensus 541 .....\*

SAT1-A. thaliana 238 -----VSILHGVTLGGTGKETG-----DRHPKIGDGLLG-----  
 SAT2-A. thaliana 201 -----VSILHGVTLGGTGKETG-----DRHPKIGEGGLLG-----  
 SAT3-A. thaliana 290 -----VSILHNVTLGGTGKCCG-----DRHPKIGDGVLLG-----  
 SAT4-A. thaliana 213 -----VSILHGVTLGGTGKQSG-----DRHPKIGDGVLLG-----  
 SAT5-A. thaliana 210 -----VSILHGVTLGGTGKCCG-----DRHPKIGDGLLG-----  
 Synechocystis 98 -----SIIYQGVTLGGTGKESG-----KRHPKIGENVVVG-----  
 Synechococcus 100 -----SIIYQGVTLGGTGKDHG-----KRHPKILANNVVG-----  
 1-C. reinhardtii 298 -----VSMLHVTLLGSGGTGKRG-----VRHPKIVGNVLLG-----  
 2-C. reinhardtii 268 -----VSILQNVTLGGTGKETG-----DRHPKIVGDNVLLG-----  
 1-T. suecica 219 -----CSILQGVTLGGTGKASC-----DRHPKIGDGVLLG-----  
 2-T. suecica 167 -----CSILQGVTLGGTGKASC-----DRHPKIGDGVLLG-----  
 3-T. suecica 215 -----VSILHVTLLGSGGTGKNG-----VRHPKIVGNVLLG-----  
 1-C. merolae 280 -----VSILHVTLLGSGTKLG-----DRHPKIVGDNVLLG-----  
 2-C. merolae 282 -----VSMLHVTLLGSGKAG-----DRHPKIVGDNVLLG-----  
 T. pseudonana 499 -----GSVLEHVTLLGSGKKG-----DRHPKIVGNVLLG-----  
 1-P. tricornutum 436 -----CSILHVTLLGSGKKG-----DRHPKIVGNVLLG-----  
 2-P. tricornutum 258 KSYLEYQGVKFLQRFDEWYVYVTEQMDSDH-----VTRQPAQSCPGTV-----  
 E. huxleyi 231 -----VIMHDVTLGATGASLHHARRTEMAGMGTSLDH-----DRHPKIGRCFLA-----  
 consensus 601 .....\*

SAT1-A. thaliana 268 ACVTILGNIRIGAGAMVAAGSIVLKDVPSHSMVAGNPAKLIGF--V-DE--Q-----DP  
 SAT2-A. thaliana 231 ACVTILGNIRIGAGAMVRAAGSIVLKDVPSHSMVAGNPAKLIRV--M-EE--Q-----DP  
 SAT3-A. thaliana 320 AGTCILGNIRIGEGAKIGAGSVLKDVPBRTAVGNPARLLGK---DN--PK--THDKLP  
 SAT4-A. thaliana 243 AGSCILGNIRIGEGAKIGAGSVVVKDVPARTAVGNPARLLGK---EN--PR--KHKLP  
 SAT5-A. thaliana 240 AGATILGNIRIGAGAVGAGSVVLDVPCRGTAAGNPARLVG-----  
 Synechocystis 128 AGAKVLGNIAGINVRIGAGSVVLDVPAFTVVGVPGRMVHPS---GERVNPLEHGKLP  
 Synechococcus 130 AGAKVLGNIIVGENTRIGAGSVVLDVPAFTVVGIPGRVHQSS---GVRINPLAHSALP  
 1-C. reinhardtii 328 AGVTVLGNIIVGAGSIVGAGSVVVDIPCHSVAVGVPARIKR--D-IV--K-----EP  
 2-C. reinhardtii 298 ACATVLGNIIVGAGSIVGAGSIVLKVPPHPTMAGVPAKIVG--V-VG--N-----EP  
 1-T. suecica 249 ANATVLGNIIVGEGACIAASLVLKDVPEPTMAGVPAKLIGR--V-EG--R-----EP  
 2-T. suecica 197 ANATVLGNIIVGEGACIAASLVLKDVPEPTMAGVPAKLIGR--V-EG--R-----EP  
 3-T. suecica 245 AGVVLGNIIVGAGSIVGAGSIVVSNLPDYCAVGVPAKIVLR--K-EG--Q-----EP  
 1-C. merolae 310 AGATILGNIRIGAGAMVAGSIVLSDLPFHSVAVGVPARVIGA--P-RT--K-----AP  
 2-C. merolae 312 AGATVLGNIIVGAGSIVGAGSIVLSDLPFHSVAVGVPARVIGA--SY--PK--G-----VYP  
 T. pseudonana 529 AGASVLGNIIVGAGSIVGAGSIVLSDLPFHSVAVGVPARVIGA--VT--A-----CP  
 1-P. tricornutum 466 AGATVLGNIIVGAGSIVGAGSIVLSDLPFHSVAVGVPARVIGA--VT--E-----CP  
 2-P. tricornutum 302 SKQVVLGNIIVGAGSIVGAGSIVLSDLPFHSVAVGVPARVIGA--H-----D  
 E. huxleyi 277 CKSTVVLGNIIVGAGSIVGAGSIVLSDLPFHSVAVGVPARVIGA--K-PN--QT--K--AA  
 consensus 661 .....\*



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SAT1-A.thaliana 317 S-----MTMEHD--ATREFFQNVAVAVRETIPNGSSVSGSCR-ERRH----
SAT2-A.thaliana 280 S-----LAKKHD--ATKEFFRHVADGYKGAQSNPGLSAGDT-EKGGH-TNS
SAT3-A.thaliana 374 G-----LTMDQT--SHISEWS-----D---Y
SAT4-A.thaliana 297 C-----LTMDQT--SYLTEWS-----D---Y
SAT5-A.thaliana 283 -KEKPTIHDEECPGESMDHT--SFISEWSD-----Y
Synechocystis 185 D-----SEGKVI--RLLLERIELLEQQVATLQQQQSEQAWES-DYRSCSET
Synechococcus 187 D-----AEANVI--RNLMERIDQLEGQVRSLSLQDNLRMTMAAAS-G-RPLREV
1-C.reinhardtii 377 V-----KEMDC-----TDYILDYT-----
2-C.reinhardtii 346 A-----LSMMHW--SQRLLSAESMDGAGGVGMNGVPLAAAMAPVNGL-ANG
1-T.suecica 297 A-----LEMHW-----
2-T.suecica 245 A-----LEMHW-----IK-----
3-T.suecica 294 N-----KTMDCI-----EYVFDYI-----
1-C.merolae 359 AFDMDQD--PTHCRKRR--TAVEHPDG-----S
2-C.merolae 364 A-----FEMDCR--AATAARVHSTNRARQHAEDSNSSTDWY---LG---D
T.pseudonana 580 S-----IGMCNL--GSKEADeg-----
1-P.tricornutum 517 S-----IGMCI--MD-ENRKI-----
2-P.tricornutum 353 GF-----LLEQECVA--AHIQHFLT-----L
E.huxleyi 324 P-----SGAPLPLDTSYVVFQGM-----
consensus 721

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SAT1-A.thaliana -----
SAT2-A.thaliana 322 TS-----
SAT3-A.thaliana 390 VI-----
SAT4-A.thaliana 313 VI-----
SAT5-A.thaliana 311 I-----
Synechocystis 228 DREPVLCRLGDREIEEFLGG-----
Synechococcus 229 RNGQAQN-LKDREIEEFLGD-----
1-C.reinhardtii 392 -----
2-C.reinhardtii 389 IAKPAKVALGKAAAAAAAAASASAPASAAASVQAAKKAAAKLGGAAKAAAAGSPAAPAG
1-T.suecica -----
2-T.suecica 254 -----
3-T.suecica 308 -----
1-C.merolae 383 SH-----SLPETGTVD----VT
2-C.merolae 401 AI-----
T.pseudonana 595 -----IV
1-P.tricornutum 531 -----VA
2-P.tricornutum 372 HE-----RPT-----
E.huxleyi 343 GI-----
consensus 781

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SAT1-A.thaliana -----
SAT2-A.thaliana -----
SAT3-A.thaliana -----
SAT4-A.thaliana -----
SAT5-A.thaliana 312 -----I-----
Synechocystis 248 -----TL-----
Synechococcus -----
1-C.reinhardtii 392 -----I-----
2-C.reinhardtii 449 KAGTPSGDVSQKQGRVARS--EVIRKKPAPEYEI
1-T.suecica -----
2-T.suecica 254 -----PIDLD-----
3-T.suecica 308 -----I-----
1-C.merolae 396 STS-----TNGHALDS-----
2-C.merolae -----
T.pseudonana 597 TFG-----MDG---I-----
1-P.tricornutum 533 TFE-----SDG---I-----
2-P.tricornutum 377 -----TI-----
E.huxleyi -----
consensus 841

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Fig. 4-4. Alignment of SAT protein of algae and *A. thaliana*.

The Cysteine residues are highlighted in yellow. Conserved amino acid was shown with dark (absolutely conserved) and grey (highly conserved residues) background. In the consensus line, asterisks indicate highly conserved amino acid. The hexapeptide motif (I/V/L)-G-XXXX-(I/V/L) is underlined in red.

The alignment sequences: SAT1 At2g17642, SAT2 At4g35642; SAT3 At5g56760; SAT4

At3g13110 SAT5 At1g55920; *Synechocystis* sp. PCC 6803 WP\_010872790.1; *Synechococcus* sp. WH 7803 WP\_011932076.1; 1-*C. reinhardtii* gi|159480774|; 2-*C. reinhardtii* gi|159484729|; 1-*C. merolae* strain 10D gi|544212076|; 2-*C. merolae* strain 10D gi|544209228|; *T. pseudonana* CCMP1335 gi|224009682|; 1-*P. tricornutum* gi|224009682|; CCAP 1055/1; 2-*P. tricornutum* CCAP 1055/1 gi|219129378|; *E. huxleyi* CCMP1516 gi|551593375|; 1, 2, 3-*T. suecica* sequences were obtained through the transcriptom.

#### 4.1.5.2. Main motives in algal OAS-TL sequences

In *A. thaliana* OAS-TL as Table. 4-7 showed, there are seven different interaction areas (Bonner et al., 2005). The OAS-TL sequences amino acid alignment data were shown in Fig. 4-5. The functional residue K (lysine, see supplementary materials for the one letter code for amino acids) was involved in the formation of the Schiff base with pyridoxal 5'-phosphate (PLP) and is absolutely conserved in all sequences. Also, the other aa residues in the K area were relatively conserved among all the species. Other portions of the protein are involved in PLP interaction (Bonner et al., 2005); they were separated into three different units: the first unit was formed by a single, highly conserved amino acid, H; the second unit was formed by five highly conserved residues, GTGGT; the last one was formed by a single amino acid, S, which was absolutely conserved. As shown in the Table. 4-7, these structures were pretty much conserved in all algal species. Only in the green algae *T. suecica*, the first unit, H, was substituted with a polar amino acid Y; in the second unit (GTGGT) the first polar amino acid, G, was substituted with the polar amino acid S.

The residues involved in substrates interaction (Bonner et al., 2005) were separated in two different units: the first one was formed by -TSGNT- (five highly conserved residues); the second unit was formed by a single amino acid -Q- which showed an absolutely conserved character. Table. 4-7 showed that, in the first unit involved in the interaction with the substrate, two residues were not highly conserved; they were i)

the second amino acid in the sequence, a -S- residue, which was a polar amino acid, and was substituted with A or -S, with the exception of the marine species *T. suecica*, in which the same position was occupied by the non-polar amino acid -V; ii) the fourth polar residues, N, which was always substituted by another polar amino acid, -S-.

The residues involved in SAT/OAS-TL interaction site were associated with a six amino acid unit, KPGPHK (Bonner et al., 2005). In algae, the first polar amino acid, K, and the last two amino acids, HK, of this motif were often substituted. In one of the sequences of the freshwater cyanobacterium *Synechocysis* sp. PCC 6803, the last two basic amino acid H and K, were substituted with the polar residues S and the non-polar I. In the OAS-TL of the marine cyanobacterium *Synechococcus* sp. WH 7803, one of the sequences showed the presence of a non-polar V in the place of the first basic amino acid K, and the terminal K was substituted by the basic amino acid R.

In *C. reinhardtii*, in one case both the first and the last K were substituted by polar residues (A and Q, respectively); in another isoform, the first K was substituted by the polar residue N, but the non-polar amino acid I, was found in the place of the terminal K.

As shown in Table. 4-7, in *T. suecica* OAS-TL, nearly all residues of the -KPGPHK- domain were substituted. In *T. suecica* OAS-TL 2-4, the first K was substituted by polar amino acids (Q in the 2 and 4 isoforms and N in isoform 3); In *T. suecica* OAS-TL 1, 3 and 4, the last two basic amino acids of the unit were substituted by YQ, TI and T, respectively.

In the OAS-TL of the red algae *C. merolae*, the first K was substituted by another basic amino acid, R. In the last two positions of the motif, the polar amino acid S and the non-polar amino acid I, were found instead of P and H.

In the diatom *T. pseudonana* CCMP 1335 OAS-TL 1 and 2, the first basic amino acid

of the motif was substituted by the polar amino acid A; in the isoform 3, it was substituted by the polar amino acid H. In the *T. pseudonana* isoform 1 and 3, the last two amino acids were substituted by a non-polar P, a polar T or non-polar F residues.

In *P. tricornutum* CCAP 1055/1 in one case the first basic amino acid K was substituted by a non-polar P residue.

In the OAS-TL of the dinoflagellate *A. klebsii*, in the six amino acid motif, nearly all residues were substituted. In the isoforms 1, 3 the K in the first position in the motif was substituted by a polar A, in the isoform 4 by a non-polar amino L. In *A. klebsii* isoforms 1, 2 and 4, the last two residues were substituted by P, TI and A, respectively.



A-A. thaliana 1 -----  
 B-A. thaliana 1 -----MAATSSSAFLLNPLTSR-----H  
 C-A. thaliana 1 MVAMIMASRFNREAKLASQILSTLLGNRSCTYTSMAATSSSALLLNPLTSS---S-SSSTL  
 Cl-A. thaliana 1 -----MASVSRLLR-----R  
 1-Synechocystis 1 -----  
 2-Synechocystis 1 -----  
 1-Synechococcus 1 -----  
 2-Synechococcus 1 -----  
 1-C. reinhardtii 1 -----MPVAAFGRSRIQSHASAGTS  
 2-C. reinhardtii 1 -----MQGR-----ASP-----MVGAVLALALAAAGT  
 3-C. reinhardtii 1 -----MQSLAKQLSAT-PRKAG  
 4-C. reinhardtii 1 -----  
 1-T. suecica 1 -----  
 2-T. suecica 1 -----  
 3-T. suecica 1 -----  
 4-T. suecica 1 -----  
 1-C. merolae 1 -----MLVSNAMY-----  
 2-C. merolae 1 -----MVQHAAN  
 1-T. pseudonana 1 -----  
 2-T. pseudonana 1 -----MKMLLASMA-----  
 3-T. pseudonana 1 -----  
 1-P. tricornutum 1 -----  
 2-P. tricornutum 1 -----  
 E. huxleyi 1 -----  
 1-A. klebsii 1 -----  
 2-A. klebsii 1 -----  
 3-A. klebsii 1 -----  
 4-A. klebsii 1 -----  
 consensus 1

A-A. thaliana 1 -----  
 B-A. thaliana 19 RPFKYSPELSSLSLSSRK---AAAFDVSS--AAFTLK-----RQSRSDVV--CKAVSIKP  
 C-A. thaliana 57 RRFRCSP EISSLSFSSAS---DFSLAMKRQSRSPADG---SERDPSVV--CEA--VKR  
 Cl-A. thaliana 12 ETIFCFS-----HTVRKLFSTVGSFSAQR-----LRDL  
 1-Synechocystis 1 -----MGFFVVR-----C--RQISL  
 2-Synechocystis 1 -----  
 1-Synechococcus 1 -----  
 2-Synechococcus 1 -----  
 1-C. reinhardtii 20 SLRHSDCIVPLRLSPVT---AAGRC-----RGSVRTVPVRAQAQATQ--AATPSIAV  
 2-C. reinhardtii 22 GAALCWW-----LLEPR-----  
 3-C. reinhardtii 17 RPQLCARERC-----A-----RV-----VRAQAAAA--AAAANSDE  
 4-C. reinhardtii 1 -----MQLQQKALRLQSTFPTRVSRVALV--PKA-VAAP  
 1-T. suecica 1 -----  
 2-T. suecica 1 -----EPIAK  
 3-T. suecica 1 -----  
 4-T. suecica 1 -----  
 1-C. merolae 9 VLVPCSLKAAQATLNRKKNSTFVRAKPEQTRILGYARRSPRARQR-----VQLA  
 2-C. merolae 8 GHAQCSF-----RPLRYK-----AETRGRVARSTRERVL-----LRCL  
 1-T. pseudonana 1 -----MA  
 2-T. pseudonana 10 -----L-----LGAASAFVPSHVPSSITSTIGSGANTQ  
 3-T. pseudonana 1 -----  
 1-P. tricornutum 1 -----  
 2-P. tricornutum 1 -----MSSSTS  
 E. huxleyi 1 -----MYRAAP-RLRSALS-----RTVS  
 1-A. klebsii 1 -----  
 2-A. klebsii 1 -----  
 3-A. klebsii 1 -----K  
 4-A. klebsii 1 -----  
 consensus 61

A-A. thaliana 1 ---MASRIAKDVTTELIGNTPLVRLNVA-----EGC--VGRVAKLEEMEPCCSVK  
 B-A. thaliana 67 EAGVEGLNIADNAAQLIGKTPMVRLNVA-----KGC--VASVAKLEEMEPCCSVK  
 C-A. thaliana 105 ETGPDGLNIADNVSLIGKTPMVRLNVA-----KGC--VANVAKLEEMEPCCSVK  
 Cl-A. thaliana 41 PKDFPSTNAKRDAQLLIGKTPVRLNVA-----EGC--EAVVAKLEEMEPCCSVK  
 1-Synechocystis 14 LGGPLPMKIASNITELIGRTPVRLNVA-----LGC--GARVAKLEEMEPCCSVK  
 2-Synechocystis 1 -----MDIKHGFVDSIGHTPLRLNVA-----DETC--ILKLEEMEPCCSVK  
 1-Synechococcus 1 -----MAIAPDITALVGCPTMVRLNVA-----ANGC--TAVVAKLEEMEPCCSVK  
 2-Synechococcus 1 -----MSRVYADNSQALIGNTPLVRLNVA-----KGC--KAVVAKLEEMEPCCSVK  
 1-C. reinhardtii 69 SVDPHVCIQPDATLVLGNTPMVRLNVA-----KGC--GARVAKLEEMEPCCSVK  
 2-C. reinhardtii 34 -KRQTGGTVRQVLDLIGNTPLVRLNVA-----EEGC--IVKLEEMEPCCSVK  
 3-C. reinhardtii 47 PKYVKNDKICKDVEVIGNTPLVRLNVA-----KGC--VAKVAKLEEMEPCCSVK  
 4-C. reinhardtii 32 EKAAVKMNIATDVTTELIGRTPVRLNVA-----KGC--HAKVAKLEEMEPCCSVK  
 1-T. suecica 1 -----ERRTRELIGRTPVRLNVA-----AKGVKLEEMEPCCSVK  
 2-T. suecica 6 PARPTGTTAESIVDLVGNTPVRLNVA-----AGC--GARVAKLEEMEPCCSVK  
 3-T. suecica 1 -----PRVHRSVLDLIGNTPLVRLNVA-----DATC--ELKLEEMEPCCSVK  
 4-T. suecica 1 -----EIGNTPLVRLNVA-----EEVRANGATLCKMEMEPCCSVK  
 1-C. merolae 59 LTAPPSVALARDVSDLVGNTPVRLNVA-----EEGV--QAVLCKLEEMEPCCSVK  
 2-C. merolae 41 FDMRLVHNVYDSFEDALIGNTPLVRLNVA-----ERTC--IVKLEEMEPCCSVK  
 1-T. pseudonana 3 NRQKGFPPLNNESEAVGNTPVRLNVA-----CPAGRTIYAKLEEMEPCCSVK  
 2-T. pseudonana 39 LFTFPRPKIAENVLELIGRTPVRLNVA-----FSC--VAVVAKLEEMEPCCSVK  
 3-T. pseudonana 1 -----KGGYESLIGNTPLVRLNVA-----SLINNNNTDANNNGVRIYAKLEEMEPCCSVK  
 1-P. tricornutum 1 ---GTRALNVAARPSDLIGNTPLVRLNVA-----AHGIDNGSRLEKLEEMEPCCSVK  
 2-P. tricornutum 7 RSPSLNAKIAENVLGLIGRTPVRLNVA-----EGC--VAVVAKLEEMEPCCSVK  
 E. huxleyi 19 SRGAGRGALVEDVTEIIGNTPLVRLNVA-----CPFGTIVAKLEEMEPCCSVK  
 1-A. klebsii 1 -----IVEDLTKIGRTPVRLNVA-----P\*AGVELYKLEEMEPCCSVK  
 2-A. klebsii 1 -----GFAGLVGNTPVRLNVA-----AATGCRVWVAKLEEMEPCCSVK  
 3-A. klebsii 2 TGGRPYEEIYESALDLVGNTPVRLNVA-----H--LDVCELVAKLEEMEPCCSVK  
 4-A. klebsii 1 -----VYSDITQLLSEDFNPTPLVRLNVA-----FKHAEVYAKLEEMEPCCSVK  
 consensus 121



A-A. thaliana 47 DRIGFSMISDAEKKGILIPGKSVLVEPTSGNIGVGLAFIAAAGYKLIITMPASMSI-ER  
 B-A. thaliana 117 DRIGYSMIDAEKGLIPGKSVLVEPTSGNIGVGLAFIAAAGYKLIITMPASMSI-ER  
 C-A. thaliana 155 DRIGYSMVDAAKCKGISPGKSVLVEPTSGNIGVGLAFIAAARGYRLIITMPASMSI-ER  
 Cl-A. thaliana 91 DRIGAMIDAEKCKLIPGKTLIEPTSGNIGVGLAFIAAARGYRLIITMPASMSI-ER  
 1-Synechocystis 66 DRIGININRAEKGILIPGKTLIEPTSGNIGVGLAFIAAARGYRLIITMPASMSI-ER  
 2-Synechocystis 45 DRIGALGHIEAEKGRKIPGKTLIEPTSGNIGVGLAFIAAARGYRLIITMPASMSI-ER  
 1-Synechococcus 47 DRIGAMVDAEAKGILIPGKTLIEPTSGNIGVGLAFIAAARGYRLIITMPASMSI-ER  
 2-Synechococcus 46 DRIGANMIDAEKGRKIPGKTLIEPTSGNIGVGLAFIAAARGYRLIITMPASMSI-ER  
 1-C. reinhardtii 119 DRIGANMIDAEKGRKIPGKTLIEPTSGNIGVGLAFIAAARGYRLIITMPASMSI-ER  
 2-C. reinhardtii 83 DRIGALCVSALADGRKIPGKTLIEPTSGNIGVGLAFIAAARGYRLIITMPASMSI-ER  
 3-C. reinhardtii 97 DRIGANMIDAEKGRKIPGKTLIEPTSGNIGVGLAFIAAARGYRLIITMPASMSI-ER  
 4-C. reinhardtii 82 DRIGYSMISDAEKKGILIPGKTLIEPTSGNIGVGLAFIAAARGYRLIITMPASMSI-ER  
 1-T. suecica 42 DRIGANMIDAEKGRKIPGKTLIEPTSGNIGVGLAFIAAARGYRLIITMPASMSI-ER  
 2-T. suecica 56 DRIGKMMIDAEKGRKIPGKTLIEPTSGNIGVGLAFIAAARGYRLIITMPASMSI-ER  
 3-T. suecica 45 DRIGALCVTDAEAKGRKIPGKTLIEPTSGNIGVGLAFIAAARGYRLIITMPASMSI-ER  
 4-T. suecica 41 DRIGAKMIDAEKGRKIPGKTLIEPTSGNIGVGLAFIAAARGYRLIITMPASMSI-ER  
 1-C. merolae 111 DRIGKMMIDAEKGRKIPGKTLIEPTSGNIGVGLAFIAAARGYRLIITMPASMSI-ER  
 2-C. merolae 91 DRIGALCVTDAEAKGRKIPGKTLIEPTSGNIGVGLAFIAAARGYRLIITMPASMSI-ER  
 1-T. pseudonana 53 DRIGALSLIEAEKGRKIPGKTLIEPTSGNIGVGLAFIAAARGYRLIITMPASMSI-ER  
 2-T. pseudonana 90 DRIGALSMIDAEKGRKIPGKTLIEPTSGNIGVGLAFIAAARGYRLIITMPASMSI-ER  
 3-T. pseudonana 52 DRIGARSMIDAEKGRKIPGKTLIEPTSGNIGVGLAFIAAARGYRLIITMPASMSI-ER  
 1-P. tricornutum 53 DRIGRSMIDAEKGRKIPGKTLIEPTSGNIGVGLAFIAAARGYRLIITMPASMSI-ER  
 2-P. tricornutum 57 DRIGALSMIDAEKGRKIPGKTLIEPTSGNIGVGLAFIAAARGYRLIITMPASMSI-ER  
 E. huxleyi 69 DRIGALAVIEAEKGRKIPGKTLIEPTSGNIGVGLAFIAAARGYRLIITMPASMSI-ER  
 1-A. klebsii 42 DRIGALAVIEAEKGRKIPGKTLIEPTSGNIGVGLAFIAAARGYRLIITMPASMSI-ER  
 2-A. klebsii 40 DRIGAVSILAEAEKGRKIPGKTLIEPTSGNIGVGLAFIAAARGYRLIITMPASMSI-ER  
 3-A. klebsii 54 DRIGKRMVIEAEKGRKIPGKTLIEPTSGNIGVGLAFIAAARGYRLIITMPASMSI-ER  
 4-A. klebsii 47 DRIGANLVIAEAEKGRKIPGKTLIEPTSGNIGVGLAFIAAARGYRLIITMPASMSI-ER  
 consensus 181

A-A. thaliana 106 RVLILAFGAEIV---LTPFAKGMGGAIA---KAEELAK---TPNGLYLOQFENPA  
 B-A. thaliana 176 RVLILAFGAEIV---LTPFAKGMGGAIA---KAEELAK---TPNGLYLOQFENPA  
 C-A. thaliana 214 RVLILAFGAEIV---LTPFAKGMGGAIA---KAEELAK---TPNGLYLOQFENPA  
 Cl-A. thaliana 150 RVTMRSEGAELV---LTPFAKGMGGAIA---KAEELAK---TPNGLYLOQFENPA  
 1-Synechocystis 125 RMLLAFGAEIV---LTPFAKGMGGAIA---KAEELAK---TPNGLYLOQFENPA  
 2-Synechocystis 103 IDLILAFGAEIV---LTPFAKGMGGAIA---KAEELAK---TPNGLYLOQFENPA  
 1-Synechococcus 106 RMLLAFGAEIV---LTPFAKGMGGAIA---KAEELAK---TPNGLYLOQFENPA  
 2-Synechococcus 104 RMLLAFGAEIV---LTPFAKGMGGAIA---KAEELAK---TPNGLYLOQFENPA  
 1-C. reinhardtii 178 RMLLAFGAEIV---LTPFAKGMGGAIA---KAEELAK---TPNGLYLOQFENPA  
 2-C. reinhardtii 141 ANMILAFGAEIV---LTPFAKGMGGAIA---KAEELAK---TPNGLYLOQFENPA  
 3-C. reinhardtii 156 RMLLAFGAEIV---LTPFAKGMGGAIA---KAEELAK---TPNGLYLOQFENPA  
 4-C. reinhardtii 141 RMLLAFGAEIV---LTPFAKGMGGAIA---KAEELAK---TPNGLYLOQFENPA  
 1-T. suecica 100 RDSMASYGGCVL---LTPFAKGMGGAIA---KAEELAK---TPNGLYLOQFENPA  
 2-T. suecica 115 RVLILAFGAEIV---LTPFAKGMGGAIA---KAEELAK---TPNGLYLOQFENPA  
 3-T. suecica 103 SQVLEAFGAEIV---LTPFAKGMGGAIA---KAEELAK---TPNGLYLOQFENPA  
 4-T. suecica 100 YTIQAFGAEIV---LTPFAKGMGGAIA---KAEELAK---TPNGLYLOQFENPA  
 1-C. merolae 170 RMLLAFGAEIV---LTPFAKGMGGAIA---KAEELAK---TPNGLYLOQFENPA  
 2-C. merolae 149 KEFLRSGAEIV---LTPFAKGMGGAIA---KAEELAK---TPNGLYLOQFENPA  
 1-T. pseudonana 111 RMLLAFGAEIV---LTPFAKGMGGAIA---KAEELAK---TPNGLYLOQFENPA  
 2-T. pseudonana 149 RMLLAFGAEIV---LTPFAKGMGGAIA---KAEELAK---TPNGLYLOQFENPA  
 3-T. pseudonana 111 KEFLRSGAEIV---LTPFAKGMGGAIA---KAEELAK---TPNGLYLOQFENPA  
 1-P. tricornutum 112 RMLLAFGAEIV---LTPFAKGMGGAIA---KAEELAK---TPNGLYLOQFENPA  
 2-P. tricornutum 116 RMLLAFGAEIV---LTPFAKGMGGAIA---KAEELAK---TPNGLYLOQFENPA  
 E. huxleyi 127 RMLLAFGAEIV---LTPFAKGMGGAIA---KAEELAK---TPNGLYLOQFENPA  
 1-A. klebsii 100 RMLLAFGAEIV---LTPFAKGMGGAIA---KAEELAK---TPNGLYLOQFENPA  
 2-A. klebsii 98 VQLLRLGAEIV---LTPFAKGMGGAIA---KAEELAK---TPNGLYLOQFENPA  
 3-A. klebsii 112 VNTMRSGAEIV---LTPFAKGMGGAIA---KAEELAK---TPNGLYLOQFENPA  
 4-A. klebsii 106 RMLLAFGAEIV---LTPFAKGMGGAIA---KAEELAK---TPNGLYLOQFENPA  
 consensus 241

A-A. thaliana 153 NPKIHYETTGPPIWDTG-KIDIFVSGGTGGIITGAGYLYKEQNA---N  
 B-A. thaliana 223 NPKIHYETTGPPIWDTG-KIDIFVSGGTGGIITGAGYLYKEQNA---E  
 C-A. thaliana 261 NPKIHYETTGPPIWDTG-KVDIFVSGGTGGIITGAGYLYKEQNA---K  
 Cl-A. thaliana 197 NPKIHYETTGPPIWDTG-KVDIFVSGGTGGIITGAGYLYKEQNA---N  
 1-Synechocystis 172 NPKIHYETTGPPIWDTG-KVDIFVSGGTGGIITGAGYLYKEQNA---S  
 2-Synechocystis 152 NPKIHYETTGPPIWDTG-KVDIFVSGGTGGIITGAGYLYKEQNA---A  
 1-Synechococcus 153 NPKIHYETTGPPIWDTG-KVDIFVSGGTGGIITGAGYLYKEQNA---K  
 2-Synechococcus 152 NPKIHYETTGPPIWDTG-KVDIFVSGGTGGIITGAGYLYKEQNA---A  
 1-C. reinhardtii 225 NPKIHYETTGPPIWDTG-KVDIFVSGGTGGIITGAGYLYKEQNA---G  
 2-C. reinhardtii 190 NPKIHYETTGPPIWDTG-KVDIFVSGGTGGIITGAGYLYKEQNA---R  
 3-C. reinhardtii 203 NPKIHYETTGPPIWDTG-KVDIFVSGGTGGIITGAGYLYKEQNA---S  
 4-C. reinhardtii 188 NPKIHYETTGPPIWDTG-KVDIFVSGGTGGIITGAGYLYKEQNA---D  
 1-T. suecica 150 NPKIHYETTGPPIWDTG-KVDIFVSGGTGGIITGAGYLYKEQNA---A  
 2-T. suecica 162 NPKIHYETTGPPIWDTG-KVDIFVSGGTGGIITGAGYLYKEQNA---G  
 3-T. suecica 152 NPKIHYETTGPPIWDTG-KVDIFVSGGTGGIITGAGYLYKEQNA---S  
 4-T. suecica 147 NPKIHYETTGPPIWDTG-KVDIFVSGGTGGIITGAGYLYKEQNA---D  
 1-C. merolae 217 NPKIHYETTGPPIWDTG-KVDIFVSGGTGGIITGAGYLYKEQNA---H  
 2-C. merolae 197 NPKIHYETTGPPIWDTG-KVDIFVSGGTGGIITGAGYLYKEQNA---G  
 1-T. pseudonana 157 NPKIHYETTGPPIWDTG-KVDIFVSGGTGGIITGAGYLYKEQNA---D  
 2-T. pseudonana 197 NPKIHYETTGPPIWDTG-KVDIFVSGGTGGIITGAGYLYKEQNA---D  
 3-T. pseudonana 165 NPKIHYETTGPPIWDTG-KVDIFVSGGTGGIITGAGYLYKEQNA---K  
 1-P. tricornutum 160 NPKIHYETTGPPIWDTG-KVDIFVSGGTGGIITGAGYLYKEQNA---LRPN  
 2-P. tricornutum 164 NPKIHYETTGPPIWDTG-KVDIFVSGGTGGIITGAGYLYKEQNA---K  
 E. huxleyi 173 NPKIHYETTGPPIWDTG-KVDIFVSGGTGGIITGAGYLYKEQNA---D  
 1-A. klebsii 146 NPKIHYETTGPPIWDTG-KVDIFVSGGTGGIITGAGYLYKEQNA---D  
 2-A. klebsii 149 NPKIHYETTGPPIWDTG-KVDIFVSGGTGGIITGAGYLYKEQNA---E  
 3-A. klebsii 161 NPKIHYETTGPPIWDTG-KVDIFVSGGTGGIITGAGYLYKEQNA---G  
 4-A. klebsii 156 NPKIHYETTGPPIWDTG-KVDIFVSGGTGGIITGAGYLYKEQNA---K  
 consensus 301



A-A. thaliana 200 VKLYGVEPESALSG-----KPGPHIQGIGAGFIPSWLN  
 B-A. thaliana 270 LKVIQVEPESALSG-----KPGPHIQGIGAGFVFKNLD  
 C-A. thaliana 308 TQVIGVEPESALSG-----KPGPHIQGIGAGFVFKNLD  
 Cl-A. thaliana 244 VKLYGVEPESALSG-----KPGPHIQGIGAGFVFKNLD  
 1-Synechocystis 219 FQAIQVEPESALSG-----KPGPHIQGIGAGFVFKNLD  
 2-Synechocystis 199 VQVVADEPMGSGSLYSFIKTGEINP-----SGNSIEGIGNSRIETANME  
 1-Synechococcus 200 LSVVAVEPESALSG-----KPGPHIQGIGAGFVFKNLD  
 2-Synechococcus 200 IESVAVEPESALSG-----KPGPHIQGIGAGFVFKNLD  
 1-C. reinhardtii 272 VQVVAVEPESALSG-----KPGPHIQGIGAGFVFKNLD  
 2-C. reinhardtii 236 VRVVEPESALSG-----KPGPHIQGIGAGFVFKNLD  
 3-C. reinhardtii 250 LQVVAVEPESALSG-----KPGPHIQGIGAGFVFKNLD  
 4-C. reinhardtii 235 VQVVAVEPESALSG-----KPGPHIQGIGAGFVFKNLD  
 1-T. suecica 197 VRYVMEPESALSG-----KPGPHIQGIGAGFVFKNLD  
 2-T. suecica 209 VQVVAVEPESALSG-----KPGPHIQGIGAGFVFKNLD  
 3-T. suecica 198 VRYVLADEPMGSGSLYSFIKTGEINP-----SGNSIEGIGNSRIETANME  
 4-T. suecica 194 IKVMAVEPESALSG-----KPGPHIQGIGAGFVFKNLD  
 1-C. merolae 244 VQVVAVEPESALSG-----KPGPHIQGIGAGFVFKNLD  
 2-C. merolae 264 IKVMAVEPESALSG-----KPGPHIQGIGAGFVFKNLD  
 1-T. pseudonana 205 TKIILAEFGAANLIGSGIKTERNA-----DGSPAGSH-----PAPAPHIQGIGAGFVFKNLD  
 2-T. pseudonana 244 MKIVAVEPESALSG-----KPGPHIQGIGAGFVFKNLD  
 3-T. pseudonana 224 PKNIVDEPESALSG-----KPGPHIQGIGAGFVFKNLD  
 1-P. tricornutum 215 LQVVAVEPESALSG-----KPGPHIQGIGAGFVFKNLD  
 2-P. tricornutum 211 LQVVAVEPESALSG-----KPGPHIQGIGAGFVFKNLD  
 E. huxleyi 221 VKIVLREPESALSG-----KPGPHIQGIGAGFVFKNLD  
 1-A. klebsii 194 LKIVLREPESALSG-----KPGPHIQGIGAGFVFKNLD  
 2-A. klebsii 196 IGVYLADEPMGSGSLYSFIKTGEINP-----SGNSIEGIGNSRIETANME  
 3-A. klebsii 208 IKIVAVEPESALSG-----KPGPHIQGIGAGFVFKNLD  
 4-A. klebsii 204 VKVCGHREPESALSG-----KPGPHIQGIGAGFVFKNLD  
 consensus 361 .....



A-A. thaliana 237 V-----DLIDEVVOVSSDESIDMARCLALKEGLLVGISSGAAARAAIKLACRP  
 B-A. thaliana 307 L-----ALVDEVTIATSSBEALBTSKCLALCEGLLVGISSGAAARAAIKVAKRP  
 C-A. thaliana 345 Q-----KIMDEVTIATSSBEALBTSKCLALKEGLLVGISSGAAARAAIKVAKRP  
 Cl-A. thaliana 281 M-----DVMESVLEVSBEALBTSKCLALKEGLLVGISSGAAARAAIKVAKRP  
 1-Synechocystis 256 V-----NLIDEVVAVDDEALBTSKCLALKEGLLVGISSGAAARAAIKVAKRP  
 2-Synechocystis 242 NVF-----HDDAVVCHDDEALBTSKCLALKEGLLVGISSGAAARAAIKVAKRP  
 1-Synechococcus 237 M-----DLIDEVVAVDDEALBTSKCLALKEGLLVGISSGAAARAAIKVAKRP  
 2-Synechococcus 243 L-----SVVDEVVECVNEESIDMARCLALKEGLLVGISSGAAARAAIKVAKRP  
 1-C. reinhardtii 309 V-----DLIDEVVKINSNEALBTSKCLALKEGLLVGISSGAAARAAIKVAKRP  
 2-C. reinhardtii 290 RAL-----HDDAFRGGDREAVEMAAVLLRNEGLLVGISSGAAARAAIKVAKRP  
 3-C. reinhardtii 287 T-----TVVDEVVKIPSDBAVEMASRLAVEEGLEGLLVGISSGAAARAAIKVAKRP  
 4-C. reinhardtii 272 T-----ALIDEVVOVSSDDAIDMARRLALKEGLLVGISSGAAARAAIKVAKRP  
 1-T. suecica 243 FGV-----VDEMLCLDCKOSFANRRVAAEDGMLLVGISSGAAARAAIKVAKRP  
 2-T. suecica 246 T-----HVVDEVVCISSDDAISMARRLALKEGLLVGISSGAAARAAIKVAKRP  
 3-T. suecica 252 QAR-----IDGAYKSSDRESVEMAHILMREGLLVGISSGAAARAAIKVAKRP  
 4-T. suecica 231 SLAPGAPLVGEGPRGHVSEIHTNSSCAIEACRMAQMGEMMVGSSGAAARAAIKVAKRP  
 1-C. merolae 301 T-----KIVNEVKQVTSMDSEMARLAVEEGLEGLLVGISSGAAARAAIKVAKRP  
 2-C. merolae 287 GFV-----PMSFEISDAEALQAVENVRHEGLLVGISSGAAARAAIKVAKRP  
 1-T. pseudonana 258 KG-----L-----DIPHEMFDIPDGAAVEFSQALARNEGTLIGISSGAAARAAIKVAKRP  
 2-T. pseudonana 281 T-----SLIDEVVOVSSDDAIDMARRLALKEGLLVGISSGAAARAAIKVAKRP  
 3-T. pseudonana 278 LGEE-----S-----IIDEVVAVDDEALBTSKCLALKEGLLVGISSGAAARAAIKVAKRP  
 1-P. tricornutum 259 L-----TLIDEVVOVSSDDAIDMARRLALKEGLLVGISSGAAARAAIKVAKRP  
 2-P. tricornutum 248 T-----SLIDEVVOVSSDDAIDMARRLALKEGLLVGISSGAAARAAIKVAKRP  
 E. huxleyi 274 DA-----PMDMLLHELVPVPGAGAIATAQSLAKEGLLVGISSGAAARAAIKVAKRP  
 1-A. klebsii 251 QG-----VDKAYHKKVILVEPKVAMEESHKLARCEGEGCGSSGAAARAAIKVAKRP  
 2-A. klebsii 250 KGLA---EHNPCGGCIDGAVVVSDEALEMAREILDHEGLLVGISSGAAARAAIKVAKRP  
 3-A. klebsii 257 Q-----DVVVYVVKTDDESFAMGRNVRHEGLLVGISSGAAARAAIKVAKRP  
 4-A. klebsii 234 T-----GIDELCEVNEEAFOMCLIRNREESLIAGFSSGAAARAAIKVAKRP  
 consensus 421 .....

A-A. thaliana 285 -ENACKLIVVLPSPFGERYLSVLFDAIRKFAEAMTFEA-----  
 B-A. thaliana 355 -ENACKLIVVLPSPFGERYLSVLFDAIRKFAEAMTFEA-----  
 C-A. thaliana 393 -ENACKLIVVLPSPFGERYLSVLFDAIRKFAEAMTFEA-----VSG  
 Cl-A. thaliana 329 -ENKKGKIVVLPSPFGERYLSVLFDAIRKFAEAMTFEA-----VD  
 1-Synechocystis 304 -ANKDKLIVVLPSPFGERYLSVLFDAIRKFAEAMTFEA-----  
 2-Synechocystis 289 -LGPCHTIVVLPSPFGERYLSVLFDAIRKFAEAMTFEA-----  
 1-Synechococcus 285 -AMEGRRIVVLPSPFGERYLSVLFDAIRKFAEAMTFEA-----  
 2-Synechococcus 291 -AYAGKIVVLPSPFGERYLSVLFDAIRKFAEAMTFEA-----  
 1-C. reinhardtii 357 -ENREKIVVLPSPFGERYLSVLFDAIRKFAEAMTFEA-----PDSWRMASGAE-RPATRE  
 2-C. reinhardtii 337 -MGPGHTIVVLPSPFGERYLSVLFDAIRKFAEAMTFEA-----L-----AFVS--  
 3-C. reinhardtii 335 -ENAGKLVVLPSPFGERYLSVLFDAIRKFAEAMTFEA-----RVKVVDAAGRE-RYVP--  
 4-C. reinhardtii 320 -ENEGKLVVLPSPFGERYLSVLFDAIRKFAEAMTFEA-----SA  
 1-T. suecica 291 -PDGSIIVVLPSPFGERYLSVLFDAIRKFAEAMTFEA-----  
 2-T. suecica 294 -ENAGKLVVLPSPFGERYLSVLFDAIRKFAEAMTFEA-----  
 3-T. suecica 299 -LGPCHTIVVLPSPFGERYLSVLFDAIRKFAEAMTFEA-----  
 4-T. suecica 291 -ESAGKIVVLPSPFGERYLSVLFDAIRKFAEAMTFEA-----PNM--  
 1-C. merolae 349 -EMKGNIVVLPSPFGERYLSVLFDAIRKFAEAMTFEA-----VET--  
 2-C. merolae 334 -LGPCHTIVVLPSPFGERYLSVLFDAIRKFAEAMTFEA-----  
 1-T. pseudonana 308 -P-EGSIVVLPSPFGERYLSVLFDAIRKFAEAMTFEA-----  
 2-T. pseudonana 329 -ENAKKIVVLPSPFGERYLSVLFDAIRKFAEAMTFEA-----  
 3-T. pseudonana 329 -MPPGSIIVVLPSPFGERYLSVLFDAIRKFAEAMTFEA-----  
 1-P. tricornutum 306 -ASAKKIVVLPSPFGERYLSVLFDAIRKFAEAMTFEA-----  
 2-P. tricornutum 296 -ENAKKIVVLPSPFGERYLSVLFDAIRKFAEAMTFEA-----VGV--  
 E. huxleyi 326 -P-EGSIVVLPSPFGERYLSVLFDAIRKFAEAMTFEA-----  
 1-A. klebsii 303 -PTMGSVVLPSPFGERYLSVLFDAIRKFAEAMTFEA-----  
 2-A. klebsii 306 -LGPCHTIVVLPSPFGERYLSVLFDAIRKFAEAMTFEA-----  
 3-A. klebsii 305 NIGAKKIVVLPSPFGERYLSVLFDAIRKFAEAMTFEA-----TVRT  
 4-A. klebsii 282 ---PENVVLPSPFGERYLSVLFDAIRKFAEAMTFEA-----ERHLPVVF  
 consensus 481 .....

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A-A. thaliana      ---
B-A. thaliana      ---
C-A. thaliana      ---
C1-A. thaliana     ---
1-Synechocystis    ---
2-Synechocystis    ---
1-Synechococcus    ---
2-Synechococcus    ---
1-C. reinhardtii   412 PRL
2-C. reinhardtii   ---
3-C. reinhardtii   ---
4-C. reinhardtii   ---
1-T. suecica       ---
2-T. suecica       ---
3-T. suecica       ---
4-T. suecica       ---
1-C. merolae       ---
2-C. merolae       ---
1-T. pseudonana    ---
2-T. pseudonana    ---
3-T. pseudonana    ---
1-P. tricornutum   ---
2-P. tricornutum   ---
E. huxleyi         383 ---
1-A. klebsii       ---
2-A. klebsii       ---
3-A. klebsii       ---
4-A. klebsii       ---
consensus          541

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Fig. 4-5. Alignment of OAS-TL of algae and *A. thaliana*.

The red rectangle identifies the PLP binding site, with the functional lysine involved in the formation of the Schiff base; the purple triangle, the green rectangle, and the blue triangle indicated the other motif involved in PLP interaction; the blue rectangle and the red triangle indicate the residues involved in substrate interaction; the yellow rectangle indicated the SAT/OAS-TL interaction site. The Cysteine residues are highlighted in yellow. Absolutely conserved amino acids are shown with dark background, highly conserved residues with grey background. At the consensus line, asterisks indicate highly conserved aminoacids.

The alignment sequences: A-At4g14880; B-At2g43750; C-AT3G59760; C1-At3g61440; 1-*Synechocystis* sp. PCC 6803 BAA17450.1; 2-*Synechocystis* sp. PCC 6803 BAA16664.1; 1-*Synechococcus* sp. WH 7803 WP\_011934122.1; 2-*Synechococcus* sp. WH 7803 WP\_011932497.1; 1-*C. reinhardtii* XP\_001696139.1; 2-*C. reinhardtii* XP\_001701454.1; 3-*C. reinhardtii* XP\_001703301.1; 4-*C. reinhardtii* XP\_001691935.1; 1-*C. merolae* strain 10D XP\_005539314.1; 2-*C. merolae* strain 10D XP\_005536023.1; 1-*T. pseudonana* CCMP 1335 XP\_002286122.1; 2-*T. pseudonana* CCMP 1335 XP\_002286707.1; 3-*T. pseudonana* XP\_002287864.1; 1-*P. tricornutum* CCAP 1055/1 XP\_002177040.1; CCAP 1055/1; 2-*P. tricornutum* CCAP 1055/1 XP\_002180648.1; *E. huxleyi* CCMP 1516 gi|485641205|; 1, 2, 3, 4-*T. suecica*; 1, 2, 3, 4-*A. klebsii* these sequences were obtained through the transcriptom.

Table. 4-7. Main binding motives in OAS-TL sequences (from the N'-terminal to C'-terminal)

	PLP binding site	Residues binding with substrate		Residues binding with PLP		OAS-TL/SAT interaction site	Residues binding with PLP
	-K-	-TSGNT-	-Q-	-H-	-GTGGT-	-KPGPHK-	-S-
<i>A-A.thaliana</i>	-K-(46)	-TSGNT-(74-78)	-Q-(147)	-H-(157)	-GTGGT-(181-185)	-KPGPHK-(217-222)	-S-(269)
<i>B-A.thaliana</i>	-K-(116)	-TSGNT-(144-148)	-Q-(207)	-H-(217)	-GTGGT-(251-255)	-KPGPHK-(287-292)	-S-(239)
<i>C-A.thaliana</i>	-K-(154)	-TSGNT-(182-186)	-Q-(255)	-H-(265)	-GTGGT-(289-293)	-KPGPHK-(325-330)	-S-(377)
<i>Cl-A.thaliana</i>	-K-(90)	-TSGNM-(118-122)	-Q-(191)	-H-(201)	-GSGGT-(225-229)	-KPGPHK-(261-266)	-S-(313)
1- <i>Synechocystis</i> sp. PCC 6803	-K-(65)	-TSGNT-(93-97)	-Q-(166)	-H-(176)	-GTGGT-(200-204)	-KPGPHK-(236-241)	-S-(288)
2- <i>Synechocystis</i> sp. PCC 6803	-K-(44)	-TAGNT-(71-75)	-Q-(146)	-H-(156)	-GTGGT-(180-184)	-SGNSI-(233-236)	-S-(274)
1- <i>Synechococcus</i> sp. WH 7803	-K-(46)	-TSGNT-(74-78)	-Q-(147)	-H-(157)	-GTGGT-(181-185)	-VAGPHK-(217-222)	-S-(269)
2- <i>Synechococcus</i> sp. WH 7803	-K-(45)	-TSGNT-(72-76)	-Q-(145)	-H-(155)	-GTGGT-(180-184)	-KPGPHK-(223-228)	-S-(275)
1- <i>C.reinhardtii</i>	-K-(118)	-TSGNT-(145-150)	-Q-(219)	-H-(229)	-GTGGT-(253-257)	-APGYHQ-(289-294)	-S-(341)
2- <i>C.reinhardtii</i>	-K-(82)	-TAGST-(109-113)	-Q-(184)	-H-(194)	-GTGGT-(217-221)	-NPFDTI-(269-274)	-S-(322)
3- <i>C.reinhardtii</i>	-K-(96)	-TSGNT-(124-128)	-Q-(197)	-H-(207)	-GTGGT-(231-235)	-KPGPHK-(270-275)	-S-(319)
4- <i>C.reinhardtii</i>	-K-(81)	-TSGNT-(109-113)	-Q-(182)	-H-(192)	-GTGGT-(216-221)	-KPGPHK-(252-257)	-S-(304)
1- <i>T.suecica</i>	-K-(41)	-TSGNT-(68-72)	-Q-(144)	-Y-(154)	-GTGGT-(178-182)	-KPSSYQ-(221-226)	-S-(375)
2- <i>T.suecica</i>	-K-(55)	-TSGNT-(83-87)	-Q-(176)	-H-(186)	-GTGGT-(190-194)	-QPGPHK-(226-231)	-S-(278)
3- <i>T.suecica</i>	-K-(44)	-TVGST-(71-75)	-Q-(146)	-H-(156)	-GTGGT-(179-183)	-NPYDTI-(240-245)	-S-(284)
4- <i>T.suecica</i>	-K-(40)	-TSGNT-(67-71)	-Q-(149)	-H-(159)	-GTGGT-(175-179)	-QHAPHI-(210-215)	-S-(275)
1- <i>C.merolae</i> strain 10D	-K-(110)	-TSGNT-(138-142)	-Q-(211)	-H-(221)	-GTGGT-(245-249)	-RPGPHK-(281-286)	-S-(333)
2- <i>C.merolae</i> strain 10D	-K-(90)	-TAGNT-(117-171)	-Q-(191)	-H-(201)	-GTGGT-(225-229)	-VGDSE-(268-271)	-S-(319)
1- <i>T.pseudonana</i> CCMP 1335	-K-(52)	-TSGNT-(79-83)	-Q-(152)	-H-(162)	-GTGGT-(185-189)	-AFAPHP-(237-242)	-S-(292)
2- <i>T.pseudonana</i> CCMP 1335	-K-(89)	-TSGNT-(117-121)	-Q-(191)	-H-(201)	-GTGGT-(225-229)	-KPGPHK-(261-266)	-S-(313)
3- <i>T.pseudonana</i> CCMP 1335	-K-(51)	-TSGST-(78-82)	-Q-(259)	-H-(169)	-GTGGT-(193-197)	-HRYDTI-(257-262)	-S-(314)
1- <i>P.tricornutum</i> CCAP 1055/1	-K-(52)	-TSGNT-(80-84)	-Q-(153)	-H-(163)	-GTGGT-(188-192)	-PQGPHK-(238-243)	-S-(290)
2- <i>P.tricornutum</i> CCAP 1055/1	-K-(56)	-TSGNT-(84-88)	-Q-(158)	-H-(168)	-GTGGT-(192-196)	-KPGPHK-(228-232)	-S-(280)
<i>E.luxleyi</i> CCMP 1516	-K-(68)	-TSGNT-(95-99)	-Q-(167)	-H-(177)	-GTGGT-(202-205)	-AFSAHP-(253-258)	-S-(320)
1- <i>A.klebsii</i>	-K-(42)	-TSGNT-(69-74)	-Q-(140)	-H-(150)	-GTGGT-(175-179)	-AWTAHP-(230-235)	-S-(287)
2- <i>A.klebsii</i>	-K-(39)	-TSGNT-(66-70)	-Q-(141)	-H-(151)	-GTGGT-(177-181)	-HRYDTI-(229-233)	-S-(291)
3- <i>A.klebsii</i>	-K-(53)	-TSGNT-(80-84)	-Q-(155)	-H-(165)	-GTGGT-(189-193)	-AYH--(239-241)	-S-(289)
4- <i>A.klebsii</i>	-K-(46)	-TSGNT-(74-78)	-Q-(150)	-H-(160)	-GTGGT-(184-188)	-PQLHA-(222-227)	-S-(266)

Highlighted residues are those that are substituted in the sequence of a given species; the number inside parenthesis indicate the position of the amino acids in the sequences.

## **4.2. OAS-TL from algae**

### **4.2.1. OAS-TL purification**

The elution patterns of OAS-TL showed differences among the experimental species (Fig. 4-6). The western blot analysis of the various fractions evidenced the presence of proteins of different sizes cross-reacting with OAS-TL antibody (Table. 4-8).



Table. 4-8. Features of purified algae OAS-TL

Species	PCP (Elution Volume of protein peak)	Immunoblot positive (+) or negative (-)	OAS-TL protein band number	OAS-TL size (kDa)
<i>Synechocystis</i> sp. PCC 6803	1 and 3 ml	(-) and (+)	2	33, 39
<i>Chlamydomonas reinhardtii</i> -TAP	3.5 ml	(+)	2	37, 40
<i>Chlamydomonas reinhardtii</i> -TP	3 ml	(+)	3	34, 44, 49
<i>Dunaliella tertiolecta</i>	2 and 3.5 ml	(-) and (+)	2	36, 38
<i>Tetraselmis suecica</i>	0.5 and 3 ml	(-) and (+)	2	33, 41
<i>Thalassiosira pseudonana</i>	2 and 3 ml	(-) and (+)	2	36, 37
<i>Phaeodactylum tricorutum</i>	3.5 ml	(+)	1	36
<i>Amphidinium klebsii</i>	2.5 ml	(+)	2	35, 39

Note: PCP, protein peak in terms of elution volume (ml) used as cross-reaction with the OAS-TL-A antibody present (+) or absence(-).



In *Synechocystis* sp. PCC 6803 (Fig. 4-6 A), a protein concentration peak appeared already after 1 ml of elution buffer passed through the column. The proteins of this peak (fraction E2), however, did not cross-react with OAS-TL antibody (Fig. 4-7). The main elution peak for *Synechocystis* sp. PCC 6803 was eluted after 3 mL of elution buffer (Fig. 4-6 A). The fractions corresponding with this following peak (fractions E6, 7, 8, 9, Fig. 4-7) were recognized by OAS-L antibody. Two proteins were detected in the western blot and had a mass of around 35 kDa (Fig. 4-7). Mobility analysis (supplemental data), indicated that these bands had sizes of 33 kDa and 39 kDa.

When *Chlamydomonas reinhardtii* was grown in TAP medium, the elution pattern only showed one protein concentration peak after 3.5 ml elution (Fig. 4-6 B). The collected fractions (E7, 8, 9, 10, Fig. 4-8) showed two signals with masses of 37 kDa and 40 kDa (Fig. 4-8). When *C. reinhardtii* was cultured in TP medium, the main elution peak appeared after 3 ml of elution buffer (Fig. 4-6 C). The western blot of the proteins in the peak fraction (E6) and in the following fractions (E7, 8, 9, Fig. 4-9) showed three bands of 34 kDa, 44 kDa and 49 kDa (Fig. 4-9).

The affinity chromatography of *Dunaliella tertiolecta* extract had a rather flat protein concentration profile, with two peaks of similar protein content after 2 and 3.5 ml elution (Fig. 4-6 D). The western blot of the protein sample obtained after 2 ml elution (E4, Fig. 4-10) showed no immunoreaction while it showed a strong band of 36 kDa and another band of 38 kDa in the fractions (E6, 7, 8, 9, Fig. 4-10) belonging to the 3.5 ml elution peak (Fig 4-6 D).

*Tetraselmis suecica* affinity chromatography afforded a peak at 0.5 ml and another at 3 ml (Fig. 4-6 E). Again, the earlier peak (E1 fraction) did not show any immunoreaction with OAS-TL antibodies (Fig. 4-11). The later peak consisting in fractions E5, 6, 7, 8, instead, showed a dense band of 41 kDa and a fainter one of 33 kDa (Fig.4-11).

*Thalassiosira pseudonana* showed an elution peak at 1 ml and a major one at 3 ml (Fig. 4-6 F). The first peak (E2 fraction) did not give a signal in the western blot. The

fractions obtained from the peak at 3 ml, instead, showed a band of 36 kDa (E7, Fig. 4-7) and of 37 kDa (E5, 6, 8, Fig. 4-12).

For *Phaeodactylum tricornutum*, one elution peak of protein concentration was observed after 3.5 ml (Fig. 4-6 G) of elution buffer. The mass of the OAS-TL contained in this peak was 36 kDa (E7, 8, 9, 10 fractions, Fig. 4-13).

OAS-TL of *Amphidinium klebsii* was eluted after 2.5 ml elution buffer (Fig. 4-6 H). A band of 35 kDa was visible in the immunoreaction (E5, 6, 7, 8 fractions, Fig. 4-14); only in the fraction E5 an additional band of 39 kDa was visible (Fig. 4-14).

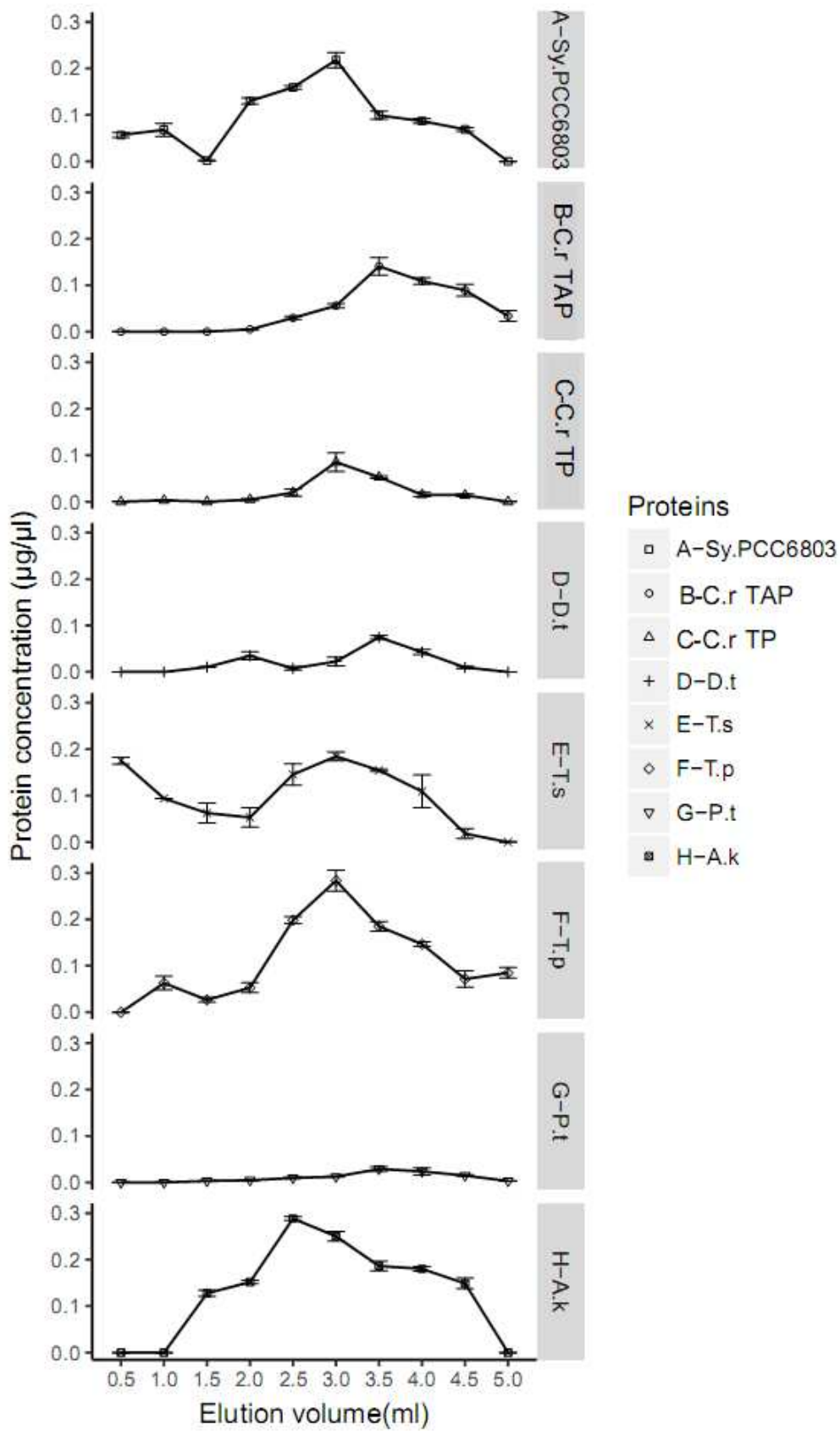


Fig. 4-6. Elution pattern of OAS-TL protein from different algal species.

From the top to the bottom different curve the algal species: A-Sy. PCC 6803: *Synechocystis* sp. PCC 6803; B-C.r-TAP: *Chlamydomonas reinhardtii* with TAP medium; C-C.r-TP:

*Chlamydomonas reinhardtii* with TP medium; D-D.t: *Dunaliella tertiolecta*; E-T.s: *Tetraselmis suecica*; F-T.p: *Thalassiosira pseudonana*; G-P.t: *Phaeodactylum tricoratum*; H-A.k: *Amphidinium klebsii*. There were three replicates the data was Mean  $\pm$  SD.

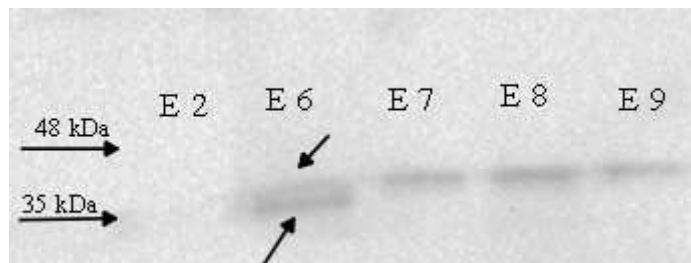


Fig. 4-7. Immunodetection of OAS-TL purified from *Synechocystis* sp. PCC 6803.

The amount of protein loaded on the gel were fraction E2 = 1.36  $\mu$ g; E7 = 2.25  $\mu$ g; E8 = 0.58  $\mu$ g; E9 = 0.58  $\mu$ g; E10 = 0.36  $\mu$ g.

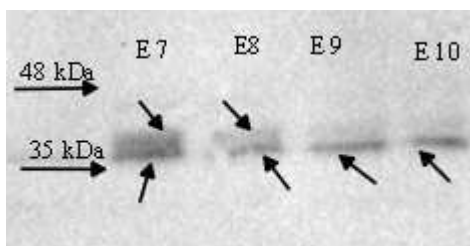


Fig. 4-8. Immunodetection of OAS-TL purified from *Chlamydomonas reinhardtii* grown in TAP medium.

The amount of protein loaded on the gel were fraction E7 = 0.62  $\mu$ g; E8 = 0.45  $\mu$ g; E9 = 0.29  $\mu$ g; E10 = 0.26  $\mu$ g.

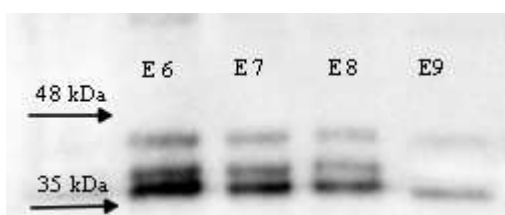


Fig. 4-9. Immunodetection of OAS-TL purified from *Chlamydomonas reinhardtii* grown in TP medium.

The amount of protein loaded on the gel were fraction E7 = 0.24  $\mu$ g; E8 = 0.58  $\mu$ g; E9 = 0.31  $\mu$ g; E10 = 0.12  $\mu$ g.

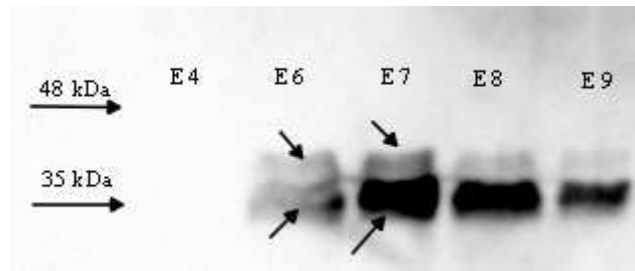


Fig. 4-10. Immunodetection of OAS-TL purified from *Dunaliella tertiolecta*.

The amount of protein loaded on the gel were fraction E4 = 0.49  $\mu\text{g}$ ; E6 = 0.12  $\mu\text{g}$ ; E7 = 0.48  $\mu\text{g}$ ; E8 = 0.35  $\mu\text{g}$ ; E9 = 0.21  $\mu\text{g}$ .

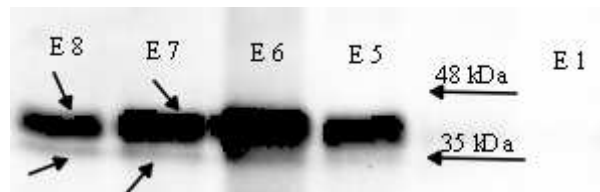


Fig. 4-11. Immunodetection of OAS-TL purified from *Tetraselmis suecica*.

The amount of protein loaded on the gel were fraction E1 = 1.35  $\mu\text{g}$ ; E5 = 1.50  $\mu\text{g}$ ; E6 = 1.75  $\mu\text{g}$ ; E7 = 1.31  $\mu\text{g}$ ; E8 = 0.92  $\mu\text{g}$ .

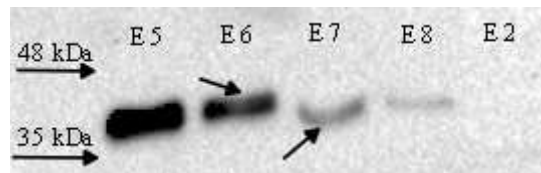


Fig. 4-12. Immunodetection of OAS-TL purified from *Thalassiosira pseudonana*.

The amount of protein loaded on the gel were fraction E2 = 1.01  $\mu\text{g}$ ; E5 = 1.43  $\mu\text{g}$ ; E6 = 1.33  $\mu\text{g}$ ; E7 = 1.01  $\mu\text{g}$ ; E8 = 0.52  $\mu\text{g}$ .

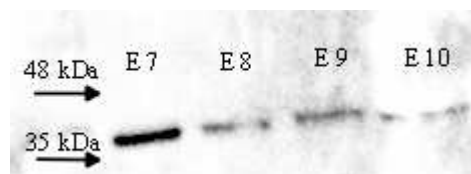


Fig. 4-13. Immunodetection of OAS-TL purified from *Phaeodactylum tricoratum*.

The amount of protein loaded on the gel were fraction E7 = 0.47  $\mu\text{g}$ ; E8 = 0.15  $\mu\text{g}$ ; E9 = 0.13  $\mu\text{g}$ ; E10 = 0.01  $\mu\text{g}$ .

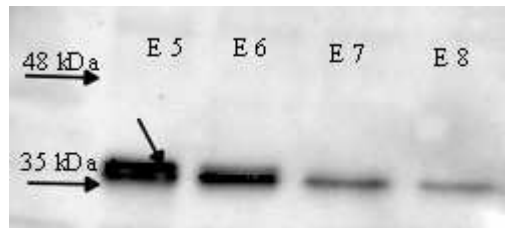


Fig. 4-14. Immunodetection of OAS-TL purified from *Amphidinium klebsii*.

The amount of protein loaded on the gel were fraction E5 = 0.87  $\mu\text{g}$ ; E6 = 0.32  $\mu\text{g}$ ; E7 = 0.31  $\mu\text{g}$ ; E8 = 0.22  $\mu\text{g}$ .

#### 4.2.2. OAS-TL enzyme activity in crude extracts

The activity of OAS-TL was present in the crude extracts of all tested algal species. Most of the species had similar OAS-TL enzyme activity on a protein basis except for *Tetraselmis suecica* (especially) and *Thalassiosira pseudonana*, which showed appreciably higher activities (Fig. 4-15, Table. 4-9).

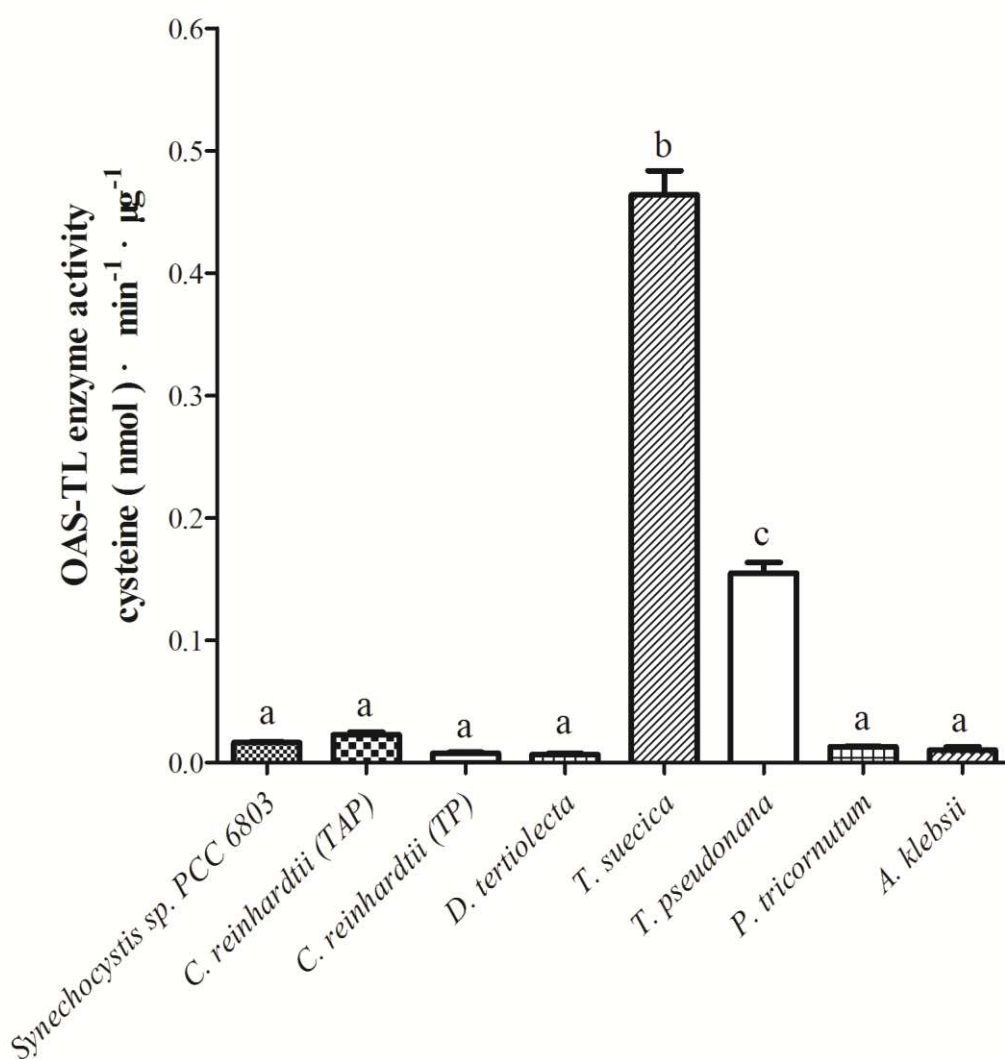


Fig. 4-15. OAS-TL enzyme activity in crude extracts of different algae.

Different letters show statistically different means ( $P < 0.05$ ). The error bars represent the standard deviations ( $n = 3$ ).

In *Synechocystis* sp. PCC 6803, the OAS-TL activity per  $\mu\text{g}$  of protein in the crude extract decreased appreciably if the crude extract was diluted 100 times (Fig. 4-16, Table. 4-9).

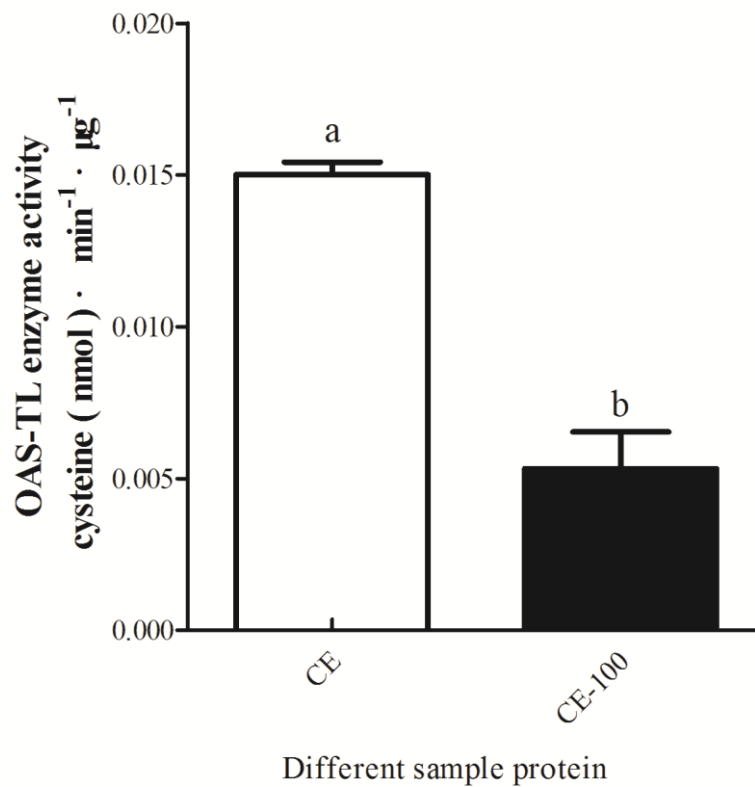


Fig. 4-16. OAS-TL activity per unit of protein in crude and diluted extracts of *Synechocystis* sp. PCC 6803.

CE is the activity of the crude extract obtained according to the extraction protocol. CE-100 is the activity in the crude extract with a 100-fold dilution. Different letters show statistically different means ( $P < 0.05$ ). The error bars show the standard deviations ( $n = 3$ ).

In the green algae *Chlamydomonas reinhardtii* cultured in TAP medium, the OAS-TL activity of the 100-fold diluted sample was similar to the activity in the undiluted extract (Fig. 4-17, Table. 4-9).



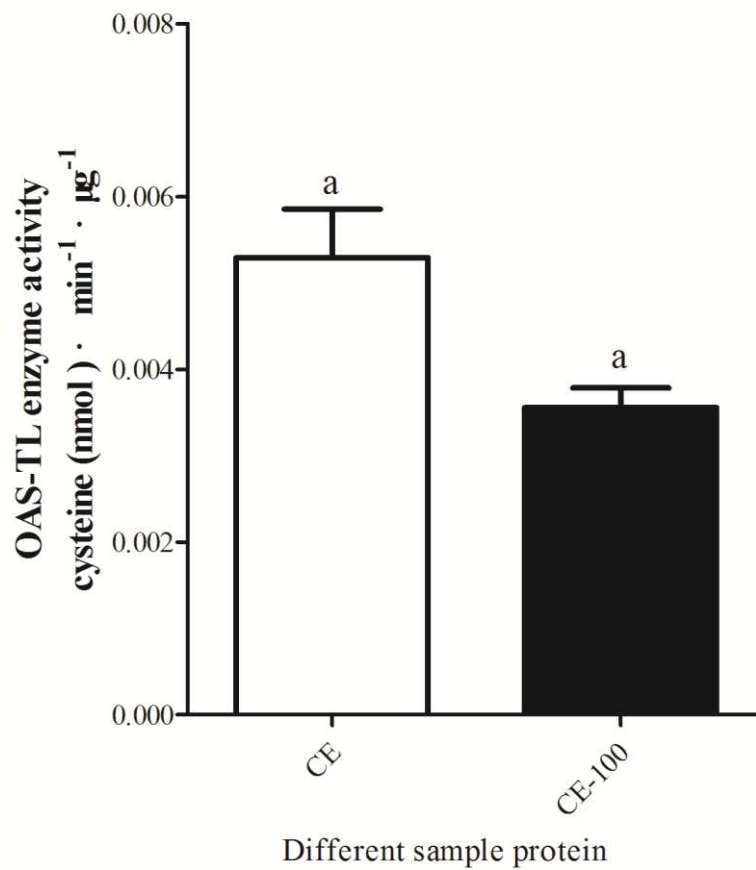


Fig. 4-17. OAS-TL activity per unit of protein in crude and diluted extracts of *Chlamydomonas reinhardtii* cultured in TAP medium.

CE is the activity of the crude extract obtained according to the extraction protocol. CE-100 is the activity in the crude extract with a 100-fold dilution. Different letters show statistically different means ( $P < 0.05$ ). The error bars show the standard deviations ( $n = 3$ ).

When *Chlamydomonas reinhardtii* was cultured in TP medium, OAS-TL activity per unit of protein was about 6.5 higher in diluted extracts (Fig. 4-18, Table. 4-9).

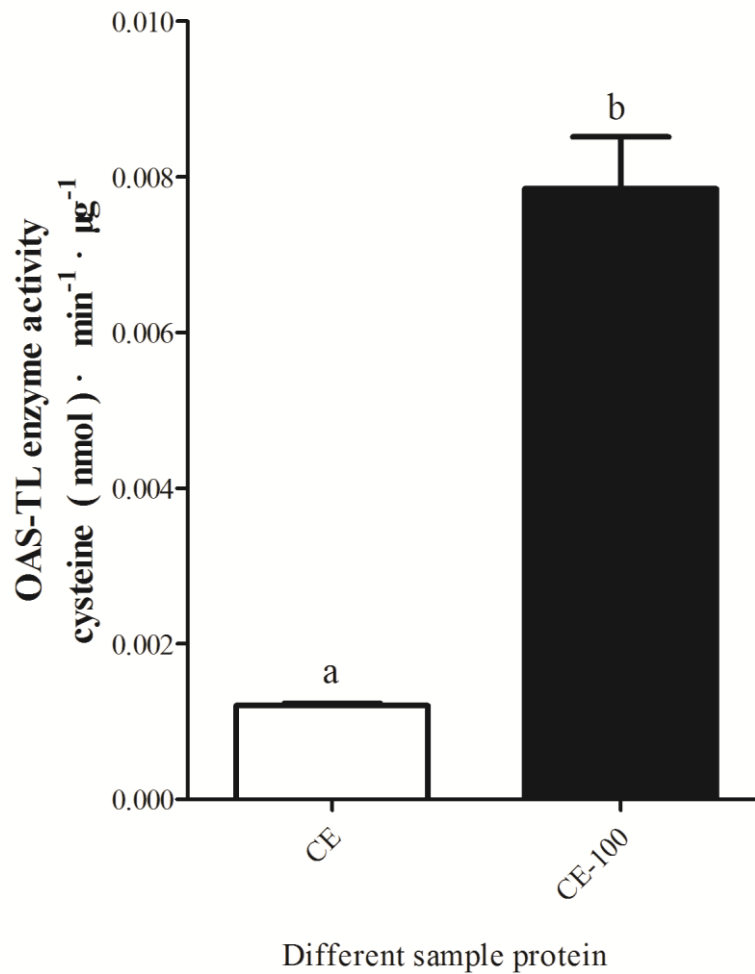


Fig. 4-18. OAS-TL activity per unit of protein in crude and diluted extracts of *Chlamydomonas reinhardtii* cultured in TP medium.

CE is the activity of the crude extract obtained according to the extraction protocol. CE-100 is the activity in the crude extract with a 100-fold dilution. Different letters show statistically different means ( $P < 0.05$ ). The error bars show the standard deviations ( $n = 3$ ).

In *Dunaliella tertiolecta*, OAS-TL activity of the 100-fold diluted sample was similar to that in the undiluted crude extract (Fig. 4-19, Table. 4-9).

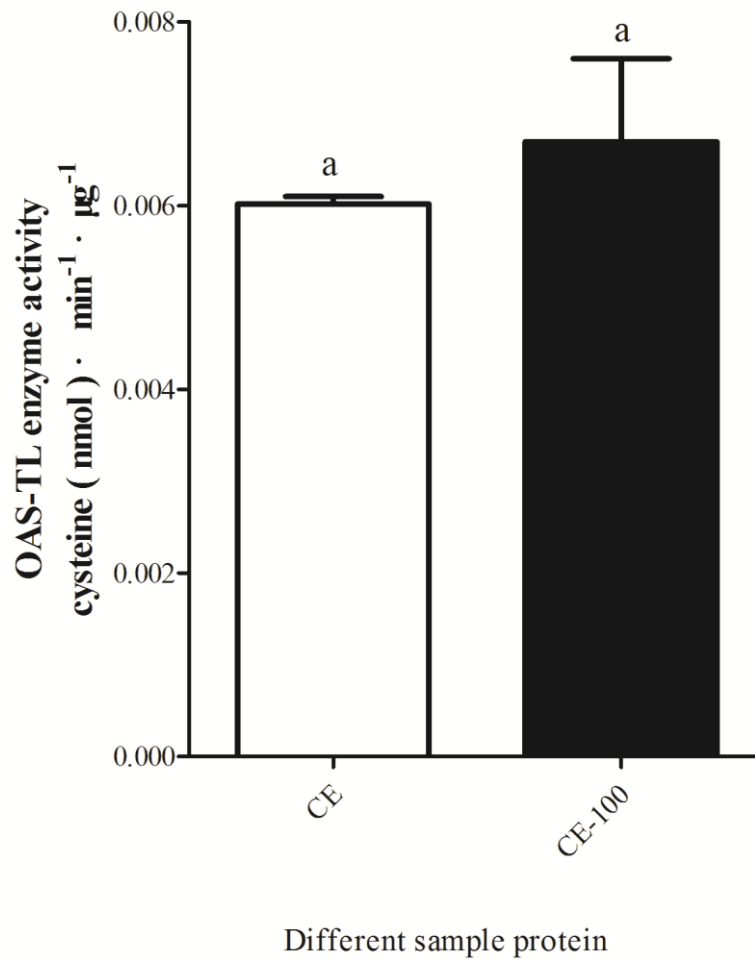


Fig. 4-19. OAS-TL activity per unit of protein in crude and diluted extracts of *Dunaliella tertiolecta*.

CE is the activity of the crude extract obtained according to the extraction protocol. CE-100 is the activity in the crude extract with a 100-fold dilution. Different letters show statistically different means ( $P < 0.05$ ). The error bars show the standard deviations ( $n = 3$ ).

In *Tetraselmis suecica*, OAS-TL enzyme activity of the 100 times diluted sample was 1.6 times higher (Fig. 4-20, Table. 4-9).

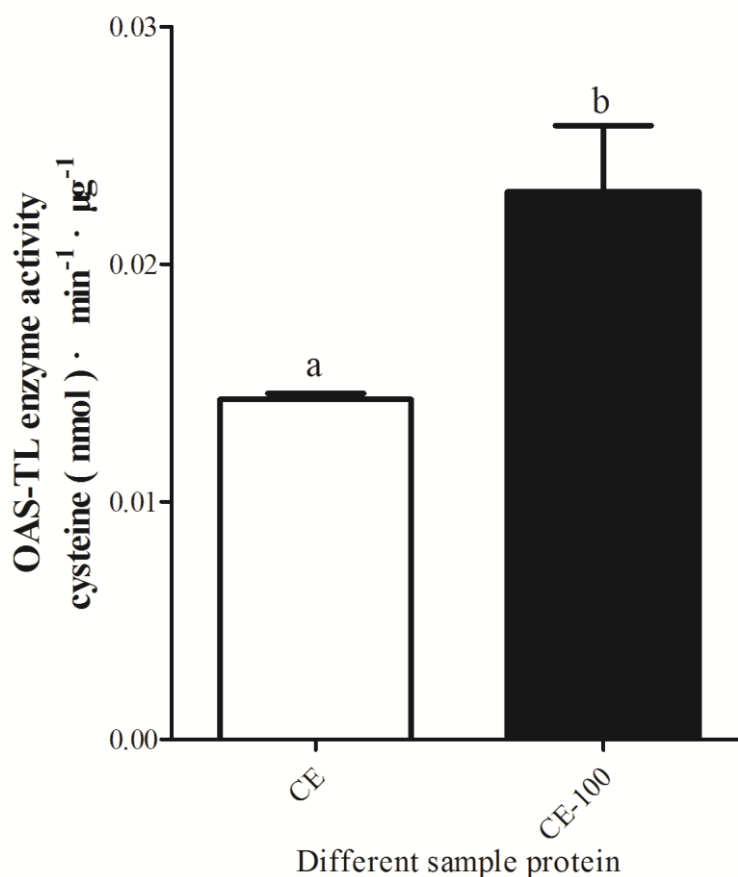


Fig. 4-20. OAS-TL activity per unit of protein in crude and diluted extracts of *Tetraselmis suecica*.

CE is the activity of the crude extract obtained according to the extraction protocol. CE-100 is the activity in the crude extract with a 100-fold dilution. Different letters show statistically different means ( $P < 0.05$ ). The error bars show the standard deviations ( $n = 3$ ).

In *Thalassiosira pseudonana*, OAS-TL enzyme activity of the 100 times diluted sample was 11.4 times higher compared to the undiluted extract (Fig. 4-21, Table. 4-9).

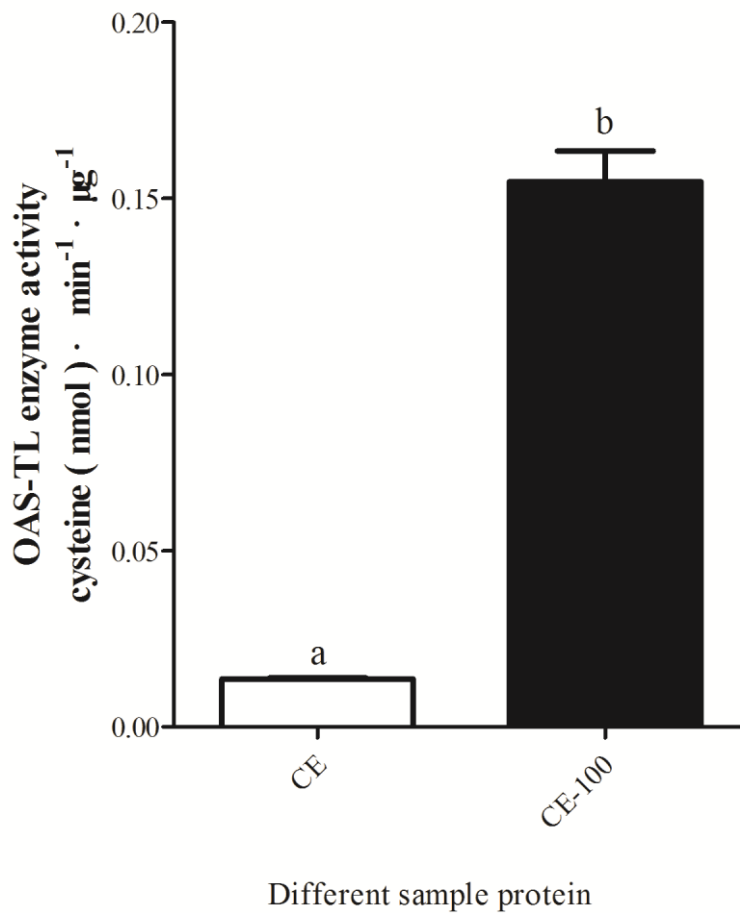


Fig. 4-21. OAS-TL activity per unit of protein in crude and diluted extracts of *Thalassiosira pseudonana*.

CE is the activity of the crude extract obtained according to the extraction protocol. CE-100 is the activity in the crude extract with a 100-fold dilution. Different letters show statistically different means ( $P < 0.05$ ). The error bars show the standard deviations ( $n = 3$ ).

In *Phaeodactylum tricornutum*, OAS-TL enzyme activity of the 100 times diluted extract was 3.3 times higher than the activity in the undiluted extract (Fig. 4-22, Table. 4-9).

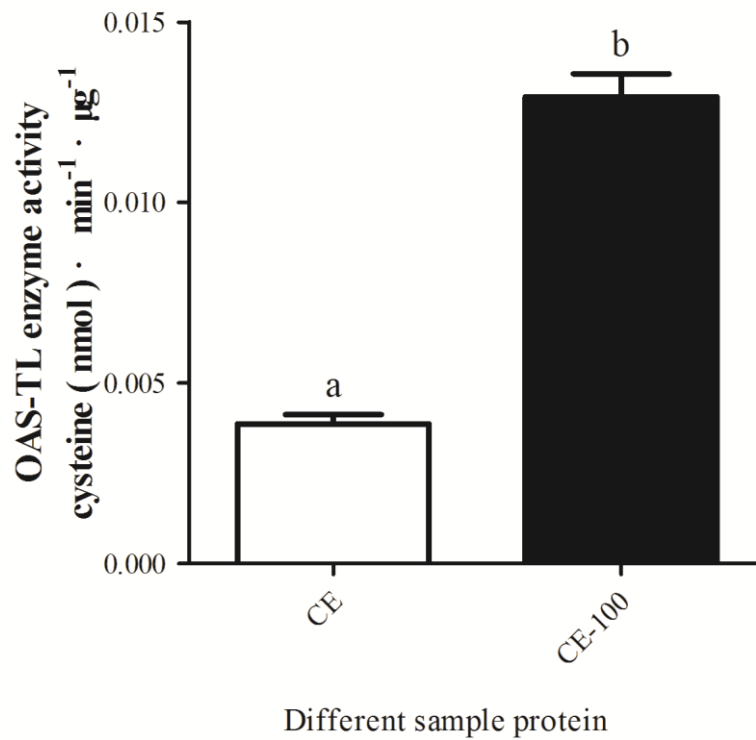


Fig. 4-22. OAS-TL activity per unit of protein in crude and diluted extracts of *Phaeodactylum tricornutum*.

CE is the activity of the crude extract obtained according to the extraction protocol. CE-100 is the activity in the crude extract with a 100-fold dilution. Different letters show statistically different means ( $P < 0.05$ ). The error bars show the standard deviations ( $n = 3$ ).

In *Amphidinium klebsii*, the OAS-TL activity of the 100 times diluted sample was 1.6 times higher than the activity in the undiluted extract (Fig. 4-23, Table. 4-9).

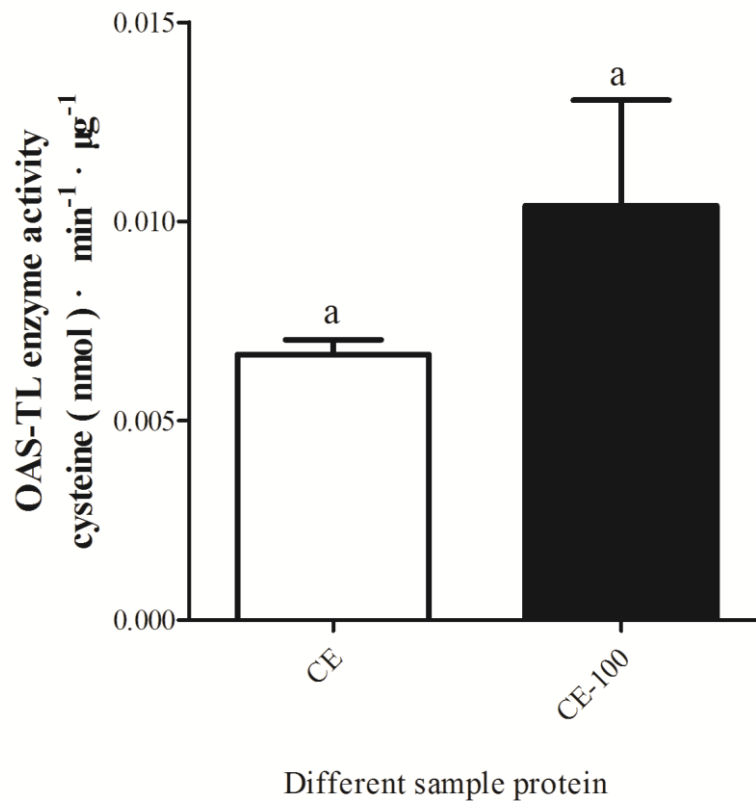


Fig. 4-23. OAS-TL activity per unit of protein in crude and diluted extracts of *Amphidinium klebsii*.

CE is the activity of the crude extract obtained according to the extraction protocol. CE-100 is the activity in the crude extract with a 100-fold dilution. Different letters show statistically different means ( $P < 0.05$ ). The error bars show the standard deviations ( $n = 3$ ).

Table. 4-9. Summary of OAS-TL activity in 100-fold diluted and undiluted crude extracts.

Species name	OAS-TL enzyme activity (Mean ±SD) nmol cysteine · min <sup>-1</sup> · ng <sup>-1</sup>	
	CE	CE-100
<i>Synechocystis</i> sp. PCC 6803	15 (0.4)	5.0 (1.2)
<i>Chlamydomonas reinhardtii</i> TAP	5.3 (0.6)	3.6 (0.2)
<i>Chlamydomonas reinhardtii</i> TP	1.2 (0.0)	8.0 (0.7)
<i>Dunaliella tertiolecta</i>	6.0 (0.0)	7.0 (0.9)
<i>Tetraselmis suecica</i>	14.3 (0.3)	23.0 (2.8)
<i>Thalassiosira pseudonana</i>	13.6 (0.4)	155 (8.8)
<i>Phaeodactylum triconutum</i>	3.9 (0.3)	12.9 (0.6)
<i>Amphidinium klebsii</i>	6.7 (0.4)	10.4 (2.7)

CE: crude extract; CE-100: 100 fold diluted crude extract. The results are shown as mean ± SD ( $n=3$ ).

### 4.2.3. OAS-TL specific activity in different microalgae

OAS-TL specific activity from the various experimental organisms was determined after purification (Fig. 4-24). The SDS-PAGEs (not shown) suggest that the purified fractions contained negligible amounts of contaminant proteins. The pure OAS-TL of the green freshwater species *Chlamydomonas reinhardtii* cultured in TP medium, and of the green marine species *Dunaliella tertiolecta* and *Tetraselmis suecica*, and especially of the marine diatom *Thalassiosira pseudonana* showed higher specific activities than the other species (Fig. 4-24). It is noteworthy that *C. reinhardtii* OAS-TL specific activity was strongly affected by the growth medium (Fig. 4-24).



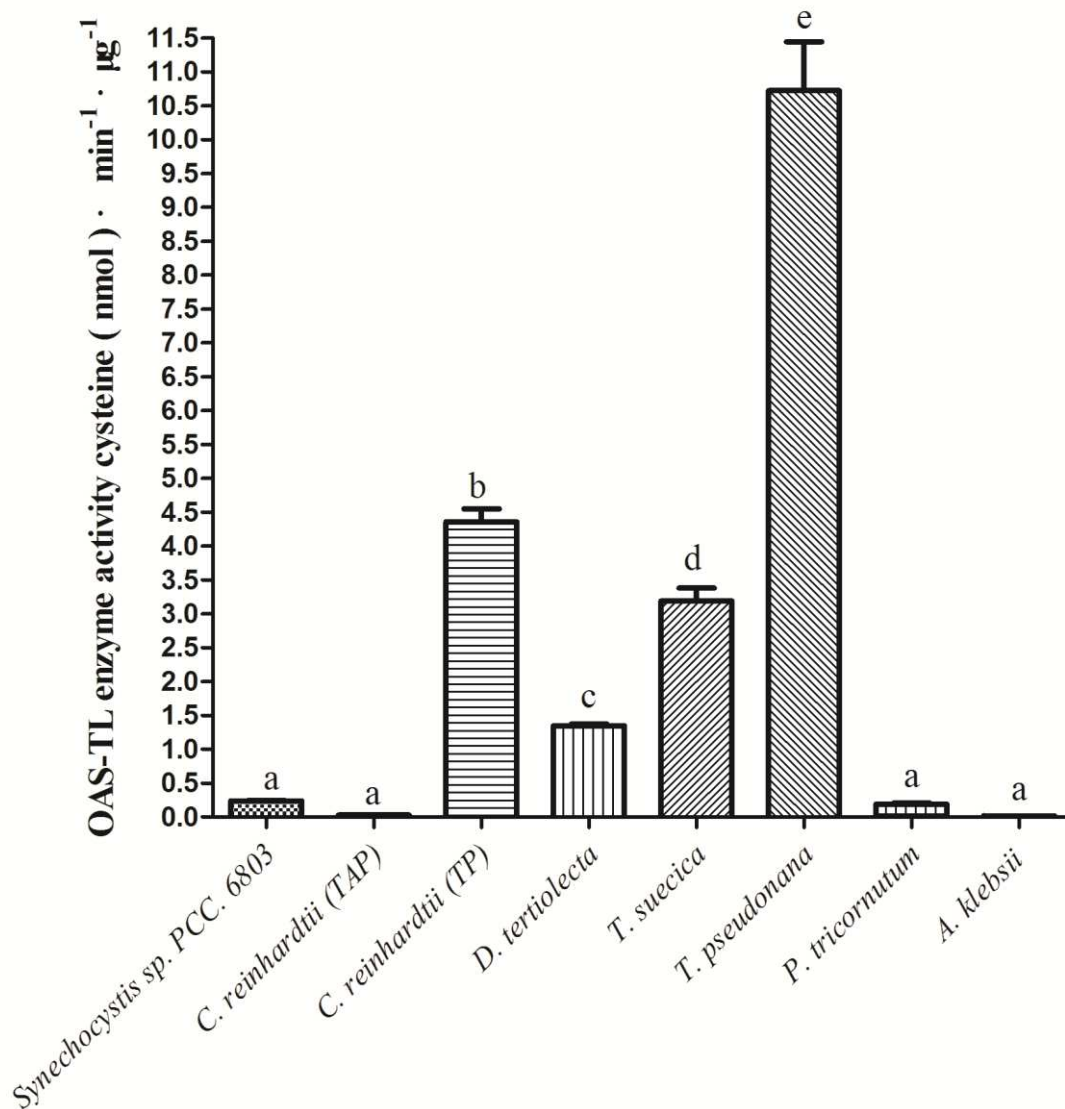


Fig. 4-24. OAS-TL enzyme activity.

Different letters show statistically different means ( $P < 0.05$ ). The error bars show the standard deviations ( $n = 3$ ).

#### 4.2.4. Cysteine Synthase Complex (CSC)

For all species tested, when purified OAS-TL was mixed with *A. thaliana* SAT5 and subject to size exclusion chromatography, a complex of more than 600 kDa was eluted (Fig. 4-25).

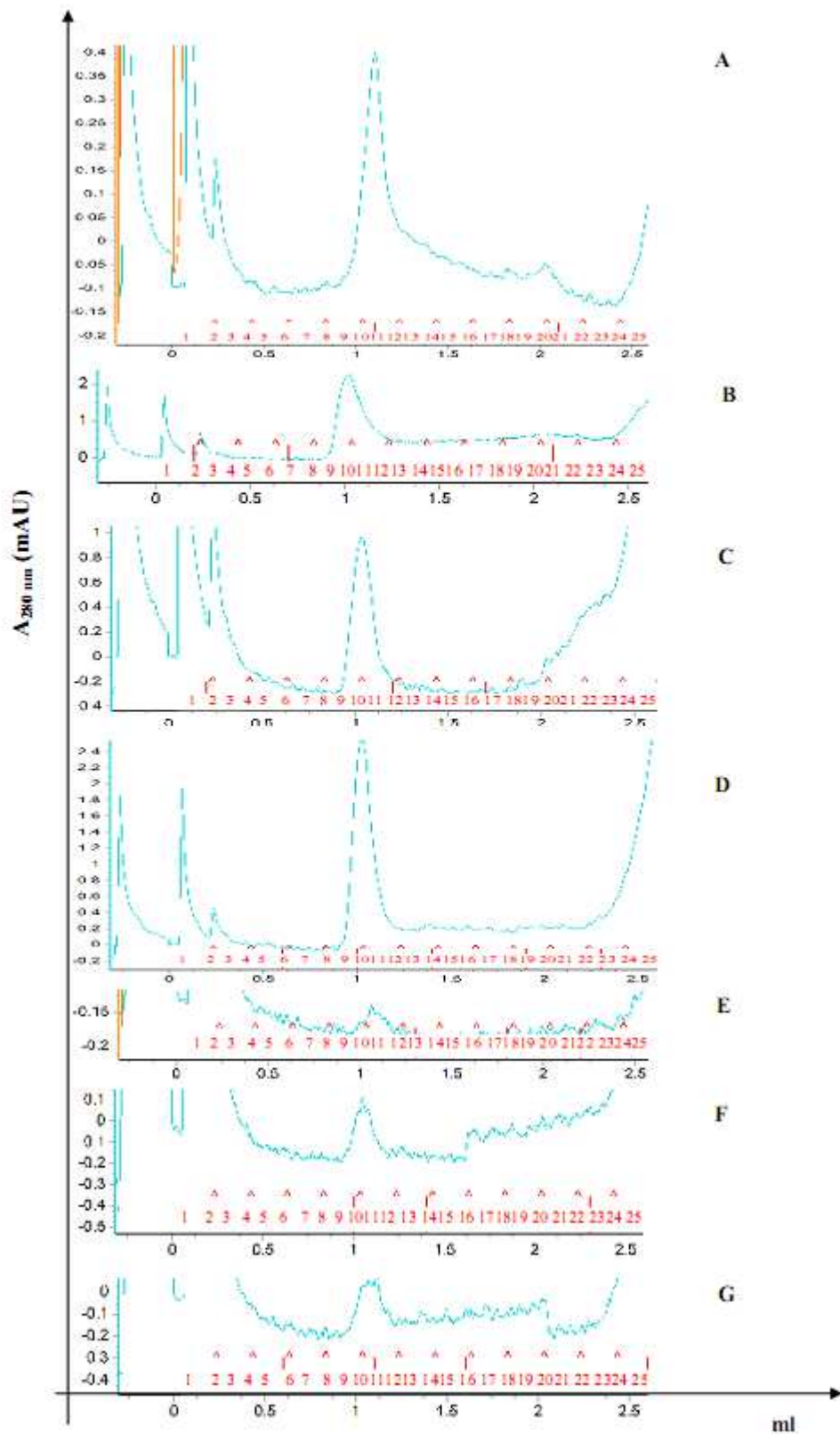


Fig. 4-25. FPLC chromatogram for OAS-TL from different algae complexed with SAT-5 from *A. thaliana*.

A: *Synechocystis* sp. PCC 6803; B: *Chlamydomonas reinhardtii*-TAP; C: *Dunaliella tertiolecta*; D: *Tetraselmis suecica*; E: *Thalassiosira pseudonana*; F: *Phaeodactylum*

*tricornutum*; G: *Amphidinium klebsii*. Red numbers represent fraction numbers in parallel with black numbers representing elution volume (ml).

In *Synechocystis*, fraction 12 (E-12) showed cross-reaction with OAS-TL antibody (Fig. 4-25 A, Fig. 4-26). The size of the protein band involved in the complex formation was 39 kDa as the size of bigger protein band found in the purification (Fig. 4-7). The same fraction also gave an immunological reaction with SAT-5 antibody (35 kDa), as shown in (Fig. 4-33 A, lane 5).

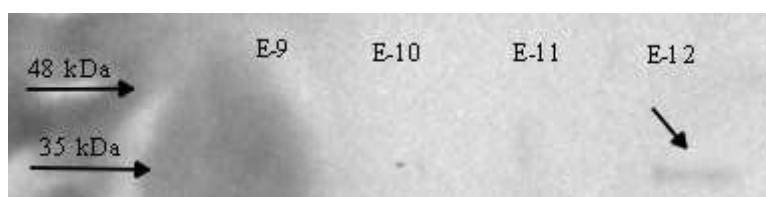


Fig. 4-26. Immunodetection of OAS-TL from *Synechocystis* sp. PCC 6803 after elution of the CSC with SAT-5 by FPLC.

E-9, E-10, E-11 and E-12 were different fractions of the FPLC separation.

The CSC of *C. reinhardtii* cultured in TAP medium was eluted in fractions E10 to E12 (Fig. 4-25 B). Both OAS-TL (Fig. 4-27) and SAT-5 immunoreactions (Fig. 4-33 B, lane 3) were present.

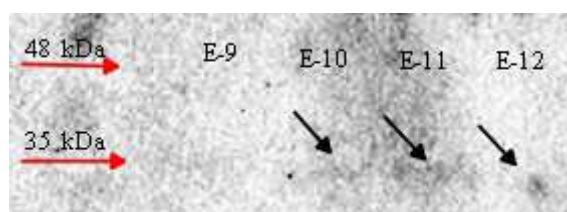


Fig. 4-27. Immunodetection of OAS-TL from *Chlamydomonas reinhardtii* in TAP medium after elution of the CSC with SAT-5 by FPLC.

E-9, E-10, E-11, E-12 were different fraction of the FPLC separation.

In *Dunaliella tertiolecta*, CSC was eluted in fractions E-10 to E-11 (Fig. 4-25 C), as confirmed by western blot analysis (Fig. 4-28 and 4-33 C). Two OAS-TL bands were present in the complex and

their sizes were consistent with the purified OAS-TL bands (Fig. 4-10).

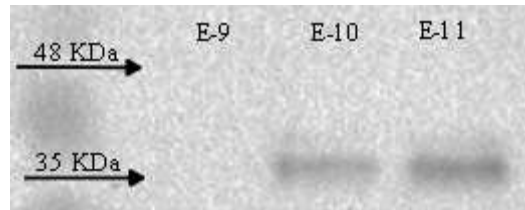


Fig. 4-28. Immunodetection of OAS-TL from *Dunaliella tertiolecta* after elution of the CSC with SAT-5 by FPLC.

E-9, E-10, E-11 were different fraction of the FPLC separation.

The CSC of *Tetraselmis suecica* was eluted in fractions E-9, 10, 11 (Fig. 4-31 D) as confirmed by the results of western blots with OAS-TL (Fig. 4-29) and SAT5 (Fig. 4-33 A, lanes 8 and 9). The OAS-TL of *T. suecica* CSC had a size of 41 kDa (Fig. 4-11), as the size of the strongest band in the western of the purified protein.

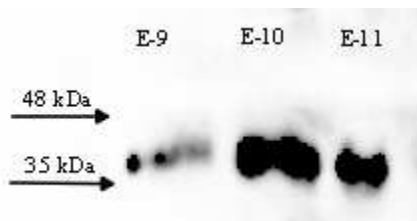


Fig. 4-29. Immunodetection of OAS-TL from *Tetraselmis suecica* after elution of the CSC with SAT-5 by FPLC.

E-9, E-10, E-11 were different fraction of the FPLC separation.

The CSC of the diatom *Thalassiosira pseudonana* was eluted in E-10, where also crossreaction with OAS-TL (Fig. 4-30) and SAT-5 (Fig. 4-33 C, lanes 5 and 6) antibodies was observed. The OAS-TL in the complex had a size of 36 kDa.

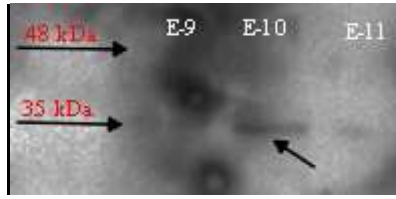


Fig. 4-30. Immunodetection of OAS-TL from *Thalassiosira pseudonana* after elution of the CSC with SAT-5 by FPLC.

E-9, E-10, E-11 were different fractions of the FPLC separation.

The CSC from *Phaeodactylum tricornutum* was eluted in fractions E-9 to E-11 (Fig. 4-25 F); the presence of the complex in these fractions was confirmed by the western blot analyses (Fig. 4-31, Fig. 4-33 C, lanes 2, 3 and 4). The OAS-TL signal in the complex corresponded to a protein of 36 kDa as shown also in the purified sample (Fig. 4-13).

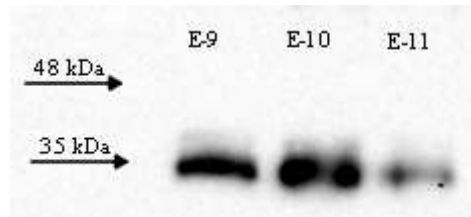


Fig. 4-31. Immunodetection of OAS-TL from *Phaeodactylum tricornutum* after elution of the CSC with SAT-5 by FPLC.

E-9, E-10, E-11 were different fractions of the FPLC separation.

In the dinoflagellate *Amphidinium klebsii*, the CSC appeared in fractions E-9 and E-10. In the same fractions, a band of 35 kDa cross reacted with OAS-TL antibody (Fig. 4-32) and a band cross reacting with SAT5 antibodies (Fig. 4-33 A, lanes 2, 3 and 4) was observed.

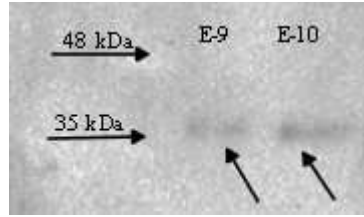


Fig. 4-32. Immunodetection of OAS-TL from *Amphidinium klebsii* after elution of the CSC with SAT-5 by FPLC.

E-9 and E-10 were different fractions of the FPLC separation.

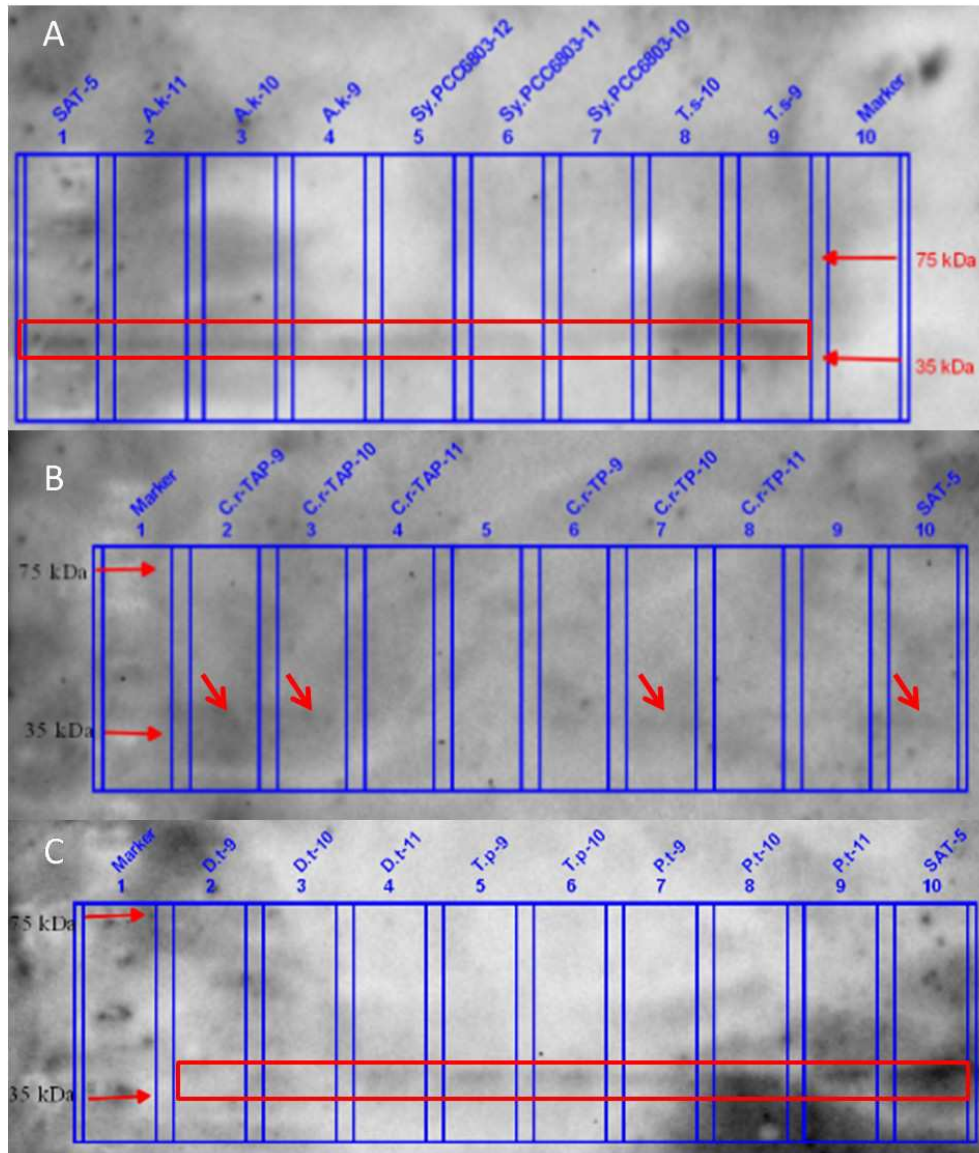


Fig. 4-33. Western blot using antibodies rose against SAT-5 from *A. thaliana*.

The samples corresponding to the peaks obtained by size exclusion chromatography for which a positive reaction with the antibody for OAS-TL was observed. Note: Marker, the protein ladder (10-180 kDa); A) from left to the right lane 1: SAT-5; lanes 2 to 4: *Amphidinium klebsii* (fractions 9-11); lanes 5 to 7: *Synechocystis sp.* PCC 6803 (fractions 10-12); lanes 8 to 9: *Tetraselmis suecica* (fractions 9-10); lane 10: Marker. B) From left to the right lane 1: Marker; lanes 2 to 4: TAP medium grown *Chlamydomonas reinhardtii* (fractions 9-11); lanes 6 to 8: TP medium grown *Chlamydomonas reinhardtii* (fractions 9-11); lane 10: SAT-5. C) From left to the right lane 1: Marker; lanes 2 to 4: *Dunaliella tertiolecta* (fractions 9-11); lanes 5 to 6: *Thalassiosira pseudonana* (fractions 9-11); lanes 7-9: *Phaeodactylum tricornutum* (fractions 9-11); lane 10: SAT-5. All the detected AtSAT-5 was showed in the red frame (A and C) and by red arrows (B).

### 4.3. In vivo redox regulation of ATP-S activity

*Thalassiosira pseudonana*, whose ATPS is believed to be redox regulated, was used for these experiments. DCMU interrupted electron transfer almost immediately at a concentration of 5  $\mu\text{M}$  (data not shown). The quantum yield of PSII was determined as Fv/Fm and its response to 0, 5 and 10  $\mu\text{M}$  DCMU was shown in Fig. 4-34. Already after 1 hour, DCMU at both 5  $\mu\text{M}$  and 10  $\mu\text{M}$  caused Fv/Fm to significantly decrease, as compared to the 0 DCMU controls ( $P < 0.001$ ). This trend was accentuated after 3 and 6 hours. The concentration of 10  $\mu\text{M}$  DCMU was chosen for the experiments.

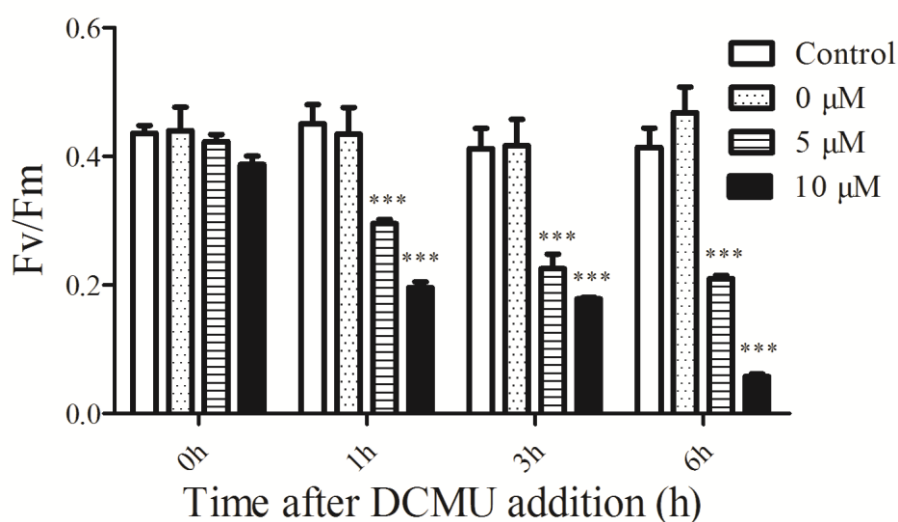


Fig. 4-34. Fv/Fm of *Thalassiosira pseudonana* incubated in the presence of 5 and 10  $\mu\text{M}$  DCMU, for 0, 1, 3 and 6 hours.

The error bars represented the standard deviations ( $n = 3$ ); asterisks indicated the significance of mean differences (\* =  $p < 0.05$ ; \*\* =  $p < 0.01$ ; \*\*\* =  $p < 0.001$ ).

DCMU (10  $\mu\text{M}$ ) had no impact on cell number and cell volume, in the course of the experiments. In the presence of 10  $\mu\text{M}$ , no significant difference in cell number and cell volume was observed (Fig. 4-35 and Fig. 4-36, respectively).



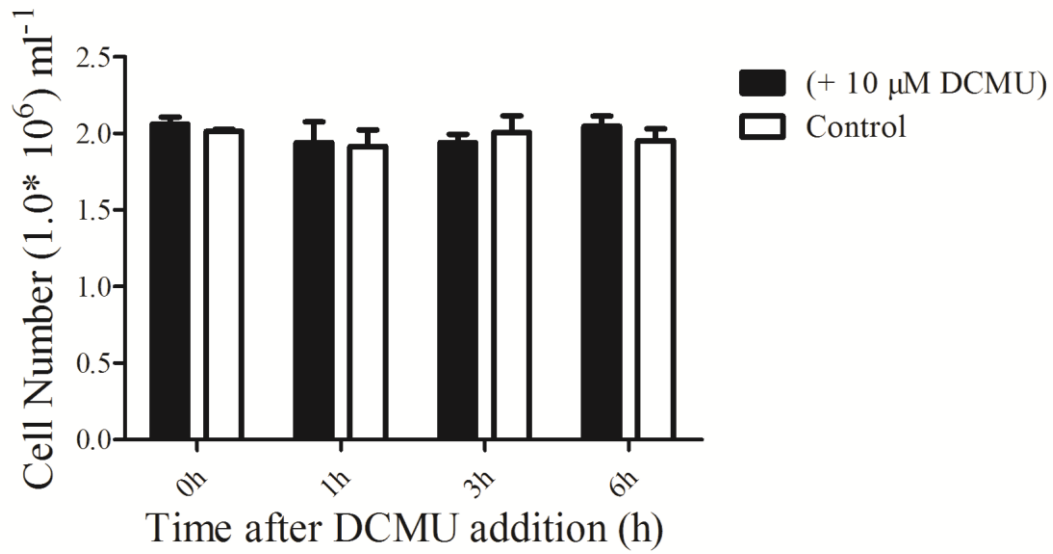


Fig. 4-35. *Thalassiosira pseudonana* cell number during incubation with 10  $\mu\text{M}$  DCMU.

The error bars represented the standard deviations ( $n = 3$ ). Means were not significantly different.

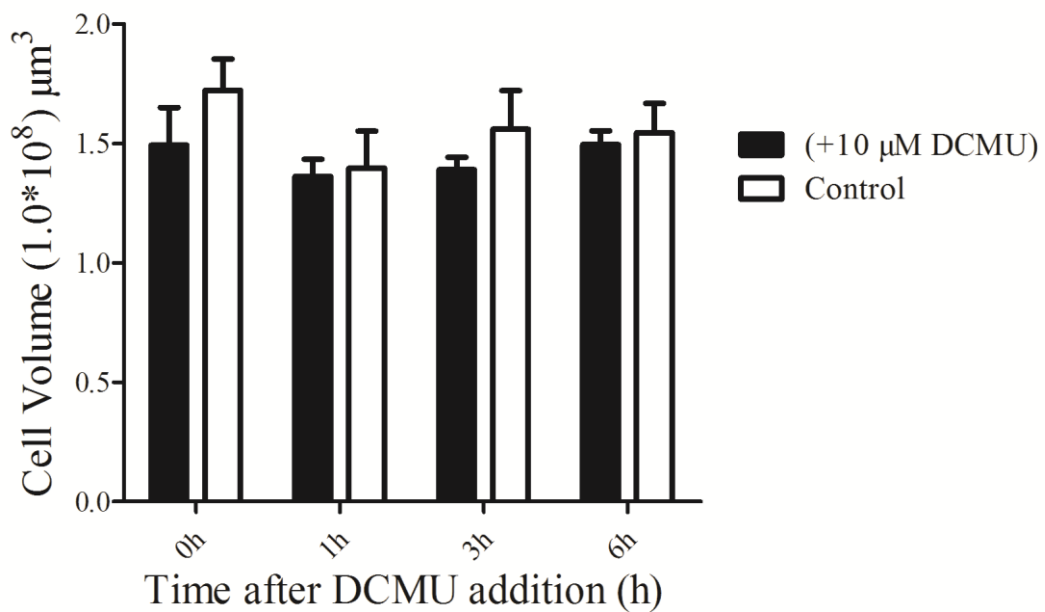


Fig. 4-36. *Thalassiosira pseudonana* cell volume during incubation with 10  $\mu\text{M}$  DCMU.

The error bars represented the standard deviations ( $n = 3$ ). Means were not significantly different.

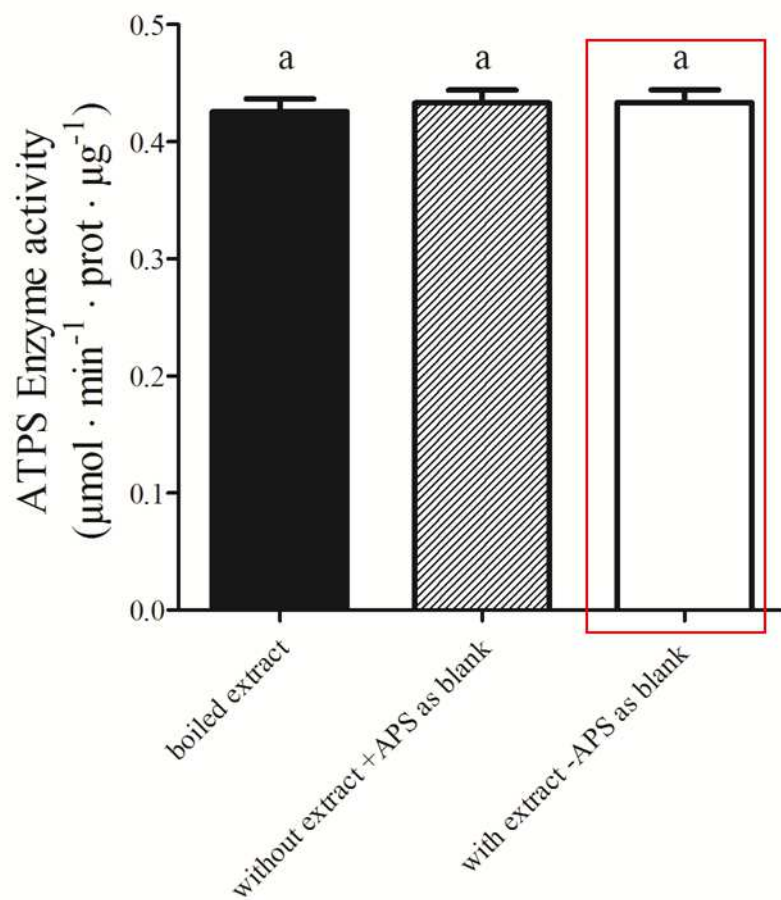


Fig. 4-37. Comparison of ATPS assay blanks obtained with different procedures. The error bars show the standard deviations (n = 3).

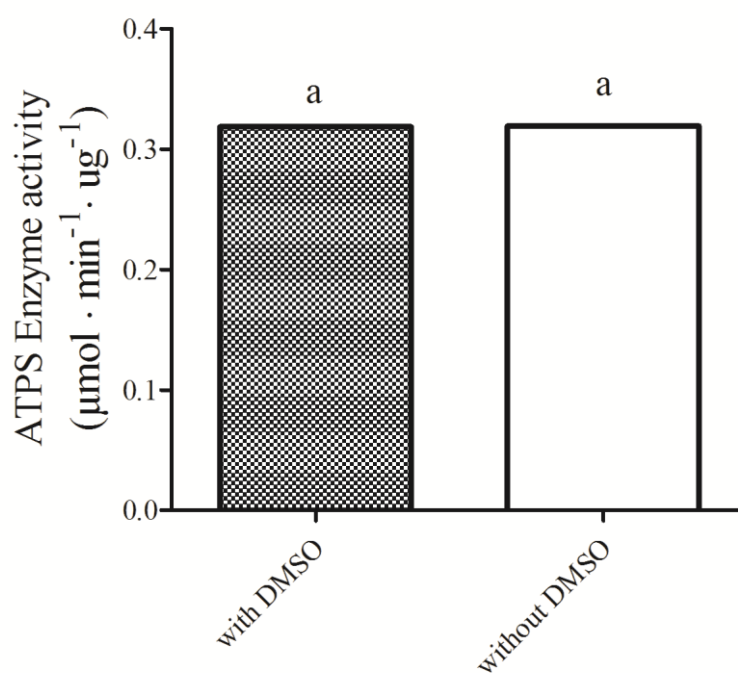


Fig. 4-38. Comparison of ATPS activity in the presence and absence of DMSO.

The error bars show the standard deviations (n = 3).

The ATP sulfurylase (ATP-S) enzyme activity was not affected by the incubation of the algae in the presence of DCMU (Fig. 4-39). Even though the activity changed over time, there was no statistically significant difference between the DCMU treated samples and the control samples.

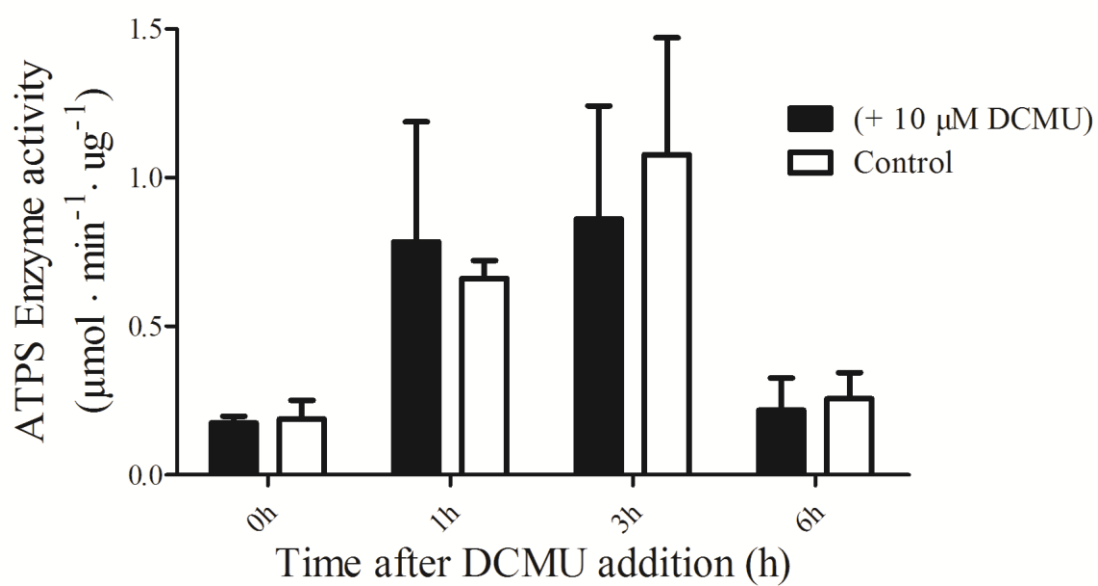


Fig. 4-39. *Thalassiosira pseudonana* ATP-S activity in the presence or absence of 10 µM DCMU over a period of 6 hours. The error bars show the standard deviations (n = 3).



## 5. Discussion

### 5.1. SAT and OAS-TL in algae

The SAT protein tree indicates that phylogenetic difference exists between the protein in green algae and vascular plants and sequences obtained from species of the red-lineages (with rare exceptions). Interestingly, cyanobacterial SAT appears to be more closely related to that of red algae. It is hard to interpret this observation. It may simply be due to the fact that extant cyanobacteria have acquired their current S assimilation complement secondarily, together with red lineage algae. The similarity between cyanobacteria and red lineage algae SATs also brings to mind the fact that cyanobacteria retained their prominence in post-Mesozoic oceans, together with red lineage algae, whereas green algae declined to a great extent. SAT phylogeny may thus be connected with the Sulfate Assimilation Hypothesis (Ratti et al., 2011), which suggests that sulfate availability in global oceans may have affected algae radiation. How SAT would affect phytoplankton radiation however, cannot be resolved with the data at hand and requires further investigations. The role of SAT in cysteine synthesis may speculatively suggest that a different control/regulation of cysteine synthesis may have repercussions on thiol metabolism and redox power management in the cell (Dickinson & Forman, 2002; Dietz & Hell, 2015).

It is also noteworthy that the C-terminus of algal SATs is rather similar to that of embryophytes with *Arabidopsis* sequence (Fig. 4-4). Thus the C-portion of algae SAT probably has a similar organization to that of *Arabidopsis* (Wirtz et al., 2001), *E.coli* (Pye et al., 2004) and *Haemophilus influenza Rd* (Gorman & Shapiro, 2004), whose SAT monomers contain a carboxyl-terminal left-handed  $\beta$ -helix (Gorman & Shapiro, 2004; Pye et al., 2004). The sequence alignments indicate that the C-terminal region of SATs is highly conserved in algae and other organisms and, based on the work

done on *E. coli* by Pye et al. (2004), it is likely to be responsible for the hetero-oligomerization with OAS-TL. The C-terminal domain is also where the catalytic site is located (Bogdanova & Hell, 1997). The idea of a bifunctional C-terminal SAT domain (involved in catalysis and oligomerization) was further strengthened by the modelling of the C-terminus of plant SAT using bacterial acyltransferase structures as template (Vuorio et al., 1991; Vaara, 1992): the C-terminus of SAT encompasses two sections, a left-handed parallel  $\beta$ - helix (L $\beta$ H) domain, which carries the catalytically active site, and a C-terminal tail that could not be modelled due to low homology to the acyltransferases (Wirtz et al., 2001). Given the fact that the C-terminus domain aminoacid sequence of algae is very similar to that of embryophytes, it may operate as in embryophytes.

The SAT-SAT interaction domain is instead located at the N-terminal  $\alpha$ -helical domain (Bogdanova & Hell, 1997). Here, homology between algae and vascular plants sequences are smaller and this may indicate possible differences in the size of the Cysteine Synthase Complex in algae and plants (see Fig. 5-1).

One thing seems to differentiate red lineage algae from green algae and their descendants: the number of cysteine residues, which are more numerous in the red lineage (especially in haptophyte). In ATPS, the number of cysteines was found to be related to redox regulation (Prioretti et al., 2016). At this stage, my data do not allow to verify if redox regulation exists in SAT and whether such regulation exists only in the SATs with more cysteine residues. In embryophytes, SAT does not appear to be redox-regulated; this is, of course, no proof that this is also the case in algae, as the work on ATPS demonstrates (Prioretti et al., 2014).

OAS-TL bioinformatic data showed a rather unclear phylogeny, possibly representing a higher variety of isoforms and a higher degree of gene exchanges, as compared to SAT. Overall the OAS-TL of algae showed a highly conserved C-terminal region. The OAS-TL protein size of algae is in the range 35-44 kDa, not too different from the enzymes of embryophytes (Table. 4-8; in *A. thaliana* OAS-TL has a mass of 33-37 kDa). The immunoreactions suggest that different sizes may be present in green algae,

although further analyses are required to confirm this. Interestingly, OAS-TL activity (as sulfide production) is higher when the enzyme is assayed in diluted crude extracts. This, in the literature (Bogdanova & Hell, 1997; Wirtz et al., 2001) is interpreted as an indication that the enzyme operates when not complexed. This is certainly a very indirect evidence, but support the idea that OAS-TL is regulated in plants as in embryophytes (Takahashi et al., 2011).

## 5.2. Cysteine Synthase Complex in algae

The main objective of my thesis was to verify whether algae form Cysteine Synthase Complexes, like vascular plants (Saito et al., 1995; Droux et al., 1998; Wirtz et al., 2001, 2010) and bacteria (Kredich & Tomkins, 1966). As reported above, the features of algae OAS-TL and SAT are suggestive that these proteins have structures similarity with the corresponding embryophyte proteins that make them capable of interacting in a Cysteine Synthase Complex. Both algae SAT and OAS-TL appeared to be located in both the chloroplast and mitochondrion. Other isoforms are possibly located in the cytosol. The algal distribution of the enzyme is thus similar to that of embryophytes, in general terms. In embryophyte, mitochondria seems the main location of OAS production, whereas cysteine is mostly produced in the cytosol (Takahashi et al., 2011). In the chloroplast, cysteine synthesis is limited by the low OAS availability and the role of this organelle in sulfur metabolism is mostly that of producing sulfide. A similar situation is thought to occur in algae.

I observed that the OAS-TL of the green algae *Chlamydomonas reinhardtii*, *Dunaliella tertiolecta* and *Tetraselmis suecica* bound to the AtSAT5 affinity column much more strongly than the OAS-TL from the diatoms *Thalassiosira pseudonana* and *Phaeodactylum tricoratum* and from the dinoflagellate *Amphidinium klebsii*. If with some degree of approximation, I consider the elution time of OAS-TL from AtSAT5 affinity column as an indication of the strength of the interaction of the proteins in the complex, this may be an indication of a lower propensity of algae of



the red lineage to form CSC complex. In embryophytes, OAS-TL only catalyzes cysteine production when it is free, whereas the complex is conducive to the synthesis of OAS. Therefore, the presumed lower tendency of red-lineage OAS-TL to bind SAT may reflect the higher tendency of red lineage algae to allocate C to cysteine, as compared to green algae. This may be linked to the appreciably higher DMSP concentration in red lineage algae than in green algae (Giordano et al., 2008), which requires a higher flux of S through cysteine and then methionine (Giordano & Prioretti, 2016). If this will be confirmed by *ad hoc* measurements, it would provide further mechanistic support to the Sulfur Facilitation Hypothesis (Ratti et al., 2011): the different organization of red algae CSC may funnel more S into DMSP. This would lead to the higher S quotas of these cells and may consequently lead to the competitive advantage afforded by the anti-grazing power of the DMSP cleavage products (Norici et al., 2005).

In *A. thaliana*, *E. coli* and *S. typhimurium*, the Cystein Synthase Complex is composed of a hexamer of SAT and two dimers of OAS-TL (Wirtz et al., 2004). My experiments results, which were consisted with the reported data (Salbitani et al., 2014) provide proof-of-concept for the biochemical analysis of the cysteine synthase complex in different microalgal species. Subsequently, my experiments in size exclusion chromatography seem to suggest that the algal complexes (although with *A. thaliana* SAT) are always larger than the mass of 320 kDa reported by Wirtz et al, (2004) for the embryophytes complex. More recently, it has been suggested that each hexamer of SAT may bind up to six dimers of OAS-TL, one for each C-terminal domain of the protein (Pye et al., 2004). Based on the estimated mass of the eluted native complex, I propose that the stoichiometry of the algae Cysteine Synthase Complex is different from that in embryophytes: the algal CSC may be made of two SAT trimer connected 'head to head' form one hexamer, with one OAS-TL dimer bound to each SAT C-terminus (Fig. 5-1 A); this gives a total mass of about 600 kDa, compatible with the size of the complex eluted from the size exclusion column. Alternatively, given that the sequence difference at the N-terminus would lead to

believe that differences between algae and plant CSC are more likely to reside in the SAT-SAT interactions, I proposed a 6 SAT (2 SAT trimers): 12 OAS-TL (6 OAS-TL dimers) stoichiometry.

In *E. coli* and possibly in *Arabidopsis*, the Cysteine Synthase Complex appears to be composed of a SAT homohexamer and two OAS-TL dimmers (Fig. 5-1 Panel B). The SAT hexamer is constituted by two trimers arranged head-to-head with the C-terminal tails of each trimer at opposite ends of the hexamer (Kumaran et al., 2009). The interaction between SAT and OAS-TL occurs through portions of SAT C-terminus, with allegedly a crucial involvement of a hexapeptide with the sequence (I/V/L)-G-XXXX-(I/V/L) (Bogdanova & Hell., 1997; Wirtz et al., 2001). This general hexapeptide is present also in algae (Fig. 4-4). On the OAS-TL side, binding with SAT takes place through a KPGPHK sequence at the C-terminus. In algae the first and last two amino acids of this sequence are often substituted (Table. 4-7). As most proteins consist of multiple domains, and domains determine the function and evolutionary relationships of proteins, it is important to understand the principles of domain combinations and interactions (Vogel et al., 2004).

The purification of the algal CSC showed that the complex was much larger in algae than in plants (Fig. 4-25), with an approximate mass of 600 kDa (Fig. 4-26 to Fig. 4-32). On the basis of the information provided by Birke et al., 2015 and Yi et al., 2013, on the size difference between algal and *Arabidopsis* CSC complex, and on the variations in both SAT and of OAS-TL sequences involved in the interaction between these two proteins in algae, I propose that the algal CSC contains two SAT trimers, as in vascular plant, but each SAT protein binds one OAS-TL dimer through its C-terminus. The proposed structure of the algal CSC is shown in Fig. 5-1 Panel A.

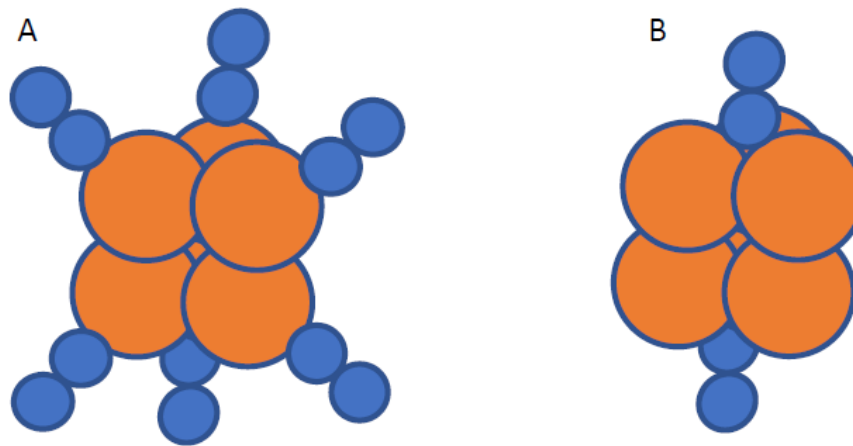


Fig. 5-1. Proposed structure of Algae Cysteine Synthase Complex.

Panel A shows the proposed structural organization of the Cysteine Synthase Complex of algae (A) in comparison to that of vascular plants (B).

### 5.3. ATP-S redox regulation

The regulatory process that I envisage for the redox-regulated ATP-S assumed that the perception of the redox state of the cell was mediated by the plastoquinone pool of the photosynthetic electron transfer chain. This hypothesis was based on the fact that other redox-regulated processes involving early stages in nutrient assimilation pathway adopt this mode of regulation. For instance, *Chlamydomonas reinhardtii* nitrate reductase expression and activity are controlled by the redox state of the plastoquinone pool (Giordano et al., 2007). I, therefore, attempted to verify whether blocking plastoquinone reduction by using Dichloromethyl Urea (DCMU) I could modulate ATP-S activity.

The results of my experiments did not show any impact of plastoquinone oxidation on ATP-S activity. The interpretation of these results is difficult. Unfortunately, the limited time at my disposal made it impossible to go deeper in the matter. It is possible that the redox state of the plastoquinone pool only operates in the activation

direction, but not in the deactivation direction. In the case of nitrate reductase, it was observed that the expression of nitrate reductase gene could be effectively blockade blocked by the oxidation of the plastoquinone pool, but the opposite result was not obtained by the over reduction of the pool. It may also be that, as for instance in the case of some diatom carbonic anhydrase (Kikutani et al., 2012), redox regulation is exerted through thioredoxin, thus using the electrons most likely donated from ferredoxin. Unfortunately, I must leave this question open for future studies.



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[https://doi.org/10.1016/0022-5193\(65\)90083-4](https://doi.org/10.1016/0022-5193(65)90083-4).



# 7. Supplemental material

## 7.1. Growth curves

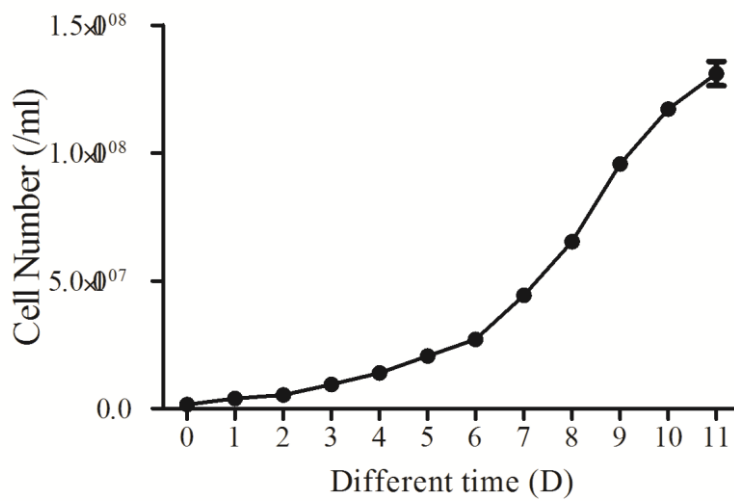


Fig. 7-1. *Synechocystis* sp. PCC6803 growth curve.

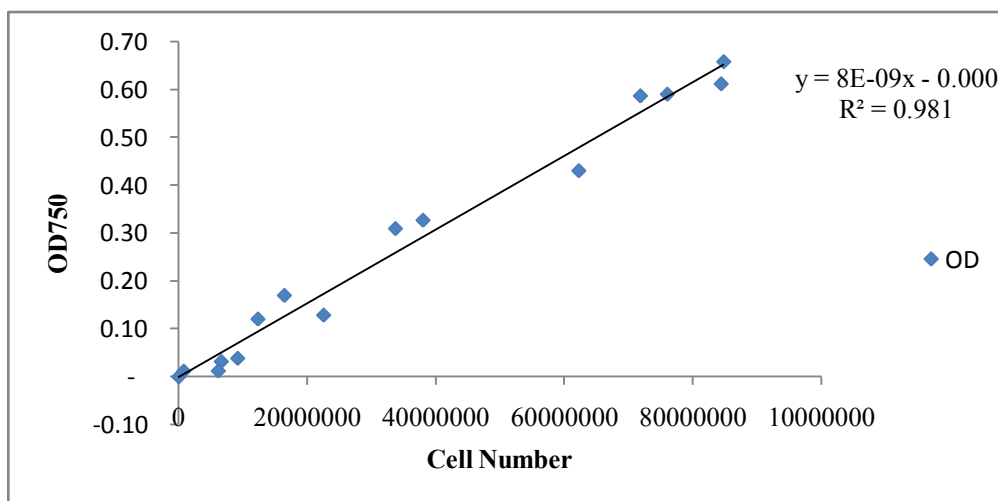


Fig. 7-2. Curve for the conversion of the OD of *Synechocystis* sp. PCC 6803 cultures to cell numbers.

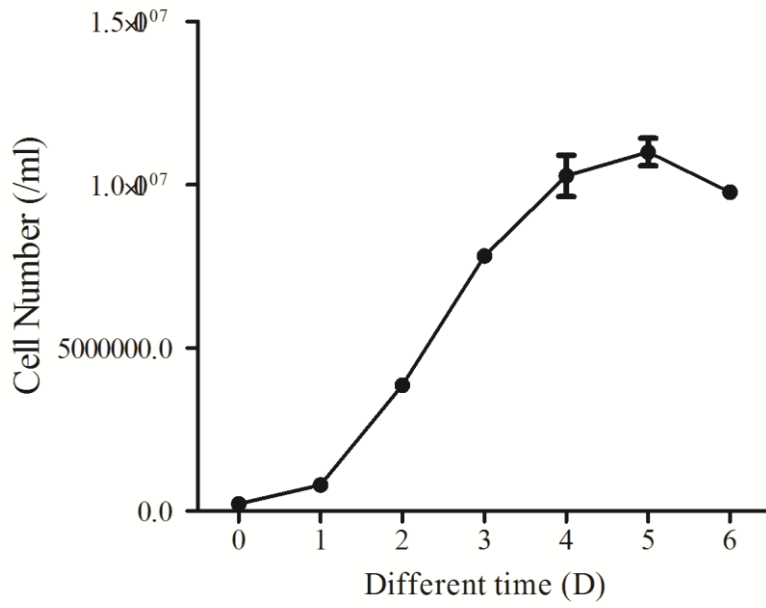


Fig. 7-3. Growth curve of *Chlamydomonas reinhardtii* cultured in TAP medium.

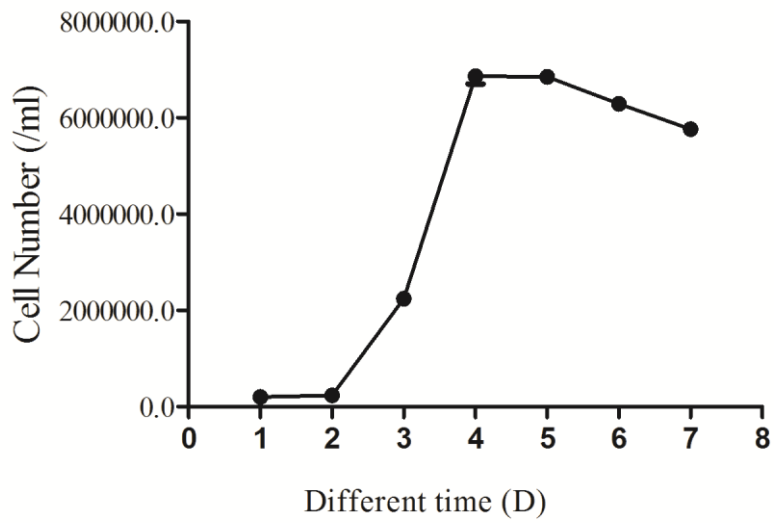


Fig. 7-4. Growth curve of *Chlamydomonas reinhardtii* cultured in TP medium.

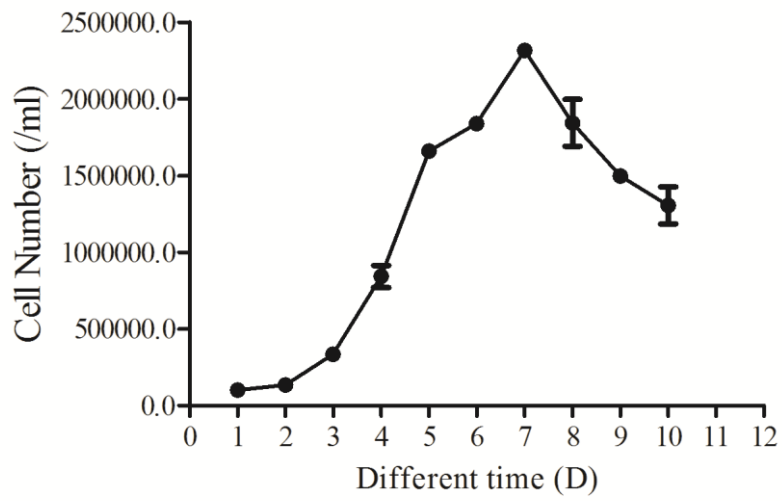


Fig. 7-5. *Dunaliella tertiolecta* growth curve.

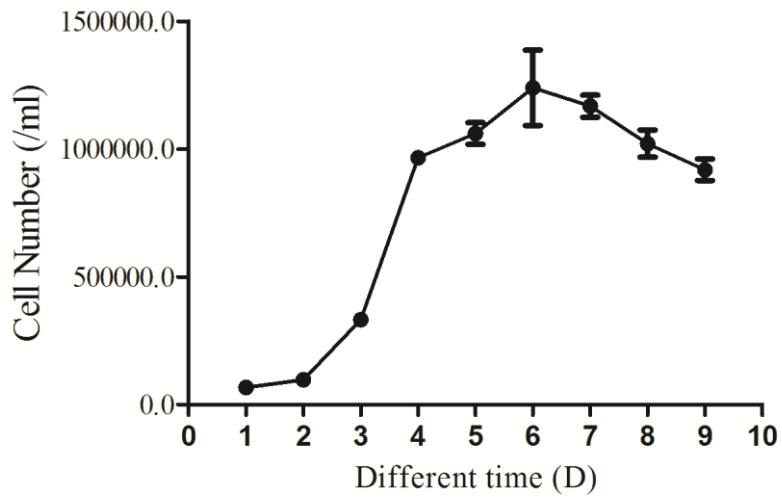


Fig. 7-6. *Tetraselmis suecica* growth curve.

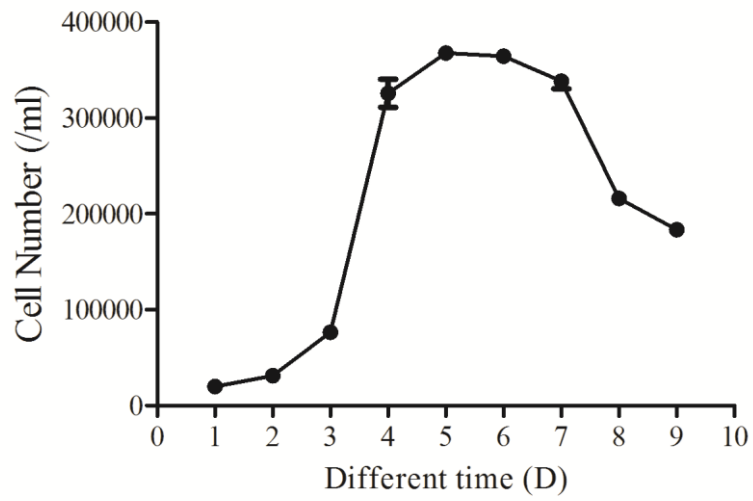


Fig. 7-7. *Thalassiosira pseudonana* growth curve.

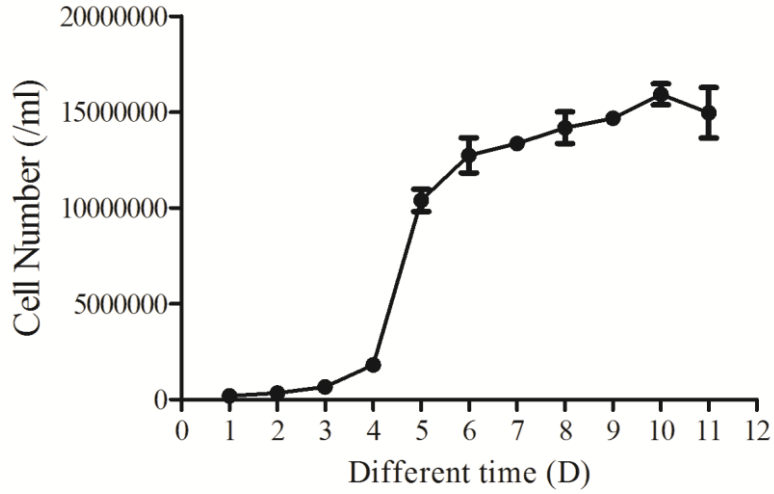


Fig. 7-8. *Phaeodactylum tricornutum* growth curve.



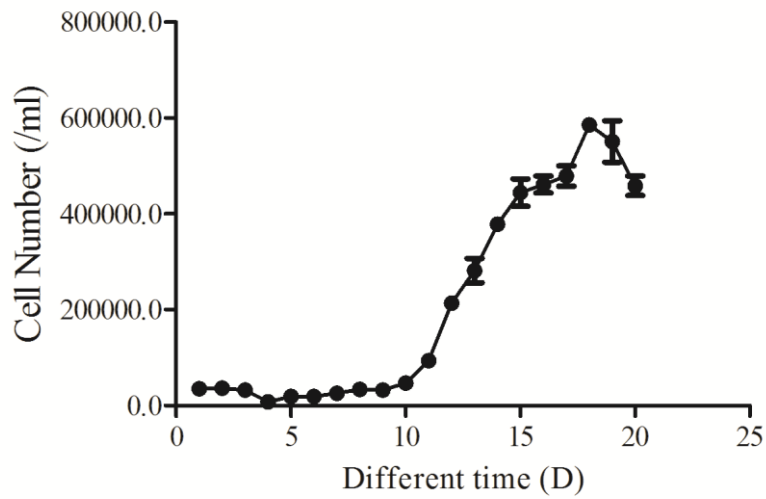


Fig. 7-9. *Amphinidinium klebsii* growth curve.

## 7.2. Correlation between relative mobility of protein with protein size

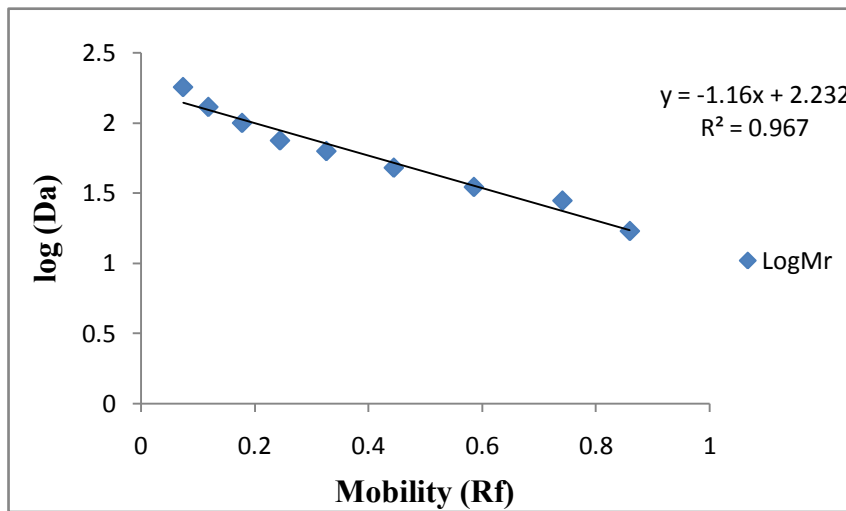


Fig. 7-10. Standard curve for the determination of protein size from the relative protein mobility in binding affinity test chromatography for *Synechocystis* sp. PCC 6803.

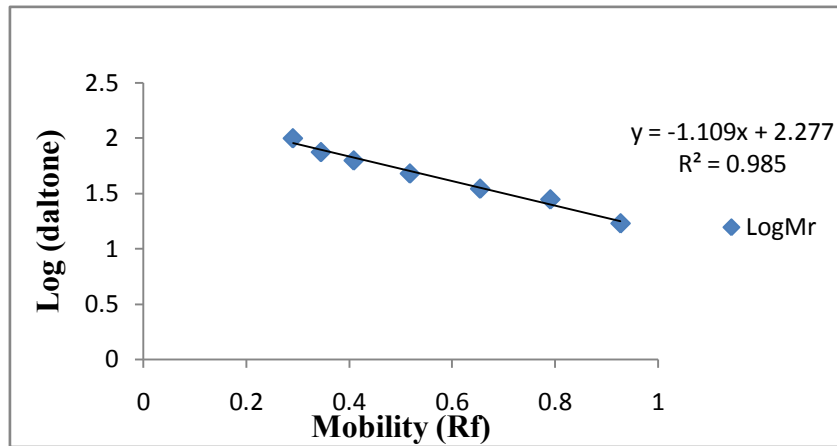


Fig. 7-11. Standard curve for the determination of protein size from relative protein mobility in binding affinity test chromatography for *Chlamydomonas reinhardtii* with TAP medium culture extract.

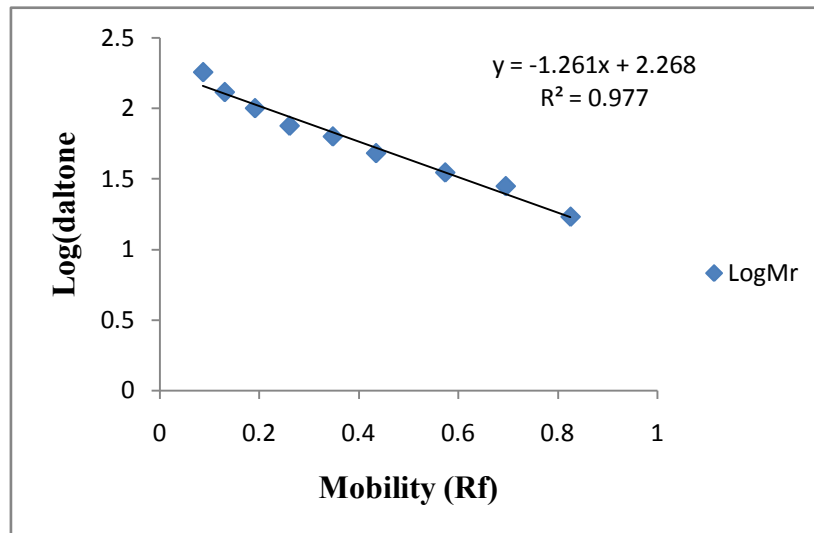


Fig. 7-12. Standard curve for the determination of protein size from the relative protein mobility in binding affinity test chromatography for *Chlamydomonas reinhardtii* with TP medium culture extract.

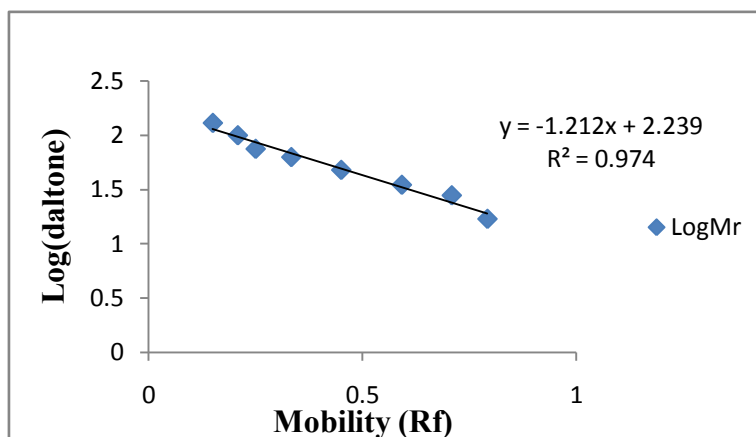


Fig. 7-13. Standard curve for the determination of protein size from the relative protein mobility in binding affinity test chromatography for *Tetraselmis suecica* extract.

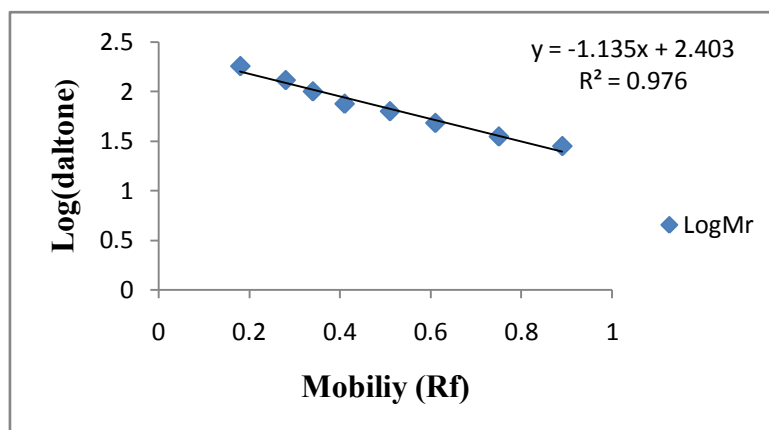


Fig. 7-14. Standard curve for the determination of protein size from the relative protein mobility in binding affinity test chromatography for *Dunaliella tertiolecta* extract.

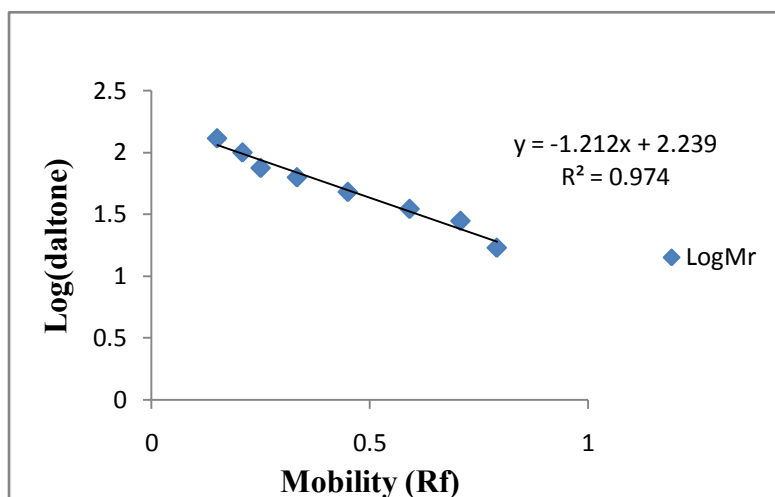


Fig. 7-15. Standard curve for the determination of protein size from the relative protein mobility in binding affinity test chromatography for *Thalassiosira pseudonana* extract.

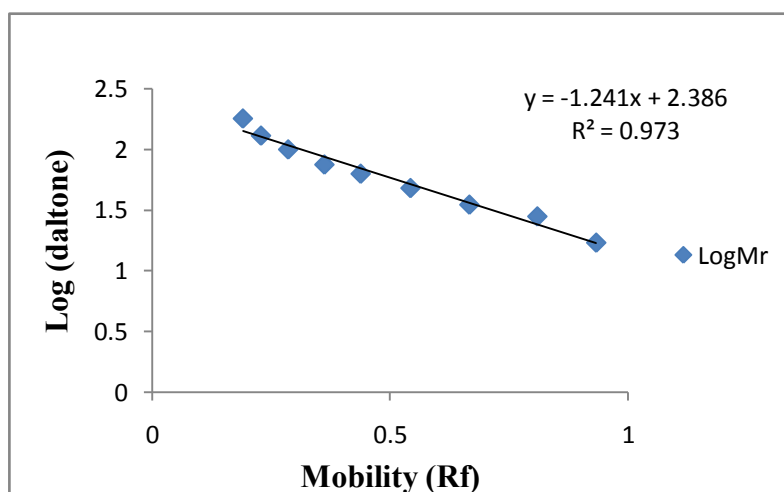


Fig. 7-16. Standard curve for the determination of protein size the relative protein mobility in binding affinity test chromatography for *Phaedactylum tricornutum* extract.

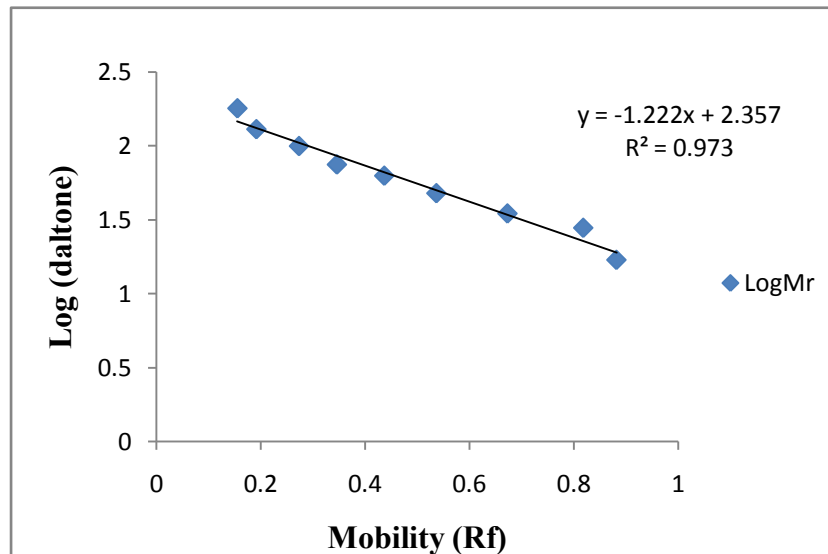


Fig. 7-17. Standard curve for the determination of protein size from the relative protein mobility in binding affinity test chromatography for *Amphidinium klebsii* extract.

### 7.3. Comparison of algae SAT and OAS-TL with *A. thaliana* sequences

#### 7.3.1. Alignment of the SAT sequences of Cyanobacteria with *A. thaliana* SAT isoforms

SAT1-A.thalia	143	SSIRLDVQAFKDRDPACLSYSSAILHLKGYLALQAYRVAHKLWKQGRKLL	192
Synechocystis	3	NSLIADFRIIFERDPAARNWLEVLFCYPGLQALLIHRFShRLYTLGLPFF	52
SAT1-A.thalia	193	ALALQSRVSEVFGIDIHPAARIGKILLDHTGTVVIGETAVIGDRVSIHLH	242
Synechocystis	53	PRLMSHLARFFFTGIEIHPGAQIQGVFIDHGMGVVIGETAIVGDYSLIYQ	102
SAT1-A.thalia	243	GVTLLGGTGKETGDRHPNIGDGALLGACVTILGNIKIGAGAMVAAGSLVLK	292
Synechocystis	103	GVTLLGGTGKESGKRHPTLGENVVVGAGAKVLGNIAIGDNVRI GAGSVVLR	152
SAT1-A.thalia	293	DVPSHSMVAGNPAKLI 308	
Synechocystis	153	DVPADFTVVGVPGRMV 168	
SAT2-A.thalia	106	SSIRHDLQAFKDRDPACLSYSSAILHLKGYHALQAYRVAHKLWNEGRKLL	155
Synechocystis	3	NSLIADFRIIFERDPAARNWLEVLFCYPGLQALLIHRFShRLYTLGLPFF	52
SAT2-A.thalia	156	ALALQSRSEVFGIDIHPAARIGEGILLDHTGTVVIGETAVIGNVSIHLH	205
Synechocystis	53	PRLMSHLARFFFTGIEIHPGAQIQGVFIDHGMGVVIGETAIVGDYSLIYQ	102
SAT2-A.thalia	206	GVTLLGGTGKETGDRHPKIGEGALLGACVTILGNISIGAGAMVAAGSLVLK	255
Synechocystis	103	GVTLLGGTGKESGKRHPTLGENVVVGAGAKVLGNIAIGDNVRI GAGSVVLR	152
SAT2-A.thalia	256	DVPSHVVAGNPAKLI RMEEQ 277	
Synechocystis	153	DVPADFTVVGVPGRMVHPSGER 174	
SAT3-A.thalia	193	IVESVKLDLLAVKERDPACISYVHCFHLFKGFLACQAHRIAHELWTQDRK	242
Synechocystis	1	MLNSLIADFRIIFERDPAARNWLEVLFCYPGLQALLIHRFShRLYTLGLP	50
SAT3-A.thalia	243	ILALLIQNRVSEFAVDFHPGAKIGTGILLDHATAIVIGETAVVGNVSI	292
Synechocystis	51	FFPRLMSHLARFFFTGIEIHPGAQIQGVFIDHGMGVVIGETAIVGDYSLI	100
SAT3-A.thalia	293	LHNVTLLGGTGKQCGDRHPKIGDGVLIAGATCILGNITIGEGAKIGAGSVV	342
Synechocystis	101	YQGVTLGGTGKESGKRHPTLGENVVVGAGAKVLGNIAIGDNVRI GAGSVV	150
SAT3-A.thalia	343	LKDVPRTTAVGNPARLL---GGKDNPKTHDKIP 373	
Synechocystis	151	LRDVPADFTVVGVPGRMVHPSGERVNP LEHGKLP 184	
SAT4-A.thalia	116	IIESTKQDLIAVKERDPACISYVHCFGLFKGFLACQAHRIAHTLWKQNRK	165
Synechocystis	1	MLNSLIADFRIIFERDPAARNWLEVLFCYPGLQALLIHRFShRLYTLGLP	50
SAT4-A.thalia	166	IVALLIQNRVSEFAVDFHPGAKIGKILLDHATGTVVIGETAVVGDVSI	215
Synechocystis	51	FFPRLMSHLARFFFTGIEIHPGAQIQGVFIDHGMGVVIGETAIVGDYSLI	100
SAT4-A.thalia	216	LHGVTLLGGTGKQSGDRHPKIGDGVLIAGAGSCILGNITIGEGAKIGSGSVV	265
Synechocystis	101	YQGVTLGGTGKESGKRHPTLGENVVVGAGAKVLGNIAIGDNVRI GAGSVV	150
SAT4-A.thalia	266	VKDVPARTTAVGNPARLI---GGKENPRKHDKIP 296	
Synechocystis	151	LRDVPADFTVVGVPGRMVHPSGERVNP LEHGKLP 184	
SAT5-A.thalia	115	NATVADLRAARVRDPACISFSSHLLNYKGLAIQAHRVSHKLWTQSRKPL	164
Synechocystis	3	NSLIADFRIIFERDPAARNWLEVLFCYPGLQALLIHRFShRLYTLGLP-PF	51
SAT5-A.thalia	165	ALALHSRISDVFA-VDIHPAAKIGKILLDHATGTVVIGETAVIGNVSIHLH	213
Synechocystis	52	FPRLMSHLARFFFTGIEIHPGAQIQGVFIDHGMGVVIGETAIVGDYSLIY	101
SAT5-A.thalia	214	HHVTLLGGTGKACGDRHPKIGDGCLIGAGATILGNVKGAGAKVAGSVVLR	263
Synechocystis	102	QGVTLGGTGKESGKRHPTLGENVVVGAGAKVLGNIAIGDNVRI GAGSVVLR	151
SAT5-A.thalia	264	IDVPCRGTAVGNPARLV 280	
Synechocystis	152	RDVPADFTVVGVPGRMV 168	

Fig. 7-18. Alignment of *Synechocystis* sp. PCC 6803 SAT amino acid sequences with *A. thaliana*.

SAT1-A.thalia	145	IRLDVQAFKDRDPACLSYSSAILHLKGYLALQAYRVAHKLW--KQGRKLL	192
Synechococcus	5	IRADLAIIRERDPAARGPLEILLCYPGFQALSLHRLSHRLWHSRLPLKLA	54
SAT1-A.thalia	193	ALALQSRVSEVFGIDIHPAARIGKGIILLDHGTGVVIGETAVIGDRVSILH	242
Synechococcus	55	ARLLSQLGRNLTGVEIHPGARIGHGVFIDHGMGVVIGETAEVGDRCLLYQ	104
SAT1-A.thalia	243	GVTLLGGTGKETGDRHPNIGDGALLGACVTILGNIKIGAGAMVAAGSLVLK	292
Synechococcus	105	GVTLLGGTGKDHGKRHPTLANNVIGAGAKVLGAIEVGTNTRIGAGSVVVR	154
SAT1-A.thalia	293	DVPSHSMVAGNPAKLI 308	
Synechococcus	155	DVEADCTVVGIPGRVI 170	
SAT2-A.thalia	108	IRHDLQAFKDRDPACLSYSSAILHLKGYHALQAYRVAHKLWNEGRKL-LA	156
Synechococcus	5	IRADLAIIRERDPAARGPLEILLCYPGFQALSLHRLSHRLWHSRLPLKLA	54
SAT2-A.thalia	157	LALQSRISE-VFGIDIHPAARIGEGILLDHGTGVVIGETAVIGNVSILH	205
Synechococcus	55	ARLLSQLGRNLTGVEIHPGARIGHGVFIDHGMGVVIGETAEVGDRCLLYQ	104
SAT2-A.thalia	206	GVTLLGGTGKETGDRHPKIGEGALLGACVTILGNISIGAGAMVAAGSLVLK	255
Synechococcus	105	GVTLLGGTGKDHGKRHPTLANNVIGAGAKVLGAIEVGTNTRIGAGSVVVR	154
SAT2-A.thalia	256	DVPSHSVAGNPAKLI 271	
Synechococcus	155	DVEADCTVVGIPGRVI 170	
SAT3-A.thalia	197	VKLDLLAVKERDPACISYVHCFLHFKGFLACQAHRIAHELWTQ--DRKIL	244
Synechococcus	5	IRADLAIIRERDPAARGPLEILLCYPGFQALSLHRLSHRLWHSRLPLKLA	54
SAT3-A.thalia	245	ALLIQNRVSEAFVDFHPGAKIGTGILLDHATAIIVIGETAVVGNVNSILH	294
Synechococcus	55	ARLLSQLGRNLTGVEIHPGARIGHGVFIDHGMGVVIGETAEVGDRCLLYQ	104
SAT3-A.thalia	295	NVTLLGGTGKQCGDRHPKIGDGVLIAGATCILGNITIGEGAKIGAGSVVLK	344
Synechococcus	105	GVTLLGGTGKDHGKRHPTLANNVIGAGAKVLGAIEVGTNTRIGAGSVVVR	154
SAT3-A.thalia	345	DVPPRTTAVGNPARLL---GGKDNPKTHDKIPGLTMDQTSHISEWSD 388	
Synechococcus	155	DVEADCTVVGIPGRVIHQSGVRINPLAHSALPDAEANVIRNLMERID 201	
SAT4-A.thalia	121	KQDLIAVKERDPACISYVHCFLGFKGFLACQAHRIAHTLWKQNR--KIVA	168
Synechococcus	6	RADLAIIRERDPAARGPLEILLCYPGFQALSLHRLSHRLWHSRLPLKLA	55
SAT4-A.thalia	169	LLIQNRVSESAFVDIHGAKIGKGIILLDHATGVVIGETAVVGDVNSILHG	218
Synechococcus	56	RLLSQLGRNLTGVEIHPGARIGHGVFIDHGMGVVIGETAEVGDRCLLYQG	105
SAT4-A.thalia	219	VTLGGTGKQSGDRHPKIGDGVLIAGASCILGNITIGEGAKIGSGSVVVKD	268
Synechococcus	106	VTLGGTGKDHGKRHPTLANNVIGAGAKVLGAIEVGTNTRIGAGSVVVRD	155
SAT4-A.thalia	269	VPARTTAVGNPARLI---GGKENPRKHKIP 296	
Synechococcus	156	VEADCTVVGIPGRVIHQSGVRINPLAHSALP 186	
SAT5-A.thalia	119	ADLRAARVRDPACISFSHCLLNYKGLAIQAHRVSHKLWTQSRKPLALA-	167
Synechococcus	7	ADLAIIRERDPAARGPLEILLCYPGFQALSLHRLSHRLW-HSRLPLKLA	55
SAT5-A.thalia	168	-LHSRIS-DVFAVDIHPAAKIGKGIILLDHATGVVIGETAVIGNVNSILHH	215
Synechococcus	56	RLLSQLGRNLTGVEIHPGARIGHGVFIDHGMGVVIGETAEVGDRCLLYQG	105
SAT5-A.thalia	216	VTLGGTGKACGDRHPKIGDGLIGAGATILGNVKIGAGAKVAGSVVLID	265
Synechococcus	106	VTLGGTGKDHGKRHPTLANNVIGAGAKVLGAIEVGTNTRIGAGSVVVRD	155
SAT5-A.thalia	266	VPCRGTAVGNPARLV 280	
Synechococcus	156	VEADCTVVGIPGRVI 170	

Fig. 7-19. Alignment of *Synechococcus* sp. WH 7803 SAT amino acid sequences with *A. thaliana*.

### **7.3.2. Alignment of the SAT sequences of green algae with *A. thaliana* SAT isoforms**



SAT1-A. thalia	73	IWDSIREEAKLEAEPEVLSFLYASILSHDCLEQALSFVLNRLQNPTL	122
1-C. reinhardt	133	LWERIRQEAQMDASSEPALASNLFSITLAHPSLEKSMAPLLANKLANPTM	182
SAT1-A. thalia	123	LATQLMDIFCNVMVHVRGIQSSIRLDVQAFKDRDPACLSYSSAILHLKGY	172
1-C. reinhardt	183	LGMQLMRLISEAYEDDAGLIEACMADLQAVYDRDPACDSFSQAMLYFKGF	232
SAT1-A. thalia	173	LALQAYRVAHKLWQGRKLLALALQSRVSEVFGIDHHPAARIGKGILLDH	222
1-C. reinhardt	233	QAIQCQRVAHWLWQGRKALALAIQSRMSEAFHVDIHPAQLGRGLLIDH	282
SAT1-A. thalia	223	GTGVVIGETAVIGDRVSIILHGVTLGGTGKETGDRHPNIGDGALLGACVTI	272
1-C. reinhardt	283	ATGVVIGETAVVGDVNSMLHHVTLGSSGTGRGVRHPTVGNVLLGAGVTV	332
SAT1-A. thalia	273	LGNIKIGAGAMVAAGSLVKDVPSSHVMVAGNPAKLI 308	
1-C. reinhardt	333	LGPI TVGAGSKVAGSVVSDIPCHSVAVGVPARII 368	
SAT2-A. thalia	36	IWDAREEAKLEAEKEPILSSFLYAGILAHDCLEQALGFVLNRLQNPTL	85
1-C. reinhardt	133	LWERIRQEAQMDASSEPALASNLFSITLAHPSLEKSMAPLLANKLANPTM	182
SAT2-A. thalia	86	LATQLLDIFYGVMHDKGIQSSIRHDLQAFKDRDPACLSYSSAILHLKGY	135
1-C. reinhardt	183	LGMQLMRLISEAYEDDAGLIEACMADLQAVYDRDPACDSFSQAMLYFKGF	232
SAT2-A. thalia	136	HALQAYRVAHKLWNEGRKLLALALQSRISEVFGIDHHPAARIGEGILLDH	185
1-C. reinhardt	233	QAIQCQRVAHWLWQGRKALALAIQSRMSEAFHVDIHPAQLGRGLLIDH	282
SAT2-A. thalia	186	GTGVVIGETAVIGNVSIILHGVTLGGTGKETGDRHPKIGEGALLGACVTI	235
1-C. reinhardt	283	ATGVVIGETAVVGDVNSMLHHVTLGSSGTGRGVRHPTVGNVLLGAGVTV	332
SAT2-A. thalia	236	LGNISIGAGAMVAAGSLVKDVPSSHVMVAGNPAKLIR 272	
1-C. reinhardt	333	LGPI TVGAGSKVAGSVVSDIPCHSVAVGVPARII 368	
SAT3-A. thalia	124	DVWAKIREEAKSDIAKEPIVSAYYHASICVRSQRSEALALNTLSVKLSNLN	173
1-C. reinhardt	132	ELWERIRQEAQMDASSEPALASNLFSITLAHPSLEKSMAPLLANKLANPT	181
SAT3-A. thalia	174	LPSNTLFDLFSGLVQNPDIVESVKLDLLAVKERDPACISYVHCFLHFYKGF	223
1-C. reinhardt	182	MLGMQLMRLISEAYEDDAGLIEACMADLQAVYDRDPACDSFSQAMLYFKGF	231
SAT3-A. thalia	224	FLACQAHRIAHLEWTQDRKILALLIQNRVSEFAVDFHPGAKIGTGILLD	273
1-C. reinhardt	232	FQAIQCQRVAHWLWQGRKALALAIQSRMSEAFHVDIHPAQLGRGLLID	281
SAT3-A. thalia	274	HATAVIGETAVVGNVSIILHNVTLGGTGKQCDRHPKIGDGVILGAGTC	323
1-C. reinhardt	282	HATGVVIGETAVVGDVNSMLHHVTLGSSGTGRGVRHPTVGNVLLGAGVT	331
SAT3-A. thalia	324	ILGNITIGEGAKIGAGSVVVKDVPRTTAVGNPARLL 360	
1-C. reinhardt	332	VLGPITVAGSKVAGSVVSDIPCHSVAVGVPARII 368	
SAT4-A. thalia	47	DVWIKMLEEAKSDVKQEPILSNYYYASITSHRSLESALAHILSVKLSNLN	96
1-C. reinhardt	132	ELWERIRQEAQMDASSEPALASNLFSITLAHPSLEKSMAPLLANKLANPT	181
SAT4-A. thalia	97	LPSNTLSELFISVLEESPEIIESTKQDLIAVKERDPACISYVHCFLGFYKGF	146
1-C. reinhardt	182	MLGMQLMRLISEAYEDDAGLIEACMADLQAVYDRDPACDSFSQAMLYFKGF	231
SAT4-A. thalia	147	FLACQAHRIAHTLWQNRKIVALLIQNRVSEFAVDIHPGAKIGKGILLD	196
1-C. reinhardt	232	FQAIQCQRVAHWLWQGRKALALAIQSRMSEAFHVDIHPAQLGRGLLID	281
SAT4-A. thalia	197	HATGVVIGETAVVGDVNSIILHGVTLGGTGKQCDRHPKIGDGVILGAGSC	246
1-C. reinhardt	282	HATGVVIGETAVVGDVNSMLHHVTLGSSGTGRGVRHPTVGNVLLGAGVT	331
SAT4-A. thalia	247	ILGNITIGEGAKIGAGSVVVKDVPARTTAVGNPARLI 283	
1-C. reinhardt	332	VLGPITVAGSKVAGSVVSDIPCHSVAVGVPARII 368	
SAT5-A. thalia	45	LWTQIKAEARRDAEAPALASYLYSTILSHSSLERSISFHLGNKLCSSTL	94
1-C. reinhardt	133	LWERIRQEAQMDASSEPALASNLFSITLAHPSLEKSMAPLLANKLANPTM	182
SAT5-A. thalia	95	LSTLLYDLFNLTFSSDPSLRNATVADLRAARVRDPACISFSHCLLNKYGKGF	144
1-C. reinhardt	183	LGMQLMRLISEAYEDDAGLIEACMADLQAVYDRDPACDSFSQAMLYFKGF	232
SAT5-A. thalia	145	LAIQAHRVSHKLWTSRKLPLALALHSRISDVFVAVDIHPAAKIGKGILLDH	194
1-C. reinhardt	233	QAIQCQRVAHWLWQGRKALALAIQSRMSEAFHVDIHPAQLGRGLLIDH	282
SAT5-A. thalia	195	ATGVVIGETAVIGNVSIILHHVTLGGTGKACDRHPKIGDGLIGAGATI	244
1-C. reinhardt	283	ATGVVIGETAVVGDVNSMLHHVTLGSSGTGRGVRHPTVGNVLLGAGVTV	332
SAT5-A. thalia	245	LGNVIGAGAKVAGSVVLDVPCRGTAVGNPARLVGGK--EKPTIHDEE	292
1-C. reinhardt	333	LGPI TVGAGSKVAGSVVSDIPCHSVAVGVPARIIKRDIVKEPVKEMDQ	382
SAT5-A. thalia	293	CPGESMDHT 301	
1-C. reinhardt	383	CTDYILDYT 391	

Fig. 7-20. Alignment of 1-C. reinhardtii SAT amino acid sequence with A. thaliana isoforms.

SAT1-A.thalia	73	IWDSIREEAKLEAEPEVLSSFLYASILSHDCLEQALSFVLNRLQNPTL	122
2-C.reinhardt	103	MWKQIRTEAQADANSEPLSSFLYASILAHDTFEQALAFVLNRLANSTM	152
SAT1-A.thalia	123	LATQLMDIFCNVMVHDRGIQSSIRLDVQAFKDRDPACLSYSSAILHLKGY	172
2-C.reinhardt	153	LSTQLFEIFHNFLSKEPDVRCALSDLAACREDPACSSYSHALLYFKGY	202
SAT1-A.thalia	173	LALQAYRVAKLWKQGRKLLALQSRVSEVFGDIHPAARIKGGILLDH	222
2-C.reinhardt	203	HAIQTQRIAHALWNRKQKVMALALQSRISEVFAVDVHPAARIKGGVLLDH	252
SAT1-A.thalia	223	GTGVVIGETAVIGDRVSLHGVTLGGTGKETGDRHPNIGDGALLGACVTI	272
2-C.reinhardt	253	GTGVVIGETAVIGNNVSLQNVTLGGTGKEIGDRHPKVGDNVLI GACATV	302
SAT1-A.thalia	273	LGNIKIGAGAMVAAGSLVKDVP SHSMVAGNPAKLIGVDEQDPSMTMEH	322
2-C.reinhardt	303	LGNIPIGEQAIAAGSLVKPVP PHTMVAGSPAKEVGPV-VGNPALSMH	351
SAT1-A.thalia	323	DATR 326	
2-C.reinhardt		WSQR 355	
SAT2-A.thalia	36	IWDAIREEAKLEAEKEPILSSFLYAGILAHDCLEQALGFVLNRLQNPTL	85
2-C.reinhardt	103	MWKQIRTEAQADANSEPLSSFLYASILAHDTFEQALAFVLNRLANSTM	152
SAT2-A.thalia	86	LATQLLDIFYGVMMDKGIQSSIRHLDQAFKDRDPACLSYSSAILHLKGY	135
2-C.reinhardt	153	LSTQLFEIFHNFLSKEPDVRCALSDLAACREDPACSSYSHALLYFKGY	202
SAT2-A.thalia	136	HALQAYRVAKLWNEGRKLLALALQSRISEVFGDIHPAARIGEGILLDH	185
2-C.reinhardt	203	HAIQTQRIAHALWNRKQKVMALALQSRISEVFAVDVHPAARIKGGVLLDH	252
SAT2-A.thalia	186	GTGVVIGETAVINGVSLHGVTLGGTGKETGDRHPKIGEGALLGACVTI	235
2-C.reinhardt	253	GTGVVIGETAVIGNNVSLQNVTLGGTGKEIGDRHPKVGDNVLI GACATV	302
SAT2-A.thalia	236	LGNISIGAGAMVAAGSLVKDVP SHSVVAGNPAKLIRVMEEQDPSLAMKH	285
2-C.reinhardt	303	LGNIPIGEQAIAAGSLVKPVP PHTMVAGSPAKEVGPVVG-NPALSMH	351
SAT2-A.thalia	286	DATKEFRHVADGYKGAQSNP SLSA 311	
2-C.reinhardt	352	WSQRLLSAESMDGAGGVGMNGVPLAA 377	
SAT3-A.thalia	125	VWAKIREEAKSDIAKEPIVSAYHASIVSQRSLAALANTLSVKLSN LNL	174
2-C.reinhardt	103	MWKQIRTEAQADANSEPLSSFLYASILAHDTFEQALAFVLNRLANSTM	152
SAT3-A.thalia	175	PSNTLFDLFSGVLPQNDIVESVKLDDLAVKERDPACISYVHCLFHKGF	224
2-C.reinhardt	153	LSTQLFEIFHNFLSKEPDVRCALSDLAACREDPACSSYSHALLYFKGY	202
SAT3-A.thalia	225	LACQAHRIAHELWTDQRKILALLIQNRVSEAFVDFHPGAKIGTGILLDH	274
2-C.reinhardt	203	HAIQTQRIAHALWNRKQKVMALALQSRISEVFAVDVHPAARIKGGVLLDH	252
SAT3-A.thalia	275	ATAIVIGETAVVGNVSLHNVTLGGTGKQCGDRHPKIGDGLVIGAGTCI	324
2-C.reinhardt	253	GTGVVIGETAVIGNNVSLQNVTLGGTGKEIGDRHPKVGDNVLI GACATV	302
SAT3-A.thalia	325	LGNITIGEGAKIGAGSVVVKDVP PRTTAVGNPARLLG 361	
2-C.reinhardt	303	LGNIPIGEQAIAAGSLVKPVP PHTMVAGSPAKEVVG 339	
SAT4-A.thalia	48	VWIKMLEEAKSDVKQEPILSNYYASITSHRSLESALAHILSVKLSN LNL	97
2-C.reinhardt	103	MWKQIRTEAQADANSEPLSSFLYASILAHDTFEQALAFVLNRLANSTM	152
SAT4-A.thalia	98	PSNTLFEFLFISVLEESPEIESTKQDLIAVKERDPACISYVHCLGFKGF	147
2-C.reinhardt	153	LSTQLFEIFHNFLSKEPDVRCALSDLAACREDPACSSYSHALLYFKGY	202
SAT4-A.thalia	148	LACQAHRIAHTLWKQNRKIVALLIQNRVSESFVADVHPGAKIGKILLDH	197
2-C.reinhardt	203	HAIQTQRIAHALWNRKQKVMALALQSRISEVFAVDVHPAARIKGGVLLDH	252
SAT4-A.thalia	198	ATGVVIGETAVVGDVNSILHGVTLGGTGKQSGDRHPKIGDGLVIGAGSCI	247
2-C.reinhardt	253	GTGVVIGETAVIGNNVSLQNVTLGGTGKEIGDRHPKVGDNVLI GACATV	302
SAT4-A.thalia	248	LGNITIGEGAKIGSGSVVVKDVP ARTTAVGNPARLLG 284	
2-C.reinhardt	303	LGNIPIGEQAIAAGSLVKPVP PHTMVAGSPAKEVVG 339	
SAT5-A.thalia	45	LWTQIKAEARRDAEAPALASYLYSTILSHSSLERSISFHLGNKLCSS TL	94
2-C.reinhardt	103	MWKQIRTEAQADANSEPLSSFLYASILAHDTFEQALAFVLNRLANSTM	152
SAT5-A.thalia	95	LSTLLYDLFLNTFSSDPSLRNATVADLRAARVDPACISFSHCLLN YKGF	144
2-C.reinhardt	153	LSTQLFEIFHNFLSKEPDVRCALSDLAACREDPACSSYSHALLYFKGY	202
SAT5-A.thalia	145	LAIQAHRVSHKLTQSRKPLALALHSRISDVFVADVHPAARIKGGILLDH	194
2-C.reinhardt	203	HAIQTQRIAHALWNRKQKVMALALQSRISEVFAVDVHPAARIKGGVLLDH	252
SAT5-A.thalia	195	ATGVVIGETAVIGNNVSLHHTVTLGGTGKACGDRHPKIGDGLVIGAGATI	244
2-C.reinhardt	253	GTGVVIGETAVIGNNVSLQNVTLGGTGKEIGDRHPKVGDNVLI GACATV	302
SAT5-A.thalia	245	LGNVKIGAGAKVAGSVVLDVPCRGTAVGNPARLVG 281	
2-C.reinhardt	303	LGNIPIGEQAIAAGSLVKPVP PHTMVAGSPAKEVVG 339	

Fig. 7-21. Alignment of 2-C. *reinhardtii* SAT amino acid sequence with *A. thaliana* isoforms.

SAT1-A.thalia	71	DPINWSIREEAKLEAEPEVLSFLYASILSHDCLEQALSFLVANLRLQNF	120
1-T.suecica	48	DALWEAIRSEARSDSDLEASLASALHSTILVHHSLAKTMAFVLANKLQSH	97
SAT1-A.thalia	121	TLLATQLMDIFCNVWVHDRGIQSSIRLDVQAFKDRDPACLSSSAILHLK	170
1-T.suecica	98	TLPATHLLHLFQEAFFNDPDI MAAVVADMNADFDRDPACEKYSCHMLNFK	147
SAT1-A.thalia	171	GYLALQAYRVAHKLWKQGRKLLALALQSRVSEVFGIDIHPAARIGKGILL	220
1-T.suecica	148	GFQAIQSYRISHWLFKRNRRALASALQSRIAELFHVLDLHPGAKLGRGIM	197
SAT1-A.thalia	221	DHGTGVVIGETAVIGDRVSI LHGVTLGGTGKETGDRHPNIGDGALLGACV	270
1-T.suecica	198	DHATGVVIGETATVGDVNSILHHVTLGSGGTGNGVRHPNIGNGVLLGAGV	247
SAT1-A.thalia	271	TILGNKIGAGAMVAAGSLVLDVPSHSMVAGNPAKLGIFVDEQDPSMTM	320
1-T.suecica	248	VCLGPITVHGSGKIGAGSLVSNLPDYCVAVGVPKVLRRKEGQDPNKTM	297
SAT1-A.thalia	321	E 321	
1-T.suecica	298	D 298	
SAT2-A.thalia	33	SDPFWDAIREEAKLEAEKEPILSSFLYAGILAHDCLEQALGFVLNRLQN	82
1-T.suecica	47	TDALWEAIRSEARSDSDLEASLASALHSTILVHHSLAKTMAFVLANKLQS	96
SAT2-A.thalia	83	PTLLATQLLDIFYGVMMHDKGIQSSIRHDLQAFKDRDPACLSSSAILHL	132
1-T.suecica	97	HTLPATHLLHLFQEAFFNDPDI MAAVVADMNADFDRDPACEKYSCHMLNF	146
SAT2-A.thalia	133	KGYHALQAYRVAHKLWNEGRKLLALALQSRSEVFGIDIHPAARIGEGIL	182
1-T.suecica	147	KGFQAIQSYRISHWLFKRNRRALASALQSRIAELFHVLDLHPGAKLGRGIM	196
SAT2-A.thalia	183	LDHGTGVVIGETAVIGNVSI LHGVTLGGTGKETGDRHPKIGEGALLGAC	232
1-T.suecica	197	IDHATGVVIGETATVGDVNSILHHVTLGSGGTGNGVRHPNIGNGVLLGAG	246
SAT2-A.thalia	233	VTILGNISIGAGAMVAAGSLVLDVPSHSMVAGNPAKLIRVMEQDPSLA	282
1-T.suecica	247	VVCLGPITVHGSGKIGAGSLVSNLPDYCVAVGVPKVLRRKEGQDPNK	296
SAT2-A.thalia	283	M 283	
1-T.suecica	297	M 297	
SAT3-A.thalia	121	EVDDVWAKIREEAKSDIAKEPIVSAIYHASIVSQRSEALANTLSVKLS	170
1-T.suecica	46	DTDALWEAIRSEARSDSDLEASLASALHSTILVHHSLAKTMAFVLANKLQ	95
SAT3-A.thalia	171	NLNLPSNTLDFLFGVQLQNPDI VESVKLDDLAVKERDPACISYVHCFH	220
1-T.suecica	96	SHTLPATHLLHLFQEAFFNDPDI MAAVVADMNADFDRDPACEKYSCHMLN	145
SAT3-A.thalia	221	FKGFLACQAHRIAHELWTQDRKILALLIQNRVSEAFVDFHPGAKIGTGI	270
1-T.suecica	146	KGFQAIQSYRISHWLFKRNRRALASALQSRIAELFHVLDLHPGAKLGRGI	195
SAT3-A.thalia	271	LLDHATAIVIGETAVVGNVSI LHNVTLGGTGKQCDRHPKIGDGVLIGA	320
1-T.suecica	196	MIDHATGVVIGETATVGDVNSILHHVTLGSGGTGNGVRHPNIGNGVLLGA	245
SAT3-A.thalia	321	GTICILGNITIGEGAKIGAGSVLKDVPRTTAVGNPARLLG---GKDNPK	367
1-T.suecica	246	GVVCLGPITVHGSGKIGAGSLVSNLPDYCVAVGVPKVLRRKEGQDPNK	295
SAT3-A.thalia	368	THDKI 372	
1-T.suecica	296	TMDQI 300	
SAT4-A.thalia	43	EDDDVWIKMLEEAKSDVKQEPILSNYYASITSHRSLESALAHILSVKL	92
1-T.suecica	45	KDLDALWEAIRSEARSDSDLEASLASALHSTILVHHSLAKTMAFVLANKL	94
SAT4-A.thalia	93	SNLNLPSNTLDFLFGVQLQNPDI VESVKLDDLAVKERDPACISYVHCFH	142
1-T.suecica	95	QSHTLPATHLLHLFQEAFFNDPDI MAAVVADMNADFDRDPACEKYSCHML	144
SAT4-A.thalia	143	GFKFLACQAHRIAHELWTQDRKILALLIQNRVSEAFVDFHPGAKIGK	192
1-T.suecica	145	NFKGFQAIQSYRISHWLFKRNRRALASALQSRIAELFHVLDLHPGAKLGRG	194
SAT4-A.thalia	193	ILLDHATGVVIGETAVVGNVSI LHGVTLGGTGKQSDRHPKIGDGVLLIG	242
1-T.suecica	195	IMIDHATGVVIGETATVGDVNSILHHVTLGSGGTGNGVRHPNIGNGVLLG	244
SAT4-A.thalia	243	AGSCILGNITIGEGAKIGSGSVVVDVFPARTTAVGNPARLLIGGKFNPKH	292
1-T.suecica	245	AGVCLGPITVHGSGKIGAGSLVSNLPDYCVAVGVPKVLRRKEGQDPNK	289
SAT4-A.thalia	293	DKIPCLTMDQTSYLTEW 309	
1-T.suecica	290	GQDPNKTMQIEYVFDY 306	
SAT5-A.thalia	45	LNTQIKAEARDAEAPALASYLYSTILSHSSLEPISFHLGNKLCSSTL	94
1-T.suecica	50	LWEAIRSEARSDSDLEASLASALHSTILVHHSLAKTMAFVLANKLQSH	99
SAT5-A.thalia	95	LSTLLYDLFLNIFSSDPLRNATVADLRAARVDRDPACISFHSCLLNKYGK	144
1-T.suecica	100	PATHLLHLFQEAFFNDPDI MAAVVADMNADFDRDPACEKYSCHMLNFKGF	149
SAT5-A.thalia	145	LAIQAHVSHKWLQSRKPLALALHRSRISDVFVAVDIHPAAKIGKGLLDH	194
1-T.suecica	150	QAIQSYRISHWLFKRNRRALASALQSRIAELFHVLDLHPGAKLGRGIMIDH	199
SAT5-A.thalia	195	ATGVVIGETAVIGNVSI LHNVTLGGTGKACGDRHPKIGDGLIGAGATI	244
1-T.suecica	200	ATGVVIGETATVGDVNSILHHVTLGSGGTGNGVRHPNIGNGVLLGAGVVC	249
SAT5-A.thalia	245	LGNVKIGAGAKVAGSVVLDVPCRTAVGNPARLVGGKE 284	
1-T.suecica	250	LGPITVHGSGKIGAGSLVSNLPDYCVAVGVPKVLRRKE 289	

Fig. 7-22. Alignment of 1-*T. suecica* SAT amino acid sequence with *A. thaliana* isoforms.



SAT1-A.thalia	72	PIWDSIREEAKLEAEPEVLSFLYASILSHDCLEQALSFLVANRLQNPT	121
2-T.suecica	53	PVWLAIARSEAGRDAAAEPILSSFLWGSILSHDTFERALAFILANRLADAT	102
SAT1-A.thalia	122	LLATQLMDIFCNVMVHDRGIQSSIRLDVQAFKDRDPACLSYSSAILHLKG	171
2-T.suecica	103	MLPTLFDIFYDTLKTSPETVFSMQDCQAAMERDPACRGYSDALLYYKG	152
SAT1-A.thalia	172	YLALQAYRVAHKLWKQGRKLLALALQSRVSEVFGIDIHFAARIGKGLLD	221
2-T.suecica	153	FHAVQAQRCAHVLWKRGRVTLALALQSKVSEVLAIHHPATRLGHGLLD	202
SAT1-A.thalia	222	HGTGVVIGETAVIGDRVSIHLGVTLGGTGKETGDRHPNIGDALLGACVT	271
2-T.suecica	203	HGTGVVIGETAVVGNYSILQGVTLGGTGKASGDRHPKIGDGVLLGANAT	252
SAT1-A.thalia	272	ILGNIKIGAGAMVAAGSLVKDVPSPHSMVAGNPAKLIGFVD	312
2-T.suecica	253	VLGNIRVGEQAQIAACSLVKDVPERTMVAGTPAKLIGRVE	293
SAT2-A.thalia	32	RSDPVDIAIREAEKLEAEKPELSSFLYAGLHDCLEQALGFVLANRLQ	81
2-T.suecica	50	KKSPVWLAIARSEAGRDAAAEPILSSFLWGSILSHDTFERALAFILANRLA	99
SAT2-A.thalia	82	NPTLLATQLLDIFYGVMHDKGIQSSIRHDLQAFKDRDPACLSYSSAILH	131
2-T.suecica	100	DATMLPTLFDIFYDTLKTSPETVFSMQDCQAAMERDPACRGYSDALLY	149
SAT2-A.thalia	132	LKGYHALQAYRVAHKLWNEGRKLLALALQSRSEVFGIDIHFAARIGEGI	181
2-T.suecica	150	YKGFHAVQAQRCAHVLWKRGRVTLALALQSKVSEVLAIHHPATRLGHGL	199
SAT2-A.thalia	182	LLDHGTGVVIGETAVIGNVSIHLGVTLGGTGKETGDRHPKIGEGALLGA	231
2-T.suecica	200	LLDHGTGVVIGETAVVGNYSILQGVTLGGTGKASGDRHPKIGDGVLLGA	249
SAT2-A.thalia	232	CVTILGNISIGAGAMVAAGSLVKDVPSPHSMVAGNPAKLIRVMEEQDPSL	281
2-T.suecica	250	NATVLGNIRVGEQAQIAACSLVKDVPERTMVAGTPAKLIGRVEGR-PAL	298
SAT2-A.thalia	282	AM 283	
2-T.suecica	299	EM 300	
SAT3-A.thalia	125	VWAKIREEAKSDIAKEPIVSAYYHASIVQRSLEAALANTLSVKLSNLM	174
2-T.suecica	54	VWLAIARSEAGRDAAAEPILSSFLWGSILSHDTFERALAFILANRLADATM	103
SAT3-A.thalia	175	PSNTLFDLFSGLVQGNPDIVESVKLLDLAVKERDPACISYVHCFLHFKGF	224
2-T.suecica	104	LPTLFDIFYDTLKTSPETVFSMQDCQAAMERDPACRGYSDALLYYKGF	153
SAT3-A.thalia	225	LACQAHRIAHELWTQDRKILALLIQNRVSEAFVDFHPGAKIGTGILLDH	274
2-T.suecica	154	HAVQAQRCAHVLWKRGRVTLALALQSKVSEVLAIHHPATRLGHGLLDH	203
SAT3-A.thalia	275	ATAIVIGETAVVGNVSIHLNVTLGGTGKQCGDRHPKIGDGVLIAGATCI	324
2-T.suecica	204	GTGVVIGETAVVGNYSILQGVTLGGTGKASGDRHPKIGDGVLLGANATV	253
SAT3-A.thalia	325	LGNITIGEGAKIGAGSVVLKDVPPERTAVGNPARLLG	361
2-T.suecica	254	LGNIRVGEQAQIAACSLVKDVPERTMVAGTPAKLIG	290
SAT4-A.thalia	48	VWIKMLEEAKSDVKQEPILSNYYYASITSHRSLESALAHILSVKLSNLM	97
2-T.suecica	54	VWLAIARSEAGRDAAAEPILSSFLWGSILSHDTFERALAFILANRLADATM	103
SAT4-A.thalia	98	PSNTLFDLFSVLEESPEIIESTKQDLIAVKERDPACISYVHCFLGFKGF	147
2-T.suecica	104	LPTLFDIFYDTLKTSPETVFSMQDCQAAMERDPACRGYSDALLYYKGF	153
SAT4-A.thalia	148	LACQAHRIAHTLWQNRKIVALLIQNRVSEAFVDFHPGAKIGKGIILLDH	197
2-T.suecica	154	HAVQAQRCAHVLWKRGRVTLALALQSKVSEVLAIHHPATRLGHGLLDH	203
SAT4-A.thalia	198	ATGVVIGETAVVGNVSIHLGVTLGGTGKQSGDRHPKIGDGVLIAGSCI	247
2-T.suecica	204	GTGVVIGETAVVGNYSILQGVTLGGTGKASGDRHPKIGDGVLLGANATV	253
SAT4-A.thalia	248	LGNITIGEGAKIGSGSVVVKDVPARTAVGNPARLIGGKE	287
2-T.suecica	254	LGNIRVGEQAQIAACSLVKDVPERTMVAGTPAKLIGRVE	293
SAT5-A.thalia	45	LWTQIKAEARRDAEAPALASYLYSTILSHSLSERSISFHLGNKLCSTL	94
2-T.suecica	54	VWLAIARSEAGRDAAAEPILSSFLWGSILSHDTFERALAFILANRLADATM	103
SAT5-A.thalia	95	LSTLLYDLFLMTFSSDPSLRNATVADLRAARVRDPACISFHSCLLNYKGF	144
2-T.suecica	104	LPTLFDIFYDTLKTSPETVFSMQDCQAAMERDPACRGYSDALLYYKGF	153
SAT5-A.thalia	145	LAIQHRVSHKLWTSRKLPLALALHSRISDVFAVDIHFAAKIGKGIILLDH	194
2-T.suecica	154	HAVQAQRCAHVLWKRGRVTLALALQSKVSEVLAIHHPATRLGHGLLDH	203
SAT5-A.thalia	195	ATGVVIGETAVIGNVSIHLHVTLGGTGKACGDRHPKIGDGLIGAGATI	244
2-T.suecica	204	GTGVVIGETAVVGNYSILQGVTLGGTGKASGDRHPKIGDGVLLGANATV	253
SAT5-A.thalia	245	LGNVKIGAGAKVAGSVLIDVPCRGTAVGNPARLVGGKE	284
2-T.suecica	254	LGNIRVGEQAQIAACSLVKDVPERTMVAGTPAKLIGRVE	293

Fig. 7-23. Alignment of 2-*T. suecica* SAT amino acid sequence with *A. thaliana* isoforms.



### **7.3.3. Alignment of the SAT sequences of red algae and red-lineage algae with *A. thaliana* SAT isoforms**

SAT1-A.thalia	71	DPIWDSIREEAKLEAEPEVLSFFLYASILSHDCLEQALSFVLNRLQNP	120
1-C.merolae	113	DLWVERVLEAEAAAREEQLLASFLYATVLNHDTEACLAFLHANKLAST	162
SAT1-A.thalia	121	TLLATQLMDFCNVMVHDRGIQSSIRLDVQAFKDRDPACLSYSSAILHLK	170
1-C.merolae	163	TLPSTMLNEIIREALEKAPEARYAIRLDDLAVADRDPACTRVIDALLFFK	212
SAT1-A.thalia	171	GYLALQAYRVAKLWKQGRKLLALALQSRVSEVFGIDHFAARIGKGIILL	220
1-C.merolae	213	GFHALQTHRVAHWLWSQNRQALAMYLHSQVCKVLQIDHFAARIGYGVFI	262
SAT1-A.thalia	221	DHGTGVVIGETAVIGDRVSIHLGVTLGGTGKTDGRHPNIGDGLGACV	270
1-C.merolae	263	DHGTGVVIGETARVGNVSLHHVTLGGTGKLDGRHPRIEDCVLIGAGA	312
SAT1-A.thalia	271	TILGNIKIGAGMVAAGSLVLDVPSHSMVAGNPAKLGIVDEQDPSMTM	320
1-C.merolae	313	TILGNITVGYGAMVACTVLTSDLPPHSTAVGVPARVIGAPRTKAPAFDM	362
SAT1-A.thalia	321	EHDAT 325	
1-C.merolae	363	DQDPT 367	
SAT2-A.thalia	34	DPIWDAIREEAKLEAEKPISSFFLYAGILAHDCLEQALGFVLNRLQNP	83
1-C.merolae	113	DLWVERVLEAEAAAREEQLLASFLYATVLNHDTEACLAFLHANKLAST	162
SAT2-A.thalia	84	TLLATQLLDIFYGVMMHDKGIQSSIRHDLQAFKDRDPACLSYSSAILHLK	133
1-C.merolae	163	TLPSTMLNEIIREALEKAPEARYAIRLDDLAVADRDPACTRVIDALLFFK	212
SAT2-A.thalia	134	GYHALQAYRVAKLWNEGRKLLALALQSRISEVFGIDHFAARIGEGILL	183
1-C.merolae	213	GFHALQTHRVAHWLWSQNRQALAMYLHSQVCKVLQIDHFAARIGYGVFI	262
SAT2-A.thalia	184	DHGTGVVIGETAVIGNVSIHLGVTLGGTGKTDGRHPKIGEGALLGACV	233
1-C.merolae	263	DHGTGVVIGETARVGNVSLHHVTLGGTGKLDGRHPRIEDCVLIGAGA	312
SAT2-A.thalia	234	TILGNISIGAGMVAAGSLVLDVPSHSMVAGNPAKLRVMEEQDPSLAM	283
1-C.merolae	313	TILGNITVGYGAMVACTVLTSDLPPHSTAVGVPARVIGAPRTKAPAFDM	362
SAT2-A.thalia	284	KHDAT 288	
1-C.merolae	363	DODPT 367	
SAT3-A.thalia	123	DDVWAKIREEAKSDIAKEPIVSAYYHASIVSQRSLAALANTLSVKLSNL	172
1-C.merolae	113	DLWVERVLEAEAAAREEQLLASFLYATVLNHDTEACLAFLHANKLAST	162
SAT3-A.thalia	173	NLPSNTLFDLFSGLVQGNPDIVESVKLDDLAVKERDPACISYVHCFHFHK	222
1-C.merolae	163	TLPSTMLNEIIREALEKAPEARYAIRLDDLAVADRDPACTRVIDALLFFK	212
SAT3-A.thalia	223	GFLACQAHRIAEHLWTQDRKILALLIQRNVSEFAVDFHFGAKIGTGILL	272
1-C.merolae	213	GFHALQTHRVAHWLWSQNRQALAMYLHSQVCKVLQIDHFAARIGYGVFI	262
SAT3-A.thalia	273	DHATAIVIGETAVVGNVSIHLHNVTGGTGKCGDRHPKIGDGVLIAGT	322
1-C.merolae	263	DHGTGVVIGETARVGNVSLHHVTLGGTGKLDGRHPRIEDCVLIGAGA	312
SAT3-A.thalia	323	CILGNITIGEGAKIGAGSVVLKDVPRTTAVGNPARLLG 361	
1-C.merolae	313	TILGNITVGYGAMVACTVLTSDLPPHSTAVGVPARVIG 351	
SAT4-A.thalia	7	TCRTGNTQDDSRFCCIKNPFRBGFESVNRKIHTQIEDDDVWIKMLEEA	56
1-C.merolae	74	TAGVFNRLKEEGACFVEFLRRAVEDCRNRVQAGGHGTEDLVWERVLEA	123
SAT4-A.thalia	57	KSDVKQEPILSNYYASITSHRLESALAHILSVKLSNLSNTLFEFL	106
1-C.merolae	124	EAAAREEQLLASFLYATVLNHDTEACLAFLHANKLASTTLPSTMLNEI	173
SAT4-A.thalia	107	ISVLEESPEIESTKQDLIAVKERDPACISYVHCFHFGKFLACQAHRIA	156
1-C.merolae	174	REALEKAPEARYAIRLDDLAVADRDPACTRVIDALLFFKGFHALQTHRVA	223
SAT4-A.thalia	157	HTLWQNRKIVALLIQRNVSEFAVDIHPGAKIGKILLDHATGVVIGET	206
1-C.merolae	224	HVLWSQNRQALAMYLHSQVCKVLQIDHFAARIGYGVFIHGTGVVIGET	273
SAT4-A.thalia	207	AVVGDVNSIHLGVTLGGTGKSGDRHPKIGDGVLIAGASCILGNITIGEG	256
1-C.merolae	274	ARVGNVSLHHVTLGGTGKLDGRHPRIEDCVLIGAGATILGNITVGYG	323
SAT4-A.thalia	257	AKIGSGSVVVKDVPARTAVGNPARLLG 284	
1-C.merolae	324	AMVGACTVLTSDLPPHSTAVGVPARVIG 351	
SAT5-A.thalia	45	LWTQIKAEARRDAEAPALASYLYSTILSHSSLERSISFHLGNKLCSTTL	94
1-C.merolae	115	VWVERVLEAEAAAREEQLLASFLYATVLNHDTEACLAFLHANKLASTTL	164
SAT5-A.thalia	95	LSTLLYDLFLNTFSSDPSLRNATVADLRAARVRDPACISFSHCLLNKYGK	144
1-C.merolae	165	PSTMLNEIIREALEKAPEARYAIRLDDLAVADRDPACTRVIDALLFFKGF	214
SAT5-A.thalia	145	LAIQHRVSHKLTQSRKPLALALHSRISDVFAVDIHPAAKIGKGIILLDH	194
1-C.merolae	215	HALQTHRVAHWLWSQNRQALAMYLHSQVCKVLQIDHFAARIGYGVFI	264
SAT5-A.thalia	195	ATGVVIGETAVIGNVSIHLHNVTGGTGKACGDRHPKIGDGLIGAGATI	244
1-C.merolae	265	GTGVVIGETARVGNVSLHHVTLGGTGKLDGRHPRIEDCVLIGAGATI	314
SAT5-A.thalia	245	LGNVKIGAGAKVAGSVVLDVPCRGTAVGNPARLVG 281	
1-C.merolae	315	LGNITVGYGAMVACTVLTSDLPPHSTAVGVPARVIG 351	

Fig. 7-25. Alignment of 1-C. merolae SAT amino acid sequence with A. thaliana isoforms.



SAT1-A.thalia	69	SYDPIWDSIREEAKLEAEEEPVLSFLYASILSHDCLEQALSFVLNRLQ	118
2-C.merolae	113	SSDPVWELVRRREAEIGAANEPQLASSLYATVLNHRCLEDTLAFHLANELA	162
SAT1-A.thalia	119	NPTLLATQLMDIFCNVMVHDRGIQSSIRLDVQAFKDRDPACLSYSSAILH	168
2-C.merolae	163	SPFFQATQYVKLFRDALYQDKSYREAIRADLLAVVRRDPAMKHCVAVLMY	212
SAT1-A.thalia	169	LKGYLALQAYRVAHKLWKQRKLLALALQSRVSEVFGIDIHFAARIGKGI	218
2-C.merolae	213	SKGYAALQAYRLAHLWRQDRKVLALFLQSEISKCFAVDIHFAARIGSGV	262
SAT1-A.thalia	219	LLDHGTGVVIGETAVIGDRVSIHGVTLGGTGKGTGRDRHPNIGDGALLGA	268
2-C.merolae	263	MIDHATGIVIGETAVVGNVSMHNVTLGGTGKEAGDRHPKVGKRVLLGA	312
SAT1-A.thalia	269	CVTILGNIKIGAGAMVAAGSLVLKDVPSHSMVAGNPAKLI	309
2-C.merolae	313	GATVGNIRIGDGAQITASSVVLKDVPPYTIIVSGVPAREVG	353
SAT2-A.thalia	33	SDPIWDAIREEAKLEAEEKPELSSFLYAGILAHDCLEQALGFVLNRLQN	82
2-C.merolae	114	SDPVWELVRRREAEIGAANEPQLASSLYATVLNHRCLEDTLAFHLANELAS	163
SAT2-A.thalia	83	PTLLATQLLDFYGVMMHDKGIQSSIRHDLQAFKDRDPACLSYSSAILHL	132
2-C.merolae	164	PFQATQYVKLFRDALYQDKSYREAIRADLLAVVRRDPAMKHCVAVLMYS	213
SAT2-A.thalia	133	KGYHALQAYRVAHKLWNEGRKLLALALQSRVSEVFGIDIHFAARIGEGIL	182
2-C.merolae	214	KGYAALQAYRLAHLWRQDRKVLALFLQSEISKCFAVDIHFAARIGSGVM	263
SAT2-A.thalia	183	LDHGTGVVIGETAVIGNVSIHGVTLGGTGKGTGRDRHPKIGEGALLGAC	232
2-C.merolae	264	IDHATGIVIGETAVVGNVSMHNVTLGGTGKEAGDRHPKVGKRVLLGAG	313
SAT2-A.thalia	233	VTILGNISIGAGAMVAAGSLVLKDVPSHSMVAGNPAK	269
2-C.merolae	314	ATVGNIRIGDGAQITASSVVLKDVPPYTIIVSGVPAR	350
SAT3-A.thalia	123	DDVWAKIREEAKSDIAKEPIVSAYYHASICVSRQSLAALANTLSVKLSNL	172
2-C.merolae	115	DPVWELVRRREAEIGAANEPQLASSLYATVLNHRCLEDTLAFHLANELASP	164
SAT3-A.thalia	173	NLPSNTLFDLFSVQLGNPDIVESVKLDDLAVKERDPACISYVHCFLHFK	222
2-C.merolae	165	FFQATQYVKLFRDALYQDKSYREAIRADLLAVVRRDPAMKHCVAVLMYSK	214
SAT3-A.thalia	223	GFLACQAHRIAHELWTQDRKILALLIQNRVSEAFVDFHPGAKIGTGILL	272
2-C.merolae	215	GYAALQAYRLAHLWRQDRKVLALFLQSEISKCFAVDIHFAARIGSGVMI	264
SAT3-A.thalia	273	DHATAIIVIGETAVVGNVSIHNVTLGGTGKQCGDRHPKIGDVLIGAGT	322
2-C.merolae	265	DHATGIVIGETAVVGNVSMHNVTLGGTGKEAGDRHPKVGKRVLLGAGA	314
SAT3-A.thalia	323	CILGNITIGEGAKIGAGSVLKDVPVPRRTAVGNPARLLGGKDNPK	367
2-C.merolae	315	TVLGNIRIGDGAQITASSVVLKDVPPYTIIVSGVPAREVGKLSYPK	359
SAT4-A.thalia	27	FRPGFSVNRKIHTQIEDDDVWIKMLEEAKSDVKQEPILLSNYYYASITS	76
2-C.merolae	97	FGPVISVDDMVR-TLTYSSDPVWELVRRREAEIGAANEPQLASSLYATVLN	145
SAT4-A.thalia	77	HRSLESALAHILSVKLSNLNPSNTLFEFISVLEESPEIIESTKQDLIA	126
2-C.merolae	146	HRCLEDTLAFHLANELASPFQATQYVKLFRDALYQDKSYREAIRADLLA	195
SAT4-A.thalia	127	VKERDPACISYVHCFLGFKGLACQAHRIAHTLWKQNRKIVALLIQNRVS	176
2-C.merolae	196	VVRRDPAMKHCVAVLMYSKGYAALQAYRLAHLWRQDRKVLALFLQSEIS	245
SAT4-A.thalia	177	ESFAVDIHFGAKIGKILLDHATGVVIGETAVVGNVSIHGVTLGGTGK	226
2-C.merolae	246	KCFAVDIHFAARIGSGVMIDHATGIVIGETAVVGNVSMHNVTLGGTGK	295
SAT4-A.thalia	227	QSGDRHPKIGDVLIGAGSCILGNITIGEGAKIGSGSVVVKDVPARTAV	276
2-C.merolae	296	EAGDRHPKVGKRVLLGAGATVGNIRIGDGAQITASSVVLKDVPPYTIIVS	345
SAT4-A.thalia	277	GNPARLIGGKENPR	290
2-C.merolae	346	GVPAREVGKLSYPK	359
SAT5-A.thalia	45	LWTQIKAEARRDAEAPALASYLYSTILSHSSSLERSISFHLGNKLCSSTL	94
2-C.merolae	117	VWELVRRREAEIGAANEPQLASSLYATVLNHRCLEDTLAFHLANELASPPF	166
SAT5-A.thalia	95	LSTLLYDLFLNFTFSSDPSLRNATVADLRAARVRDPACISFSHCLLNKYGK	144
2-C.merolae	167	QATQYVKLFRDALYQDKSYREAIRADLLAVVRRDPAMKHCVAVLMYSKGY	216
SAT5-A.thalia	145	LAIQAHVSHKLTQSRKPLALALHSRISDVFAVDIHFAARIGKGIILLDH	194
2-C.merolae	217	AALQAYRLAHLWRQDRKVLALFLQSEISKCFAVDIHFAARIGSGVMIDH	266
SAT5-A.thalia	195	ATGVVIGETAVIGNVSIHNVTLGGTGKACGDRHPKIGDGLIGAGATI	244
2-C.merolae	267	ATGIVIGETAVVGNVSMHNVTLGGTGKEAGDRHPKVGKRVLLGAGATV	316
SAT5-A.thalia	245	LGNVKIGAGAKVAGSVVLIDVPCRGTAAGNPARLVGGKEKP	286
2-C.merolae	317	LGNIRIGDGAQITASSVVLKDVPPYTIIVSGVPAREVGKLSYP	358

Fig. 7-26. Alignment of 2-C. merolae SAT amino acid sequence with A. thaliana isoforms.



SAT1-A. thalia	71	DPiWDSIREEAKLEAEPEVLSFLYASILSHDCLEQALSFVLANRLQNP	120
T. pseudonana	332	DLVWDLMRHEAQIEAQREPLLVSFYLYSTILNHPTLEAALAFHLANRLESS	381
SAT1-A. thalia	121	TLLATQLMDFCNVMVHDRGIQSSIRLDVQAFKDRDPACLSYSSAILHLK	170
T. pseudonana	382	AMLSTQVMELVREALDGDDEEFQRNLRADIMAVRDRDPACTCLPDVFLYFK	431
SAT1-A. thalia	171	GYLALQAYRVAHLKWKQGRKLLALALQSRVSEVFGIDIHPAARIGKGILL	220
T. pseudonana	432	GFHALQSYRVSNYLWRSGRRLAHYLSQVSTFQIDIHPNATIGSGVML	481
SAT1-A. thalia	221	DHGTGVVIGETAVIGDRVSIHGVTLGGTGKGTGDRHPNIGDGALLGACV	270
T. pseudonana	482	DHGTGIVIGETAHLGHNCVLLHHVTLGSGGKGVDRHPKIGNGVLLGAGA	531
SAT1-A. thalia	271	TILGNIKIGAGAMVAAGSLVLDVPSHSMVAGNPAKLIG-FVD-EQDPSM	318
T. pseudonana	532	SVLGNIHIGDGCQVAGATLVVEDLPPRSVAVGVPAKIIGRFVDVTAQPSL	581
SAT1-A. thalia	319	TMEHDATRE 327	
T. pseudonana	582	GMNQLGSKE 590	
SAT2-A. thalia	34	DPiWDAIREEAKLEAEKEPILSSFLYAGILAHDCLEQALGFVLANRLQNP	83
T. pseudonana	332	DLVWDLMRHEAQIEAQREPLLVSFYLYSTILNHPTLEAALAFHLANRLESS	381
SAT2-A. thalia	84	TLLATQLLDIFYGVMMDKGIQSSIRHDLQAFKDRDPACLSYSSAILHLK	133
T. pseudonana	382	AMLSTQVMELVREALDGDDEEFQRNLRADIMAVRDRDPACTCLPDVFLYFK	431
SAT2-A. thalia	134	GYHALQAYRVAHLKLNWENGRKLLALALQSRISEVFGIDIHPAARIGEGILL	183
T. pseudonana	432	GFHALQSYRVSNYLWRSGRRLAHYLSQVSTFQIDIHPNATIGSGVML	481
SAT2-A. thalia	184	DHGTGVVIGETAVIGNVSIHGVTLGGTGKGTGDRHPKIGEGALLGACV	233
T. pseudonana	482	DHGTGIVIGETAHLGHNCVLLHHVTLGSGGKGVDRHPKIGNGVLLGAGA	531
SAT2-A. thalia	234	TILGNISIGAGAMVAAGSLVLDVPSHVVAGNPAKLI-RVME-EQDPSL	281
T. pseudonana	532	SVLGNIHIGDGCQVAGATLVVEDLPPRSVAVGVPAKIIGRFVDVTAQPSL	581
SAT2-A. thalia	282	AMKHATKE 290	
T. pseudonana	582	GMNQLGSKE 590	
SAT3-A. thalia	122	VDDVWAKIREEAKSDIAKEPIVSAYYHASIVSORSLEAALANTLSVKLSN	171
T. pseudonana	331	VDLVWDLMRHEAQIEAQREPLLVSFYLYSTILNHPTLEAALAFHLANRLES	380
SAT3-A. thalia	172	LNLPNTLFDLFSGVLQGNPDIVESVKLDDLAVKERDPACISYVHCFLHF	221
T. pseudonana	381	SAMLSTQVMELVREALDGDDEEFQRNLRADIMAVRDRDPACTCLPDVFLYFK	430
SAT3-A. thalia	222	KGFLACQAHRIAHELWTQDRKILALLIQNRVSEFAVDFHPGAKIGTGIL	271
T. pseudonana	431	KGFLALQSYRVSNYLWRSGRRLAHYLSQVSTFQIDIHPNATIGSGVM	480
SAT3-A. thalia	272	LDHATAIVIGETAVVGNVSIHNVTLGGTGKQCGDRHPKIGDGVLLIGAG	321
T. pseudonana	481	LDHGTGIVIGETAHLGHNCVLLHHVTLGSGGKGVDRHPKIGNGVLLGAG	530
SAT3-A. thalia	322	TCILGNITIGEGAKIGAGSVVLDKDVPPRTTAVGNPARLLG 361	
T. pseudonana	531	ASVLGNIHIGDGCQVAGATLVVEDLPPRSVAVGVPAKIIG 570	
SAT4-A. thalia	46	DDVWIKMLEEAKSDVKQEPILSNYYASITSHRSLESALAHILSVKLSNL	95
T. pseudonana	332	DLVWDLMRHEAQIEAQREPLLVSFYLYSTILNHPTLEAALAFHLANRLESS	381
SAT4-A. thalia	96	NLPNTLFLFELFISVLEESPEIIESTKQDLIAVKERDPACISYVHCFLGFK	145
T. pseudonana	382	AMLSTQVMELVREALDGDDEEFQRNLRADIMAVRDRDPACTCLPDVFLYFK	431
SAT4-A. thalia	146	GFLACQAHRIAHTLWKQNRKIVALLIQNRVSEFAVDIHPGAKIGKGILL	195
T. pseudonana	432	GFHALQSYRVSNYLWRSGRRLAHYLSQVSTFQIDIHPNATIGSGVML	481
SAT4-A. thalia	196	DHATGVVIGETAVVGDVNSILHGVTLGGTGKQSGDRHPKIGDGVLLIGAGS	245
T. pseudonana	482	DHGTGIVIGETAHLGHNCVLLHHVTLGSGGKGVDRHPKIGNGVLLGAGA	531
SAT4-A. thalia	246	CILGNITIGEGAKIGSGSVVVKDVPARTTAVGNPARLLG 284	
T. pseudonana	532	SVLGNIHIGDGCQVAGATLVVEDLPPRSVAVGVPAKIIG 570	
SAT5-A. thalia	45	LWTQIKAEARRDAEAEAPALASYLYSTILSHSSLERSISFHLGNKLCSSSTL	94
T. pseudonana	334	VWDLMRHEAQIEAQREPLLVSFYLYSTILNHPTLEAALAFHLANRLESSAM	383
SAT5-A. thalia	95	LSTLLYDLFLNFTSSDPSLRNATVADLRAARVDRDPACISFSHCLLNKYGK	144
T. pseudonana	384	LSTQVMELVREALDGDDEEFQRNLRADIMAVRDRDPACTCLPDVFLYFKGF	433
SAT5-A. thalia	145	LAIQAHVRVSHKLWTQSRKPLALALHSRISDVFAVDIHPAAKIGKGILLDH	194
T. pseudonana	434	HALQSYRVSNYLWRSGRRLAHYLSQVSTFQIDIHPNATIGSGVMLDH	483
SAT5-A. thalia	195	ATGVVIGETAVIGNVSIHNVTLGGTGKACGDRHPKIGDGCCLIGAGATI	244
T. pseudonana	484	GTGIVIGETAHLGHNCVLLHHVTLGSGGKGVDRHPKIGNGVLLGAGASV	533
SAT5-A. thalia	245	LGNVIGAGAKVAGSVVLDVPCRGTAVGNPARLVG 281	
T. pseudonana	534	LGNIHIGDGCQVAGATLVVEDLPPRSVAVGVPAKIIG 570	

Fig. 7-27. Alignment of *T. pseudonana* SAT amino acid sequence with *A. thaliana* isoforms.

SAT1-A.thalia	67	NSSYDPIWDSIREAKLEAEPEVLSFLYASILSHDCLEQALSFLVLANR	116
1-P.tricornnut	263	DGSVDLWVDDLWRDAYQEAQREPLLVFLYSTILNHPFSLESSLSFLLANR	312
SAT1-A.thalia	117	LQNPTLL-ATQLMDFICNVVMVHDRGIQSSIRLDVQAFKDRDPACLSSYSSA	165
1-P.tricornnut	313	LQSPAMMISTQLQSLIYASLQRCPIFRRALRADLMAVRDRDPVAVQSLPVDV	362
SAT1-A.thalia	166	ILHLKGYLALQAYRVAHLWK-QGRKLLALALQSRVSEVFGIDHFAARI	214
1-P.tricornnut	363	FLYFKGFHALESHRVAHTLWKKQNKRVLAQYLQSQVSTFTQIDHFNATF	412
SAT1-A.thalia	215	GKGIILLDHGTGVVIGETAVIGDRVSI LHGVTLGGTGKGTGDRHPNIGDGA	264
1-P.tricornnut	413	MGIMLDHGTGIVVGETAAVGHNCISLHHVTLGGSGKKGVDHRPRVGNV	462
SAT1-A.thalia	265	LLGACVITLGNIKIGAGAMVAAGSLVLDKVPVSHMVAAGNPAKLI	312
1-P.tricornnut	463	LLGAGATVLPVHIGDGSQVAGTLVSDLPVSHCAVAVGPARIIGSFIDV	512
SAT1-A.thalia	313	EQDPSMTM 320	
1-P.tricornnut	513	TEQPSIGM 520	
SAT2-A.thalia	30	DPRSDFIWDIAIREAKLEAEKEPILSSFLYAGILAHDCLEQALGFVLANR	79
1-P.tricornnut	263	DGSVDLWVDDLWRDAYQEAQREPLLVFLYSTILNHPFSLESSLSFLLANR	312
SAT2-A.thalia	80	LQNPTLL-ATQLLDIFYGMMHDKGIQSSIRHDLQAFKDRDPACLSSYSSA	128
1-P.tricornnut	313	LQSPAMMISTQLQSLIYASLQRCPIFRRALRADLMAVRDRDPVAVQSLPVDV	362
SAT2-A.thalia	129	ILHLKGYHALQAYRVAHLKLN-EGRKLLALALQSRVSEVFGIDHFAARI	177
1-P.tricornnut	363	FLYFKGFHALESHRVAHTLWKKQNKRVLAQYLQSQVSTFTQIDHFNATF	412
SAT2-A.thalia	178	GEGILLDHGTGVVIGETAVIGNVSI LHGVTLGGTGKGTGDRHPKIGEGA	227
1-P.tricornnut	413	MGIMLDHGTGIVVGETAAVGHNCISLHHVTLGGSGKKGVDHRPRVGNV	462
SAT2-A.thalia	228	LLGACVITLGNISIGAGAMVAAGSLVLDKVPVSHMVAAGNPAKLI	271
1-P.tricornnut	463	LLGAGATVLPVHIGDGSQVAGTLVSDLPVSHCAVAVGPARIIG	506
SAT3-A.thalia	119	DAEVDDVWAKIREAKSDIAKEPIVSAYYHASIVSQRSLAALANTLSVK	168
1-P.tricornnut	263	DGSVDLWVDDLWRDAYQEAQREPLLVFLYSTILNHPFSLESSLSFLLANR	312
SAT3-A.thalia	169	L-SNINLPSNTLFDLFSGLQGNPDIVSEVKLDDLAVKERDPACISYVHC	217
1-P.tricornnut	313	LQSPAMMISTQLQSLIYASLQRCPIFRRALRADLMAVRDRDPVAVQSLPVDV	362
SAT3-A.thalia	218	FLHFKGFACQAHRIAEHLWT-QDRKILALLIQNRVSEAFVDFHPGAKI	266
1-P.tricornnut	363	FLYFKGFHALESHRVAHTLWKKQNKRVLAQYLQSQVSTFTQIDHFNATF	412
SAT3-A.thalia	267	GTGILLDHATAIVIGETAVVGNVSI LHNVTLGGTGKQGDHRHPKIGDGV	316
1-P.tricornnut	413	MGIMLDHGTGIVVGETAAVGHNCISLHHVTLGGSGKKGVDHRPRVGNV	462
SAT3-A.thalia	317	LIGAGTCILGNITIGEGAKIGAGSVVLDKVPVPRRTAVGNPARLLG	361
1-P.tricornnut	463	LLGAGATVLPVHIGDGSQVAGTLVSDLPVSHCAVAVGPARIIG	507
SAT4-A.thalia	46	DDVWIKMLEEAKSDVKQEPILSNYYIASITSHRSLESALAHILSVKL-SN	94
1-P.tricornnut	267	DLVWDDLWRDAYQEAQREPLLVFLYSTILNHPFSLESSLSFLLANRLQSP	316
SAT4-A.thalia	95	LNLPSNTLFEFISVLEESPEIESTKQDILAVKERDPACISYVHCFGLF	144
1-P.tricornnut	317	AMMISTQLQSLIYASLQRCPIFRRALRADLMAVRDRDPVAVQSLPVDVFLYF	366
SAT4-A.thalia	145	KGFLACQAHRIAEHLWT-QNRKIVALLIQNRVSEAFVDFHPGAKIGKI	193
1-P.tricornnut	367	KGFHALESHRVAHTLWKKQNKRVLAQYLQSQVSTFTQIDHFNATFGMGI	416
SAT4-A.thalia	194	LLDHATGVVIGETAVVGNVSI LHGVTLGGTGKQGDHRHPKIGDGLIGA	243
1-P.tricornnut	417	MLDHGTGIVVGETAAVGHNCISLHHVTLGGSGKKGVDHRPRVGNVLLGA	466
SAT4-A.thalia	244	GSCILGNITIGEGAKIGSGSVVVDKVPARTTAVGNPARLIG 284	
SAT5-A.thalia	34	AAAAAEEAAGLWTOIKAEARRDAEAPALASYLYSTILSHSSLSERSISF	83
1-P.tricornnut	258	SVYADDSVDLWVDDLWRDAYQEAQREPLLVFLYSTILNHPFSLESSLSF	307
SAT5-A.thalia	84	HLGNKLCs-STLLSTLLYDLFLNTFSSDPSLRNATVADLRAARVRDPACI	132
1-P.tricornnut	308	LLANRLQSPAMMISTQLQSLIYASLQRCPIFRRALRADLMAVRDRDPVAVQ	357
SAT5-A.thalia	133	SFHSCLLNKGFALQAHRVSHKLWT-QSRKPLALALHSRISDVFAVDIH	181
1-P.tricornnut	358	SLPDVFLYFKGFHALESHRVAHTLWKKQNKRVLAQYLQSQVSTFTQIDH	407
SAT5-A.thalia	182	PAKIKGILLDHATGVVIGETAVIGNVSI LHNVTLGGTGKQGDHRHPK	231
1-P.tricornnut	408	PNATFGMIMLDHGTGIVVGETAAVGHNCISLHHVTLGGSGKKGVDHRPR	457
SAT5-A.thalia	232	IGDGCLIGAGATILGNVIGAGAKVAGSVVLDVPCRGTAVGNPARLVG	281
1-P.tricornnut	458	VGNVLLGAGATVLPVHIGDGSQVAGTLVSDLPVSHCAVAVGPARIIG	507
SAT2-A.thalia	76	LANRLQNPTLLATQLLDIFYGMMHDKGIQSSIRHDL	112
2-P.tricornnut	333	LARLLPNATLEVIHSDDGHDGFLLEQEQVAAHIQHFL	369
SAT1-A.thalia	276	IKIGAGAMVAAGSLVLDKDV	294
2-P.tricornnut	170	VPIACGAQHSAAVQIAISEV	188
SAT3-A.thalia	73	RDSSKHDDDESGFRYMNYFRYPDRS	97
2-P.tricornnut	235	RQRGRGDDDDTGGPAYGSHARWQVKS	259
SAT4-A.thalia	133	ACISYVHCFGLFKGFACQAHRIAH	157
2-P.tricornnut	340	ATLEVIHSDDGHDGFLLEQEQVAAH	364
SAT5-A.thalia	124	ARVRDPACISFHSCLLNKGFALQAHRVSH	154
2-P.tricornnut	334	ARLLPNATLEVIHSDDGHDGFLLEQEQVAAH	364

Fig. 7-28. Alignment of *P. tricornutum* SAT amino acid sequence with *A. thaliana* isoforms.



SAT1-A.thalia	156	DPACLSYSSAILHLKGYLALQAYRVAHKLWKQGR---KLLALALQSRVSE	202
E.huxleyi	146	DAAFAGFLRIYLFKGFHVSQCARVAHFWWNPNGSGRWIALALQSEMDS	195
SAT1-A.thalia	203	VFGIDIHPAARIGKGIILLDHTGTVVIGETAVIGDRVSIHGVTLGGTGKE	252
E.huxleyi	196	AFGVDIHPAARWGRGITMDHGTGCVIGETAVIGDNVYIMHDVTLGATGAS	245
SAT1-A.thalia	253	TG-----DRHPNIGDGALLGACVTILGNIKIGAGAMVAA	286
E.huxleyi	246	LHHARTEMAGMGTSLDHHRPKIGRGAFLACKSTVLGNIQVGAGAT---	292
SAT1-A.thalia	287	GSLVLKDVPSHSMVAGNPAKLI 308	
E.huxleyi	293	-ALVNKVPVAGYTAVGSPARML 313	
SAT2-A.thalia	119	DPACLSYSSAILHLKGYHALQAYRVAHKLWNEGR---KLLALALQSRVSE	165
E.huxleyi	146	DAAFAGFLRIYLFKGFHVSQCARVAHFWWNPNGSGRWIALALQSEMDS	195
SAT2-A.thalia	166	VFGIDIHPAARIGEGILLDHTGTVVIGETAVIGNVSIHGVTLGGTGKE	215
E.huxleyi	196	AFGVDIHPAARWGRGITMDHGTGCVIGETAVIGDNVYIMHDVTLGATGAS	245
SAT2-A.thalia	216	TG-----DRHPKIGEGALLGACVTILGNISIGAGAMVAA	249
E.huxleyi	246	LHHARTEMAGMGTSLDHHRPKIGRGAFLACKSTVLGNIQVGAGAT---	292
SAT2-A.thalia	250	GSLVLKDVPSHVVAGNPAKLI 271	
E.huxleyi	293	-ALVNKVPVAGYTAVGSPARML 313	
SAT3-A.thalia	208	DPACISYVHCFHFKGFLACQAHRIAHELWTQDR---KILALLIQNRVSE	254
E.huxleyi	146	DAAFAGFLRIYLFKGFHVSQCARVAHFWWNPNGSGRWIALALQSEMDS	195
SAT3-A.thalia	255	AFAVDFHPGAKIGTGILLDHATAIVIGETAVVGNVSIHNVTLGGTG--	302
E.huxleyi	196	AFGVDIHPAARWGRGITMDHGTGCVIGETAVIGDNVYIMHDVTLGATGAS	245
SAT3-A.thalia	303	-----KQCG-----DRHPKIGDGVILIGAGTCILGNITIGEGAKIGA	338
E.huxleyi	246	LHHARTEMAGMGTSLDHHRPKIGRGAFLACKSTVLGNIQVGAGAT---	292
SAT3-A.thalia	339	GSVVLKDVPPRTTAVGNPARLLGGKDN 365	
E.huxleyi	293	-ALVNKVPVAGYTAVGSPARMLPPKPN 318	
SAT4-A.thalia	131	DPACISYVHCFHFKGFLACQAHRIAHTLWKQ---NRKIVALLIQNRVSE	177
E.huxleyi	146	DAAFAGFLRIYLFKGFHVSQCARVAHFWWNPNGSGRWIALALQSEMDS	195
SAT4-A.thalia	178	SFAVDIHPGAKIGKGIILLDHTGTVVIGETAVVGNVSIHGVTLGGTG--	225
E.huxleyi	196	AFGVDIHPAARWGRGITMDHGTGCVIGETAVIGDNVYIMHDVTLGATGAS	245
SAT4-A.thalia	226	-----KQSG-----DRHPKIGDGVILIGAGSCILGNITIGEGAKIGS	261
E.huxleyi	246	LHHARTEMAGMGTSLDHHRPKIGRGAFLACKSTVLGNIQVGAGAT---	292
SAT4-A.thalia	262	GSVVVKDVPARTAVGNPARLIGGKENPRK 291	
E.huxleyi	293	-ALVNKVPVAGYTAVGSPARMLPPKPNQTK 321	
SAT5-A.thalia	126	VRDPACISFSHCLLNKGYFLAIQAHRVSHKLWTQ---SRKPLALALHSRI	172
E.huxleyi	144	VPDAAFAGFLRIYLFKGFHVSQCARVAHFWWNPNGSGRWIALALQSEM	193
SAT5-A.thalia	173	SDVFAVDIHPAAKIGKGIILLDHTGTVVIGETAVIGNVSIHVVTLGGTG	222
E.huxleyi	194	SDAFGVDIHPAARWGRGITMDHGTGCVIGETAVIGDNVYIMHDVTLGATG	243
SAT5-A.thalia	223	KACG-----DRHPKIGDGLIGAGATILGNVIGAGAKV	256
E.huxleyi	244	ASLHHARTEMAGMGTSLDHHRPKIGRGAFLACKSTVLGNIQVGAGAT-	292
SAT5-A.thalia	257	GAGSVVLIDVPCRGTAVGNPARLVGGKEKPT 287	
E.huxleyi	293	---ALVNKVPVAGYTAVGSPARMLPPKPNQTK 320	

Fig. 7-29. Alignment of *E. huxleyi* SAT amino acid sequence with *A. thaliana* isoforms.

#### **7.3.4. Alignment of the OAS-TL sequence of Cyanobacteria with *A. thaliana* OAS-TL isoforms**

A-A. thaliana	4	RIAKDVTELI	NTPLVVLNNVA--EGCVGRVAAKLEMMPEPCSSVKDRIGF	51	
1-Synechocyst	21	KIASNITELIGRTPLVRLNRIPLLEGCGAKIVVKLEGMNPAASVKDRIGI		70	
A-A. thaliana	52	SMISDAEKKGLIKPGESVLI	EPTSGNTGVGLAFTAAAKGYKLIITMPASM	101	
1-Synechocyst	71	NMINRAEQGLIEPGKTLLE	EPTSGNTGIALAMVAAAKGYQLIITMPETM	120	
A-A. thaliana	102	STERRIILLAFVVELVLT	DPKMGKGAIAKAEELAKTNGYMLQQFENF	151	
1-Synechocyst	121	SQERRAMLKAYGAKLELT	PGSEGSGGCIIRRAQELAESLFNAYMLQQFDNF	170	
A-A. thaliana	152	ANPKIHVETTGPEIWKGT	GGKIDGIVSGIGTGGTITGAGKYLKEQNAVVK	201	
1-Synechocyst	171	ANPQIHQQTTALEI	WQDTDGAIDFLVAGVGTGGTITGVASVLKPKKPSFQ	220	
A-A. thaliana	202	LYGVEPVESAILSGGKPG	PHKIQGIAGGFI	PSVNLVDLIDEVIVQVSSDES	251
1-Synechocyst	221	AI	AVEPQNSFVLSGGKPGPHKIQGIAGGFIPEVLDVNLIDEVIAVTDEEA	270	
A-A. thaliana	252	IDMARQLALKEGLLVG	ISSGAAAAAIIKLAQRFENAGKLFVAIFPSFGER	301	
1-Synechocyst	271	IAYGRRLAREEGILSG	ISTGAALAAAIKVAKRPANKDKLIVMIQPSFGER	320	
A-A. thaliana	302	VLSTVLF	308		
1-Synechocyst	321	VLSTPLF	327		
B-A. thaliana	73	LNIADNAQLIGKTPM	VVLNNV--VKGCVA	VAAKLEIMEPCSSVKDRIG	120
1-Synechocyst	20	MKIASNITELIGRTPLVRLNRIPLLEGCGAKIVVKLEGMNPAASVKDRIG		69	
B-A. thaliana	121	YSMITDAEKKGLITPGK	SVLVESTSGNTGIGLAFIAASKGYKLIITMPAS	170	
1-Synechocyst	70	INMINRAEQGLIEPGKTLLE	EPTSGNTGIALAMVAAAKGYQLIITMPET	119	
B-A. thaliana	171	MSLERRVLLRAF	GAEVLVTEPAKMGMTGAIQKAEELKKTNSYMLQQFDN	220	
1-Synechocyst	120	MSQERRAMLKAYGAKLELT	PGSEGSGGCIIRRAQELAESLFNAYMLQQFDN	169	
B-A. thaliana	221	FANPKIHVETTGPEI	WEDTRGKIDILVAGIGTGGTITGVGRFIKERKPEL	270	
1-Synechocyst	170	FANPQIHQQTTALEI	WQDTDGAIDFLVAGVGTGGTITGVASVLKPKKPSF	219	
B-A. thaliana	271	KVIGVEPTESAILSGGKPG	PHKIQGIAGGFVFNKLDLAI	VDEYIAISSEE	320
1-Synechocyst	220	QATAVEPQNSFVLSGGKPG	PHKIQGIAGGFIPEVLDVNLIDEVIAVTDEE	269	
B-A. thaliana	321	AIETSQKQALQEGLLV	GISSGAAAAAIQVAKRPNAGKLI	AVVPSFGE	370
1-Synechocyst	270	AIAYGRRLAREEGILSG	ISTGAALAAAIKVAKRPANKDKLIVMIQPSFGE	319	
B-A. thaliana	371	RYLSTQLFQSI	381		
1-Synechocyst	320	RYLSTPLFQDL	330		
C-A. thaliana	97	VVCEAVKRETGPDGLNI	ADNVSQLIGKTPMVLNLSIA--KGCVANIAAKL	144	
1-Synechocyst	6	VRCRQISLLGGFLPMKIASNITELIGRTPLVRLNRIPLLEGCGAKIVVKL		55	
C-A. thaliana	145	EIMEPCSSVKDRIGYS	MVTDAAEQKGFISPGKSVLVEPTSGNTGIGLAFIA	194	
1-Synechocyst	56	EGMNPAAASVKDRIGINMINRAEQGLIEPGKTLLE	EPTSGNTGIALAMVA	105	
C-A. thaliana	195	ASRGYRLIITMPASMS	MERRVLLKAFGAEVLVTDPAKMGTVGAVQKAEEL	244	
1-Synechocyst	106	AAKGYQLIITMPETMSQ	ERRAMLKAYGAKLELT	PGSEGSGGCIIRRAQELA	155
C-A. thaliana	245	KNTPDAYMLQQFDN	FANPKIHVETTGPEI	WDDTKGKVDIFVAGIGTGGTI	294
1-Synechocyst	156	ESLFNAYMLQQFDN	FANPQIHQQTTALEI	WQDTDGAIDFLVAGVGTGGTI	205
C-A. thaliana	295	TGVGRFIKKNPKTQV	IGVEPTESDILSGGKPGPHKIQGIAGGFIPK	NLD	344
1-Synechocyst	206	TGVASVLKPKKPSFQ	AI	AVEPQNSFVLSGGKPGPHKIQGIAGGFIPEVLD	255
C-A. thaliana	345	QKIMDEVIAISSEEA	IETAKQLALKEGLLVG	ISSGAAAAAIKVAKRPN	394
1-Synechocyst	256	VNLIDEVIAVTDEEA	IAYGRRLAREEGILSG	ISTGAALAAAIKVAKRPN	305
C-A. thaliana	395	AGKLI	AVVPSFGERYLSTPLFQSI	419	
1-Synechocyst	306	KDKLIVMIQPSFGERYLSTPLFQDL	330		
Cl-A. thaliana	56	LIGKTPLVFLNKVT--	EGCEAVVAAKQEHFQPTCSIKDRPAIAMIADAEK	103	
1-Synechocyst	29	LIGRTPLVRLNRIPLLEGCGAKIVVKLEGMNPAASVKDRIGINMINRAEQ		78	
Cl-A. thaliana	104	KKLIIPGKTLLEPTSG	NMGISLAFMAAMKGYRIIMTMSVTSLE	RRVVM	153
1-Synechocyst	79	EGLIEPGKTLLEPTSGNTGIALAMVAAAKGYQLIITMPETMSQERRAML		128	
Cl-A. thaliana	154	RSFGAEVLVTDPAKMG	GGTVKAYDILLDSTPDAFMCCQFANPANTQIHFD	203	
1-Synechocyst	129	KAYGAKLELT	PGSEGSGGCIIRRAQELAESLFNAYMLQQFDN	FANPQIHQQ	178
Cl-A. thaliana	204	TGPEI	WEDTLGNVDIFVMGIGSGGTVSGVGRYLKSKNPNVKIY	GVPAE	253
1-Synechocyst	179	TTALEI	WQDTDGAIDFLVAGVGTGGTITGVASVLKPKKPSFQAI	AVEPQN	228
Cl-A. thaliana	254	SNILNGGKPGPHAITG	NGVGFKPEILEMDVMSVLEVSSEDAIK	MARELA	303
1-Synechocyst	229	SPVLSGGKPGPHKIQGI	GAGFIPEVLDVNLIDEVIAVTDEEA	IAYGRRLA	278
Cl-A. thaliana	304	LKEGLMVGISGANTVA	IRLAKMPENKGLIVT	HASGERVLSVLF	353
1-Synechocyst	279	REEGILSGISTGAALAAAIKVAKRPN	KDKLIVMIQPSFGERYLSTPLFQ		328
Cl-A. thaliana	354	EL	355		
1-Synechocyst	329	DL	330		

Fig. 7-30. Alignment of 1-Synechocystis sp. PCC 6803 OAS-TL amino acid with *A. thaliana* isoforms.



A-A. thaliana	13	IGNTPLVLYLNNVAEGCVGRVAAKLEMMPCSSVKDRIGFSMISDAEKKGL	62
2-Synechocyst	11	IGHTPLIRLNSFSDETGCCELLGKAEFMNPGGSVKDRAALGIIETAEKEGK	60
A-A. thaliana	63	IKPGESVLIPTSGNTGVLAFIAAAGYKLIITMPASMTERRILLAF	112
2-Synechocyst	61	LKPGGTV-VEGTAGNTGIGLAHICNAKGYKCLIVIPDTQSQEKIDLLRTL	109
A-A. thaliana	113	GVEL--VLTDPKMGKGAIAKAEELAKTPNGYMLQQFENPANPKIHYET	160
2-Synechocyst	110	GAEVRTVPAVYRDPNNYVKLSGRIAAELDNAIWANQFDNLANRDAHHT	159
A-A. thaliana	161	TGPEIWKGTGGKIDGFVSGIGTGGTITGAGKYLKEQANVKLYGVEPVES	210
2-Synechocyst	160	TGPEIWQQTGKVDAWVAATGTGGTYAGVALYLKEQSEAVQCVVADPMGS	209
A-A. thaliana	211	AILSGGKPGPHK-----IQGIGAGFIPSVLNVLDLDEVVQVSSDESIDM	254
2-Synechocyst	210	GLYSFIKTGEINPSGNSITEGIGNSRITANMENVPIDDAVQIDDPALRV	259
A-A. thaliana	255	ARQLALKEGLLVGISSGAAAAAIAKLAQRPENAGKLFVAIFPSPFGERYLS	304
2-Synechocyst	260	VYQLLRDGLFMGGSVGINVGAAYQLAKKL-GPGHTIVTVLDCDGGARYQS	308
B-A. thaliana	73	LNIADNAAQLIGKTPMVYLNNVKGCVASVAAKLEIMEPCCSVKDRIGYS	122
2-Synechocyst	1	MDIKHGFVDSIGHTPLIRLNSFSDETGCCELLGKAEFMNPGGSVKDRAALG	50
B-A. thaliana	123	MITDAEKEGLITPGKSVLVESTSGNTGIGLAFIAASKGYKLIITMPASMS	172
2-Synechocyst	51	IIETAEKEGKXKPGGTV-VEGTAGNTGIGLAHICNAKGYKCLIVIPDTQS	99
B-A. thaliana	173	LERRVLLRAFPAEL--VLTEPAKGMTGAIQKAEELKKTTPNSYMLQQFDN	220
2-Synechocyst	100	QEKIDLLRTLGAEVRTVPAVYRDPNNYVKLSGRIAAELDNAIWANQFDN	149
B-A. thaliana	221	PANPKIHYETTGPFIWEDTRGKIDILVAGIGTGGTITGVGRFIKERKPEL	270
2-Synechocyst	150	LANRDAHHTTGPFIWQQTGKVDAWVAATGTGGTYAGVALYLKEQSEAV	199
B-A. thaliana	271	KVIGVEPTESAILSGGKPGPHK-----IQGIGAGFVFNKLDLAIVDEYI	314
2-Synechocyst	200	QCVVADPMGSGLYSFIKTGEINPSGNSITEGIGNSRITANMENVPIDDAV	249
B-A. thaliana	315	AISSEEAIEISKQLALQEGLLVGISSGAAAAAIAQVAKRPNAGKLIIVV	364
2-Synechocyst	250	QIDDPALRVVYQLLRDGLFMGGSVGINVGAAYQLAKKL-GPGHTIVTV	298
B-A. thaliana	365	FSPFGERYLS	374
2-Synechocyst	299	LDCDGGARYQS	308
C-A. thaliana	121	IGKTPMVYLNNSIAKGCVANIAAKLEIMEPCCSVKDRIGYSMVTDAEQKGF	170
2-Synechocyst	11	IGHTPLIRLNSFSDETGCCELLGKAEFMNPGGSVKDRAALGIIETAEKEGK	60
C-A. thaliana	171	ISPGKSVLVEPTSGNTGIGLAFIAASRGYRLIITMPASMSMERRVLLKAF	220
2-Synechocyst	61	LKPGGTV-VEGTAGNTGIGLAHICNAKGYKCLIVIPDTQSQEKIDLLRTL	109
C-A. thaliana	221	GAEL--VLTDPKGMTGAVQKAEELKNTPDAYMLQQFDNANPKIHYET	268
2-Synechocyst	110	GAEVRTVPAVYRDPNNYVKLSGRIAAELDNAIWANQFDNLANRDAHHT	159
C-A. thaliana	269	TGPEIWDITGKVDIFVAGIGTGGTITGVGRFIKEKNPKTQVIGVEPTES	318
2-Synechocyst	160	TGPEIWQQTGKVDAWVAATGTGGTYAGVALYLKEQSEAVQCVVADPMGS	209
C-A. thaliana	319	DILSGGKPGPHK-----IQGIGAGFIPKNDLQKIMDEVIAISSEEAIEI	362
2-Synechocyst	210	GLYSFIKTGEINPSGNSITEGIGNSRITANMENVPIDDAVQIDDPALRV	259
C-A. thaliana	363	AKQLALKEGLMVGISSGAAAAAIAKVAKRPNAGKLIIVVFPSPFGERYLS	412
C1-A. thaliana	260	IGKTPLVFLNKVTEGCEAVAAKQEHFQPTCSFKDRPAIAMIADAEKXKL	308
2-Synechocyst	11	IGHTPLIRLNSFSDETGCCELLGKAEFMNPGGSVKDRAALGIIETAEKEGK	60
C1-A. thaliana	107	IIPGKTTLIEPTSGNMGISLAFMAAMKGYRIITMPYSYTLERRVTMRSF	156
2-Synechocyst	61	LKPGGT-VVEGTAGNTGIGLAHICNAKGYKCLIVIPDTQSQEKIDLLRTL	109
C1-A. thaliana	157	GAEL--VLTDPKMGMTVKKAYDLLDSTPDAFMCQQFANPANTQIHFDI	204
2-Synechocyst	110	GAEVRTVPAVYRDPNNYVKLSGRIAAELDNAIWANQFDNLANRDAHHT	159
C1-A. thaliana	205	TGPEIWEIDLGNVDIFVMGIGSGGTVSGVGRYLSKKNPNVKIYGVPEPAES	254
2-Synechocyst	160	TGPEIWQQTGKVDAWVAATGTGGTYAGVALYLKEQSEAVQCVVADPMGS	209
C1-A. thaliana	255	NILNGGKPGPHAITGN---GVGFKPEILDMD--VMESVLEVSSEDAIKM	298
2-Synechocyst	210	GLYSFIKTGEINPSGNSITEGIGNSRITANMENVPIDDAVQIDDPALRV	259
C1-A. thaliana	299	ARELALKEGLMVGISSGANTVAAIRLAKMPENKGLIVTIHASFGERYLS	348
2-Synechocyst	260	VYQLLRDGLFMGGSVGINVGAAYQLAKKL-GPGHTIVTVLDCDGGARYQS	308
C1-A. thaliana	349	SVLFDE	354
2-Synechocyst	309	RLYNQE	314

Fig. 7-31. Alignment of 2-Synechocystis sp. PCC 6803 OAS-TL amino acid with *A. thaliana* isoforms.

A-A. thaliana	5	IAXDVTELGIGTFLVVLNNV--AEGCVGRVAAKLEMEPCSSVKDRIGFS	52
1-Synechococcus	3	IAPDITLVGCTPMVRLNRLPKAWGCTAEIVAKLESFNPTASVKDRIAGA	52
A-A. thaliana	53	MISDAEKKGLIKPGESVLIEPTSGNTGVGLAFTAAAGYKLIITMPASMS	102
1-Synechococcus	53	MVEAAESAGTIAPERTVLVEPTSGNTGIALAMVAAARGVRLIITMPDTMS	102
A-A. thaliana	103	TERRIILLAFGVLEVLTDPAKGMKGAIAKAEIILAKTPNGVMLQQFNPA	152
1-Synechococcus	103	TERRAMLRAVGAEQLTTPGMEGMQGAIERARELVDEIPGAYLLQQFDNPA	152
A-A. thaliana	153	NPKIHVETTGPEIWKGTGGKIDGFVSGIGTGGTITGAGKYLKEQNAVVKL	202
1-Synechococcus	153	NPAVHAASTAEIHWADTEGSLDAVVAGVGTGGTITGCAVRLKERQPKLSV	202
A-A. thaliana	203	YGVPEVSAIISGGKPGPHKIQQIGAGFIPSVLNVLDLIDEVQVSSDESI	252
1-Synechococcus	203	VAVEPAASPVLAGGVAGPHRLQGIAGFIPVLEMDLIDEIIAVSDDEAM	252
A-A. thaliana	253	DMARQLALKEGLLVGISSGAAAAAIAKLAQRPENAGKLFVAIFPSPFGERY	302
1-Synechococcus	253	DVGRRLAREEGLLCGVSSGAAVAAALRLGQRPAMEGRRIVVILASFGERY	302
A-A. thaliana	303	LSTVLF 308	
1-Synechococcus	303	LSTPMF 308	
B-A. thaliana	75	IADNAAQLIGKTPMVYLNNVK--GCVASVAAKLEIMEPCSSVKDRIGYS	122
1-Synechococcus	3	IAPDITLVGCTPMVRLNRLPKAWGCTAEIVAKLESFNPTASVKDRIAGA	52
B-A. thaliana	123	MITDAEEKGLITPGKSVLVEPTSGNTGIGLAFIAASKGVKLIITMPASMS	172
1-Synechococcus	53	MVEAAESAGTIAPERTVLVEPTSGNTGIALAMVAAARGVRLIITMPDTMS	102
B-A. thaliana	173	LERRVLLRAFGAELVLTPEAKGMTGAIQKAEIILKKTNPNSYMLQQFDNPA	222
1-Synechococcus	103	TERRAMLRAVGAEQLTTPGMEGMQGAIERARELVDEIPGAYLLQQFDNPA	152
B-A. thaliana	223	NPKIHVETTGPEIHWEDTRGKIDILVAGIGTGGTITGVGRFIERKPKELKV	272
1-Synechococcus	153	NPAVHAASTAEIHWADTEGSLDAVVAGVGTGGTITGCAVRLKERQPKLSV	202
B-A. thaliana	273	IGVEPTESAILSGGKPGPHKIQQIGAGFVFPKNLDLAIVDEVIASSEEAI	322
1-Synechococcus	203	VAVEPAASPVLAGGVAGPHRLQGIAGFIPVLEMDLIDEIIAVSDDEAM	252
B-A. thaliana	323	ETSKQLALQEGLLVGISSGAAAAAIAQVAKRPENAGKLIIVVFPSPFGERY	372
1-Synechococcus	253	DVGRRLAREEGLLCGVSSGAAVAAALRLGQRPAMEGRRIVVILASFGERY	302
B-A. thaliana	373	LSTQLFQS 380	
1-Synechococcus	303	LSTPMFST 310	
C-A. thaliana	113	IADNVSQLIGKTPMVYLNNSIAK--GCVANIAAKLEIMEPCSSVKDRIGYS	160
1-Synechococcus	3	IAPDITLVGCTPMVRLNRLPKAWGCTAEIVAKLESFNPTASVKDRIAGA	52
C-A. thaliana	161	MVTDAEQKGFISPGKSVLVEPTSGNTGIGLAFIAASRGVRLIITMPASMS	210
1-Synechococcus	53	MVEAAESAGTIAPERTVLVEPTSGNTGIALAMVAAARGVRLIITMPDTMS	102
C-A. thaliana	211	MERRVLLKAFGAELVLTDPKGMTGAVQKAEIILKNTPDAYMLQQFDNPA	260
1-Synechococcus	103	TERRAMLRAVGAEQLTTPGMEGMQGAIERARELVDEIPGAYLLQQFDNPA	152
C-A. thaliana	261	NPKIHVETTGPEIWDGKGVDFVAGIGTGGTITGVGRFIERKPKTKQV	310
1-Synechococcus	153	NPAVHAASTAEIHWADTEGSLDAVVAGVGTGGTITGCAVRLKERQPKLSV	202
C-A. thaliana	311	IGVEPTESDILSGGKPGPHKIQQIGAGFIPKNLDQKIMDEVIASSEEAI	360
1-Synechococcus	203	VAVEPAASPVLAGGVAGPHRLQGIAGFIPVLEMDLIDEIIAVSDDEAM	252
C-A. thaliana	361	ETAKQLALKEGLMVGISSGAAAAAIAKVAKRPENAGKLIIVVFPSPFGERY	410
1-Synechococcus	253	DVGRRLAREEGLLCGVSSGAAVAAALRLGQRPAMEGRRIVVILASFGERY	302
C-A. thaliana	411	LSTPLFQS 418	
1-Synechococcus	303	LSTPMFST 310	
C1-A. thaliana	52	DASLLIGKTPVFLNKVTE--GCEAYVAAKQEHFQPTCSIKDRPATAMIA	99
1-Synechococcus	6	DITLVGCTPMVRLNRLPKAWGCTAEIVAKLESFNPTASVKDRIAGAMVE	55
C1-A. thaliana	100	DAEKKKLIIPGKTTLIEPTSGNMGISLAFMAAMKGYRIIMTPSYTSLER	149
1-Synechococcus	56	AAESAGTIAPERTVLVEPTSGNTGIALAMVAAARGVRLIITMPDTMSTER	105
C1-A. thaliana	150	RVTMRSFGAELVLTDPKGMGIVKAYDLDLSTPDAFMQQFANPANTQ	249
1-Synechococcus	106	RAMLRAVGAEQLTTPGMEGMQGAIERARELVDEIPGAYLLQQFDNPA	155
C1-A. thaliana	200	IHFDTTGPEIHWEDTLGNVDIFVMIGSGGTVSGVGRYLSKSNPNVKIYGV	299
1-Synechococcus	156	VHAASTAEIHWADTEGSLDAVVAGVGTGGTITGCAVRLKERQPKLSVVAV	205
C1-A. thaliana	250	EPAESNINLGGKPGPHAITGNVGFKPEILDMDVMESVLEVSSEDAIKMA	299
1-Synechococcus	206	EPAASPVLAGGVAGPHRLQGIAGFIPVLEMDLIDEIIAVSDDEAMVVG	255
C1-A. thaliana	300	RELALKEGLMVGISSGANTVAAIRLAKMPENKGLIVTHASFGERVLS	349
1-Synechococcus	256	RRLAREEGLLCGVSSGAAVAAALRLGQRPAMEGRRIVVILASFGERYLST	305
C1-A. thaliana	350	VLF 352	
1-Synechococcus	306	PMF 308	

Fig. 7-32. Alignment of 1-*Synechococcus* sp. WH 7803 OAS-TL amino acid with *A. thaliana* isoforms.



A- <i>A. thaliana</i>	3	SRIAKDVTELGNTPLVYLNNVAEGCVGRVAAKLEMMPEPCSSVKDRIGFS	52
2- <i>Synechococc</i>	2	SRVYADNSQAIGNTPLVRLNHVTKGCKATVLAKVEGRNPAYSVKCRIGAN	51
A- <i>A. thaliana</i>	53	MISDAEKKGLIKPGESVLIPTSGNTGVGLAFTAAAKGYKLIITMPASMS	102
2- <i>Synechococc</i>	52	MIWDAEKRGALTEGK-VIVEPTSGNTGIALAFTAAARGYKLVLTMPESMS	100
A- <i>A. thaliana</i>	103	TERRIILLAFGVELVLTDFPAKGMKGATAKAEIILAKTFNGYMLQ-QFENP	151
2- <i>Synechococc</i>	101	IERRRVMAVLGAEIVLTEAAKGMFGATAKAKEIAASDPAKYFMPGQFENP	150
A- <i>A. thaliana</i>	152	ANPKIHYETTGPPIWKGTGGKIDGFVSGIGTGGTITGAGKYLK-EQNAV	200
2- <i>Synechococc</i>	151	ANPEIHFKTTPPEIWNDCDGAIDVLVSGVGTGGTITGVSRYIKNEAGKAI	200
A- <i>A. thaliana</i>	201	KLYGVEPVESAILSGG-----KPGPHKIQQIGAGFIPSVLNVLDIDEVV	244
2- <i>Synechococc</i>	201	ESVAVEPSSHSPVITQTLNGEELKPGPHKIQQIGAGFIPENLDLSVVDKVE	250
A- <i>A. thaliana</i>	245	QVSSDESIDMARQLALKEGLLVGISSGAAAAAAIKLAQRPNAGKLFVAI	294
2- <i>Synechococc</i>	251	QVTNEESIAMAQRLAKEEGLLVGISCGAAAAAAIRLAQQDAYAGKTIVVV	300
A- <i>A. thaliana</i>	295	FPSFGERVLSVLF 308	
2- <i>Synechococc</i>	301	LPDLAERYLSSVMF 314	
B- <i>A. thaliana</i>	76	ADNAAQLIGKTPMVYLNNVVKGCVASVAAKLEIMEPCSSVKDRIGYSMIT	125
2- <i>Synechococc</i>	6	ADNS-QAIGNTPLVRLNHVTKGCKATVLAKVEGRNPAYSVKCRIGANMIW	54
B- <i>A. thaliana</i>	126	DAEEKGLITPGKSVLVESTSGNTGIGLAFIAASKGYKLIITMPASMSLER	175
2- <i>Synechococc</i>	55	DAEKRGALTEGK-VIVEPTSGNTGIALAFTAAARGYKLVLTMPESMSIER	103
B- <i>A. thaliana</i>	176	RVLLEAFGAELVLTTEPAKGMTGAIQKAEIILKKTIPNSYMLQ-QFDNPANP	224
2- <i>Synechococc</i>	104	RRVMAVLGAEIVLTEAAKGMFGATAKAKEIAASDPAKYFMPGQFENPANP	153
B- <i>A. thaliana</i>	225	KIHVETTGPPIWEDTRGKIDILVAGIGTGGTITGVGRFIK-ERKPELKV	273
2- <i>Synechococc</i>	154	EIHFKTTGPEIWNDCDGAIDVLVSGVGTGGTITGVSRYIKNEAGKAI	203
B- <i>A. thaliana</i>	274	GVEPTESAILSGG-----KPGPHKIQQIGAGFVFNLDLAIVDEYIAIS	317
2- <i>Synechococc</i>	204	AVEPSSHSPVITQTLNGEELKPGPHKIQQIGAGFIPENLDLSVVDKVEQVT	253
B- <i>A. thaliana</i>	318	SEEAITETSKQLALQEGLLVGISSGAAAAAAIQVAKRPNAGKLIIVVFP	367
2- <i>Synechococc</i>	254	NEESIAMAQRLAKEEGLLVGISCGAAAAAAIRLAQQDAYAGKTIVVVLFD	303
B- <i>A. thaliana</i>	368	FGERYLSTQLFQSI 381	
2- <i>Synechococc</i>	304	LAERYLSSVMFADV 317	
C- <i>A. thaliana</i>	114	ADNVSQLIGKTPMVYLNNSIAGKCVANIAAKLEIMEPCSSVKDRIGYSMVT	163
2- <i>Synechococc</i>	6	ADN-SQAIGNTPLVRLNHVTKGCKATVLAKVEGRNPAYSVKCRIGANMIW	54
C- <i>A. thaliana</i>	164	DAEQKGFISPGKSVLVEPTSGNTGIGLAFIAASRGYRILITMPASMSMER	213
2- <i>Synechococc</i>	55	DAEKRGALTEGK-VIVEPTSGNTGIALAFTAAARGYKLVLTMPESMSIER	103
C- <i>A. thaliana</i>	214	RVLLEAFGAELVLTDPKGMTGAVQKAEIILKNTPDAYMLQ-QFDNPANP	262
2- <i>Synechococc</i>	104	RRVMAVLGAEIVLTEAAKGMFGATAKAKEIAASDPAKYFMPGQFENPANP	153
C- <i>A. thaliana</i>	263	KIHVETTGPPIWDDTKGKVDIFVAGIGTGGTITGVGRFIKNEKPKT-QVI	311
2- <i>Synechococc</i>	154	EIHFKTTGPEIWNDCDGAIDVLVSGVGTGGTITGVSRYIKNEAGKAI	203
C- <i>A. thaliana</i>	312	GVEPTESDILSGG-----KPGPHKIQQIGAGFIPKNLDQKIMDEYIAIS	355
2- <i>Synechococc</i>	204	AVEPSSHSPVITQTLNGEELKPGPHKIQQIGAGFIPENLDLSVVDKVEQVT	253
C- <i>A. thaliana</i>	356	SEEAITETAKQLALKEGLMVGISSGAAAAAAIKVAKRPNAGKLIIVVFP	405
2- <i>Synechococc</i>	254	NEESIAMAQRLAKEEGLLVGISCGAAAAAAIRLAQQDAYAGKTIVVVLFD	303
C- <i>A. thaliana</i>	406	FGERYLSTPLFQSI 419	
2- <i>Synechococc</i>	304	LAERYLSSVMFADV 317	
Cl- <i>A. thaliana</i>	52	DASLLIGKTPLVFLNKVTEGCEAYVAAKQEHFQPTCSIKDRPAIAMIADA	101
2- <i>Synechococc</i>	7	DNSQAIGNTPLVRLNHVTKGCKATVLAKVEGRNPAYSVKCRIGANMIWDA	56
Cl- <i>A. thaliana</i>	102	EKKKLIIPGKTTLIEPTSGNMGISLAFMAAMKGYRIIMTSPSYTSLERRV	151
2- <i>Synechococc</i>	57	EKRGALETEGKV-IVEPTSGNTGIALAFTAAARGYKLVLTMPESMSIERRR	105
Cl- <i>A. thaliana</i>	152	TMRSFGAELVLTDPKMGGTVKKAYDILLDSTPDA-FMCQQFANPANTQI	200
2- <i>Synechococc</i>	106	VMAVLGAEIVLTEAAKGMFGATAKAKEIAASDPAKYFMPGQFENPANPEI	155
Cl- <i>A. thaliana</i>	201	HFDTTGPEIWEEDTLGNVDIFVMGIGSGGTVSGVGRYLSK-NPNVKIYGV	249
2- <i>Synechococc</i>	156	HFKTTGPEIWNDCDGAIDVLVSGVGTGGTITGVSRYIKNEAGKAI	205
Cl- <i>A. thaliana</i>	250	EPAESNI----LNGG--KPGPHAITGNGVGFKPEIILDMVMSVLEVSSE	293
2- <i>Synechococc</i>	206	EPSSHSPVITQTLNGEELKPGPHKIQQIGAGFIPENLDLSVVDKVEQVTNE	255
Cl- <i>A. thaliana</i>	294	DAIKMARELALKEGLMVGISSGANTVAAIRLAKMPENKGLIVTIHASF	343
2- <i>Synechococc</i>	256	ESIAMAQRLAKEEGLLVGISCGAAAAAAIRLAQQDAYAGKTIVVVLFDLA	305
Cl- <i>A. thaliana</i>	344	ERYLSSVLFDEL 355	
2- <i>Synechococc</i>	306	ERYLSSVMFADV 317	

Fig. 7-33. Alignment of 2-*Synechococcus* sp. WH 7803 OAS-TL amino acid with *A. thaliana* isoforms.



**7.3.5. Alignment of the OAS-TL sequence of green algae with  
*A. thaliana* OAS-TL isoforms**

A-A. thaliana	5	IAKDVTELIQNTPLVVLNNVAEGCVGRVAAKLEMEMEPCSSVKDRIGFSMI	54
1-C. reinhardtii	77	IQPDATRLVGNTPMVFVLSVTRGCGARIAAKLESFEPCCSVKDRIALNMI	126
A-A. thaliana	55	SDAEKKGLIKPGESVLIPTSGNTGVGLAFTAAAKGYKLIITMPASMSTE	104
1-C. reinhardtii	127	ERAEQAGQISPGVTTLIEPTSGNTGVALAYVAAAKGYRLALTMPETMSIE	176
A-A. thaliana	105	RRIIILAFGVVELVLTDPKAGMKGAIKAEIILAKTPNGVYLQQFENPANP	154
1-C. reinhardtii	177	RRVLLKAFGAELVLTFGRLGMTGAIKAEEMVRSTPNAFMLQQFDNPNP	226
A-A. thaliana	155	KIHVETTGPEIWKGTGGKIDGTVSGIGTGGTITGAGKYLKEQANVNLVYG	204
1-C. reinhardtii	227	EVHLKTTGPEIWRDTAGNIDMFVAGVGTGGTISGVGQYLKEQKPGVQVVA	276
A-A. thaliana	205	VEPVESAILSGGKPGPHKIQQIGAGFIPSVLNVLDIDEVVQVSSDESIDM	254
1-C. reinhardtii	277	VEPAESFVISGGAPGVHQIQIGAGFVFNLRVLDLDEVVKINSNEAIEIEM	326
A-A. thaliana	255	ARQLALKEGLLVGISSGAAAAATKLAQRPENAGKLFVAIFPSPGGERYLS	304
1-C. reinhardtii	327	ARRLAVEEGLLCGISSGAAVAAAIKAKRPNRDKLIVTVLPSFGERYLS	376
A-A. thaliana	305	TVLFD 309	
1-C. reinhardtii	377	TVLFN 361	
B-A. thaliana	73	LNIADNAAQLIGKTPMVYLNNVKCVASVAAKLEIMEPCCSVKDRIGYS	122
1-C. reinhardtii	75	VGIQPDATRLVGNTPMVFVLSVTRGCGARIAAKLESFEPCCSVKDRIALN	124
B-A. thaliana	123	MITDAEEKGLITPGKSVLVEPTSGNTGIGLAFIAASKGYKLIITMPASMS	172
1-C. reinhardtii	125	MIERAEQAGQISPGVTTLIEPTSGNTGVALAYVAAAKGYRLALTMPETMS	174
B-A. thaliana	173	LERRVLLKAFGAELVLTDPKAGMTGAIKAEIILKKTNSYMLQQFDNPA	222
1-C. reinhardtii	175	IERRVLLKAFGAELVLTFGRLGMTGAIKAEEMVRSTPNAFMLQQFDNPA	224
B-A. thaliana	223	NPKIHVETTGPEIWDTRGKIDILVAGIGTGGTITGVGRFIERKPKELKV	272
1-C. reinhardtii	225	NPEVHLKTTGPEIWRDTAGNIDMFVAGVGTGGTISGVGQYLKEQKPGVQV	274
B-A. thaliana	273	IGVEPTESAILSGGKPGPHKIQQIGAGFVFNLRVLDLIDEVYIAISSEAI	322
1-C. reinhardtii	275	VAVEPAESFVISGGAPGVHQIQIGAGFVFNLRVLDLDEVVKINSNEAI	324
B-A. thaliana	323	ETSKQLALQEGLLVGISSGAAAAAIQVAKRPNENAGKLIIVVFPFGERY	372
1-C. reinhardtii	325	EMARRLAVEEGLLCGISSGAAVAAAIKAKRPNRDKLIVTVLPSFGERY	374
B-A. thaliana	373	LSTQLFQSI 381	
1-C. reinhardtii	375	LSTVLFNTL 363	
C-A. thaliana	111	LNIADNVSQLIGKTPMVYLNNSIAKGCVANIAAKLEIMEPCCSVKDRIGYS	160
1-C. reinhardtii	75	VGIQPDATRLVGNTPMVFVLSVTRGCGARIAAKLESFEPCCSVKDRIALN	124
C-A. thaliana	161	MVTDAEQKGFISPGKSVLVEPTSGNTGIGLAFIAASRGYRLIITMPASMS	210
1-C. reinhardtii	125	MIERAEQAGQISPGVTTLIEPTSGNTGVALAYVAAAKGYRLALTMPETMS	174
C-A. thaliana	211	MERRVLLKAFGAELVLTDPKAGMTGAVQKAEIILKNTPDAYMLQQFDNPA	260
1-C. reinhardtii	175	IERRVLLKAFGAELVLTFGRLGMTGAIKAEEMVRSTPNAFMLQQFDNPA	224
C-A. thaliana	261	NPKIHVETTGPEIWDTRGKVDIFVAGIGTGGTITGVGRFIERKPKTKQV	310
1-C. reinhardtii	225	NPEVHLKTTGPEIWRDTAGNIDMFVAGVGTGGTISGVGQYLKEQKPGVQV	274
C-A. thaliana	311	IGVEPTESDILSGGKPGPHKIQQIGAGFIPKLNLDKIMDEVIAISSEAI	360
1-C. reinhardtii	275	VAVEPAESFVISGGAPGVHQIQIGAGFVFNLRVLDLDEVVKINSNEAI	324
C-A. thaliana	361	ETAKQLALKEGLMVGISSGAAAAAIKVAKRPNENAGKLIIVVFPFGERY	410
1-C. reinhardtii	325	EMARRLAVEEGLLCGISSGAAVAAAIKAKRPNRDKLIVTVLPSFGERY	374
C-A. thaliana	411	LSTPLFQSI 419	
1-C. reinhardtii	375	LSTVLFNTL 383	
Cl-A. thaliana	45	PSTNAKRDAQLLIGKTPLVFLNKVTEGCEAYVAAKQEHFQPTCSIKDRPA	94
1-C. reinhardtii	73	PHVGIQPDATRLVGNTPMVFVLSVTRGCGARIAAKLESFEPCCSVKDRIA	122
Cl-A. thaliana	95	IAMIADAEEKKLIIPGKTTLIEPTSGNMGISLAFMAAMKGYRIIMTSPSY	144
1-C. reinhardtii	123	LNMIERAEQAGQISPGVTTLIEPTSGNTGVALAYVAAAKGYRLALTMPET	172
Cl-A. thaliana	145	TSLERRVTRMRSFGAELVLTDPKAGMGGTVKKAYDILLDSTPDAFMCQQFAN	194
1-C. reinhardtii	173	MSIERRVLLKAFGAELVLTFGRLGMTGAIKAEEMVRSTPNAFMLQQFDN	222
Cl-A. thaliana	195	PANTQIHFDTTGPEIWDTRGKVDIFVMGIGSGGTVSGVGRYLSKSNPNV	244
1-C. reinhardtii	223	PANPEVHLKTTGPEIWRDTAGNIDMFVAGVGTGGTISGVGQYLKEQKPGV	272
Cl-A. thaliana	245	KIYGVPEAESNIILGGKPGPHAITGNVGVFKPEILDMDVMESVLEVSSD	294
1-C. reinhardtii	273	QVVAVEPAESFVISGGAPGVHQIQIGAGFVFNLRVLDLDEVVKINSNE	322
Cl-A. thaliana	295	AIKVAARELALKEGLMVGISSGANTVAAIRLAKMPENKGLIVTIHASFGE	344
1-C. reinhardtii	323	AIEMARRLAVEEGLLCGISSGAAVAAAIKAKRPNRDKLIVTVLPSFGE	372
Cl-A. thaliana	345	RYLSSVLFDEL 355	
1-C. reinhardtii	373	RYLSTVLFNTL 383	

Fig. 7-34. Alignment of 1-C. reinhardtii OAS-TL amino acid with A. thaliana isoforms.

A-A. thaliana	5	IAKDVTELIGNTPLVVLNNVAEGCVGRVAAKLEMMFPCSSVKDRIGFSMI	54
2-C. reinhardtii	41	VRQGVLDLIGNTPLVRVASLSEETGCEIIVWKAEMLNPGGSVKDRVALQIV	90
A-A. thaliana	55	SDAEKKGLIKPGESVLIPTSGNTGVGLAFTAAAKGYKLIITPMASMS	104
2-C. reinhardtii	91	SEALADGRLRPG-GLITEGTAGSTGVSLAMVAAAYGCRCSITMPDDAAIE	139
A-A. thaliana	105	RRIIILAFGVVELVLTDPKAGM--KGATAKABEILAKTNGVYMLQQFENFA	152
2-C. reinhardtii	140	KANMIQAYGASVRRVRFVSIHVHPEHPVNVARREAASTPGALFADQFENE	189
A-A. thaliana	153	NPKIHVYETTGPEIWKGTGGKIDGFVSGIGTGGTITGAGKYLKEQANVVKL	202
2-C. reinhardtii	190	NFRAHLKT-GEEIQQQTQGRVHAFVSGAGTGGTVAGVSTALKARNFRV	238
A-A. thaliana	203	YGVFVESAIISSGGKPG-----PHKI--QGIGAGFIPSVL	235
2-C. reinhardtii	239	FLVDFPGSSLFNKKVGRVMTSEEAEGRKLNPFDTITEGIGINRLTANF	288
A-A. thaliana	236	NVDLIDEVQVSSDESIDMARQLALKEGLLVGSSGAAAAAIKLAQRPE	285
2-C. reinhardtii	289	NRALIDDAFRGTDREAVEMAAYLLRNEGLWVGSSAAMNCVGAVK--AARAM	337
A-A. thaliana	286	NAGKLFVAIFPSFGERYLS 304	
2-C. reinhardtii	338	GPHTIVTLLCDGGHRHLS 356	
B-A. thaliana	64	IKPEAGVEGLNIADNAAQLIGKTPMVYLVNNUVKGCVASVAAKLEIMEFCC	113
2-C. reinhardtii	30	LEPRKRQTGGTVRQGVLDLIGNTPLVRVASLSEETGCEIIVWKAEMLNPGG	79
B-A. thaliana	114	SVKDRIGYSMITDAEEKGLITPGKSVLVESTSGNTGIGLAFIAASKGYKL	163
2-C. reinhardtii	80	SVKDRVALQIVSEALADGRLRPG-GLITEGTAGSTGVSLAMVAAAYGCR	128
B-A. thaliana	164	ILTMPASMSLERRVLLRAFGAELVLTPEAKGM--TGAIQKABEILKKT	211
2-C. reinhardtii	129	SITMPDDAAIEKANMIQAYGASVRRVRFVSIHVHPEHPVNVARREAASTPG	178
B-A. thaliana	212	SYMLQQFDNPNFPIHYETTGPEIWDTRGKIDILVAGIGTGGTITGVGR	261
2-C. reinhardtii	179	ALFADQFENEANFRAHLKT-GEEIQQQTQGRVHAFVSGAGTGGTVAGVST	227
B-A. thaliana	262	FIKERKPELVIGVEPTESAILSSGGKPG-----PHKI--Q	294
2-C. reinhardtii	228	ALKARNFRVRFVFLVDFPGSSLFNKKVGRVMTSEEAEGRKLNPFDTITE	277
B-A. thaliana	295	GIGAGFVFNKLDLAIVDEYIATSSSEAIETSKQLALQEGLLVGISSGAAA	344
2-C. reinhardtii	278	GIGINRLTANFNRALIDDAFRGTDREAVEMAAYLLRNEGLWVGSSAAMNC	327
B-A. thaliana	345	AAAIQVAKRPNAGKLIIVFVPSFGERYLS 374	
2-C. reinhardtii	328	VGAVKAA-RAMGPHTIVTLLCDGGHRHLS 356	
C-A. thaliana	110	GLNIADNVSQLIGKTPMVYLVNLSIAKGCVANIAAKLEIMEFCCSVKDRIGY	159
2-C. reinhardtii	38	GGTVRQGVLDLIGNTPLVRVASLSEETGCEIIVWKAEMLNPGGSVKDRVAL	87
C-A. thaliana	160	SMVTDAEQKGFISPGKSVLVEPTSGNTGIGLAFIAASRGVRLITMPASM	209
2-C. reinhardtii	88	QIVSEALADGRLRPG-GLITEGTAGSTGVSLAMVAAAYGCRCSITMPDDA	136
C-A. thaliana	210	SMERRVLLKAFGAELVLTDPKAGM--TGAVQKABEILKNTFDAYMLQQFD	257
2-C. reinhardtii	137	AIEKANMIQAYGASVRRVRFVSIHVHPEHPVNVARREAASTPGALFADQFE	186
C-A. thaliana	258	NPNFPIHYETTGPEIWDTRGKIDILVAGIGTGGTITGVGRFIEKKNPK	307
2-C. reinhardtii	187	NEANFRAHLKT-GEEIQQQTQGRVHAFVSGAGTGGTVAGVSTALKARNFR	235
C-A. thaliana	308	TQVIGVEPTESDILSSGGKPG-----PHKI--QGIGAGFIP	340
2-C. reinhardtii	236	VRVFLVDFPGSSLFNKKVGRVMTSEEAEGRKLNPFDTITEGIGINRLT	285
C-A. thaliana	341	KNLDQKIMDEVIATSSSEAIETAKQLALKEGLLVGSSGAAAAAIKVAK	390
2-C. reinhardtii	286	ANFNRALIDDAFRGTDREAVEMAAYLLRNEGLWVGSSAAMNCVGAVKAA-	334
C-A. thaliana	391	RPNAGKLIIVFVPSFGERYLS 412	
2-C. reinhardtii	335	RAMGPHTIVTLLCDGGHRHLS 356	
Cl-A. thaliana	56	LIGKTPLVFLNKVTEGCEAYVAAKQEHFQPTCSIKDRPAIAMIADAEXKK	105
2-C. reinhardtii	48	LIGNTPLVRVASLSEETGCEIIVWKAEMLNPGGSVKDRVALQIVSEALADG	97
Cl-A. thaliana	106	LIIPGKTTLIEPTSGNMGISLAFMAAMKGVRIIMTTPSYTSLERRVTMRS	155
2-C. reinhardtii	98	RLRPGGL-ITEGTAGSTGVSLAMVAAAYGCRCSITMPDDAAIEKANMIQA	146
Cl-A. thaliana	156	FGAELVLTDPKAGMGGT--VKKAYDLLDSTPDAFMCCQFANFANTQIHFD	203
2-C. reinhardtii	147	YGASVRRVRFVSIHVHPEHPVNVARREAASTPGALFADQFENEANFRAHLK	196
Cl-A. thaliana	204	TGPEIWDTRGKIDILVAGIGTGGTITGVGRVLSKPNVVIYGVPEAE	253
2-C. reinhardtii	197	T-GEEIQQQTQGRVHAFVSGAGTGGTVAGVSTALKARNFRVRFVFLVDFPG	245
Cl-A. thaliana	254	SNILNGGKPG-----PHAITGNVGVFKPEILDMD--VMES	286
2-C. reinhardtii	246	SSLFNKKVGRVMTSEEAEGRKLNPFDTITEGIGINRLTANFNRALIDD	295
Cl-A. thaliana	287	VLEVSEDAIKMARELALKEGLLVGSSGANTVAAIRLAKMPENKGLIV	336
2-C. reinhardtii	296	AFRGTDREAVEMAAYLLRNEGLWVGSSAAMNCVGAVKAAARAM-GPHTIV	344
Cl-A. thaliana	337	TIHASFGERYLS 348	
2-C. reinhardtii	345	TLLCDGGHRHLS 356	

Fig. 7-35. Alignment of 2-C. reinhardtii OAS-TL amino acid with A. thaliana isoforms.



A-A. thaliana	4	RIAKDVTELGNTPLVYLNVAEGCVGRVAAKLEMMPECCSSVKDRIGFSM	53
3-C. reinhardtii	54	KICKDVTEVIGNTPMVYLNVRTRGCVAKVAAKLEIMQPCSSVKDRIGRNM	103
A-A. thaliana	54	ISDAEKKGLIKPGESVLIPTSGNTGVGLAFTAAAKGYKLIITMPASMS	103
3-C. reinhardtii	104	IEDAEKRGMIKPGVTTLVEPTSGNTGIGLAFTAAARGYKLIITMPASMSL	153
A-A. thaliana	104	ERRILLAFGVELVLTDPAKGMKGAIAKAEELAKTPNGVMLQQFENPAN	153
3-C. reinhardtii	154	ERRVLLRAFGAELVLTDPAKGMRGAVEKCNETIAAKTPNSYILQQFENPAN	203
A-A. thaliana	154	PKIHVETTGPEIWKGTGGKIDGFVSGIGTGGTITGAGKYLKEQANVKLY	203
3-C. reinhardtii	204	PEIHRLTTGPEIWRDTAGTVDILVAGVGTGGTITGTEFLKSKKPSLQVI	253
A-A. thaliana	204	GVFVPSAIISSGGKPGPHKIQQIGAGFIPSVLNVLDIDEVVQVSSDESID	253
3-C. reinhardtii	254	AVFVPSFVLSGGKPGPHKIQQIGAGFVPAIINTVYDEVIKIPSDDAVR	303
A-A. thaliana	254	MARQLALKEGLLVGISSGAAAAAIAKLAQRPNAGKLFVAIFPSFGERYL	303
3-C. reinhardtii	304	MASRLAVEEGLFCGISSGAAVLAAVQVAARPENAGKLVAVVLPFSFGERYL	353
A-A. thaliana	304	STVLFDAATRKEAEM 318	
3-C. reinhardtii	354	SSVLFNDRLECEQL 368	
B-A. thaliana	46	AFTLKRQSRSDVVCVAVSIKPEAGVEGLNIADNAAQLIGKTFMVYLNNVV	95
3-C. reinhardtii	27	ARVVRAQAAAAAANSDEPKY-VKNDKICKDVTEVIGNTPMVYLNVRV	75
B-A. thaliana	96	KGCVASVAAKLEIMEPCSSVKDRIGYSMITDAEEKGLITPGKSVLVESTS	145
3-C. reinhardtii	76	RGCVAKVAKLEIMQPCSSVKDRIGRNMIEDAEKRGMIKPGVTTLVEPTS	125
B-A. thaliana	146	GNTGIGLAFIAASKGYKLIITMPASMSLERRVLLRAFGAELVLTDPAKGM	195
3-C. reinhardtii	126	GNTGIGLAFTAAARGYKLIITMPASMSLERRVLLRAFGAELVLTDPAKGM	175
B-A. thaliana	196	TGAIQKABEILKKTTPNSYMLQQFDNPNANPKIHYETTGPEIWEDETRGKIDI	245
3-C. reinhardtii	176	RGAVEKCNETIAAKTPNSYILQQFENPANPEIHRLTTGPEIWRDTAGTVDI	225
B-A. thaliana	246	LVAGIGTGGTITGVGRFIKERKPELVIGVEPTESAILSSGGKPGPHKIQQ	295
3-C. reinhardtii	226	LVAGVGTGGTITGTEFLKSKKPSLQVIAVEPSESFVLSGGKPGPHKIQQ	275
B-A. thaliana	296	IGAGFVFNLDLAIIVDEYIAISSEEAETSKQLALQEGLLVGISSGAAA	345
3-C. reinhardtii	276	IGAGFVPAIINTVYDEVIKIPSDDAVRMASRLAVEEGLFCGISSGAAVL	325
B-A. thaliana	346	AAIQVAKRPNAGKLIIVVFPFSFGERVLSQFLQSIREECEQMQPE 391	
3-C. reinhardtii	326	AAVQVAARPENAGKLVAVVLPFSFGERVLSVLFNDRLECEQLKQD 371	
C-A. thaliana	113	IADNVSQILIGKTFMVYLNVAIAKGCVANIAAKLEIMEPCSSVKDRIGYSMV	162
3-C. reinhardtii	55	ICKDVTEVIGNTPMVYLNVRTRGCVAKVAAKLEIMQPCSSVKDRIGRNM	104
C-A. thaliana	163	TDAEQKGFISPGKSVLVEPTSGNTGIGLAFIAASRGYKLIITMPASMSME	212
3-C. reinhardtii	105	EDAEKRGMIKPGVTTLVEPTSGNTGIGLAFTAAARGYKLIITMPASMSLE	154
C-A. thaliana	213	RRVLLKAFGAELVLTDPAKGMTGAVQKABEILKNTFPDAMYLQQFDNPNANP	262
3-C. reinhardtii	155	RRVLLRAFGAELVLTDPAKGMRGAVEKCNETIAAKTPNSYILQQFENPANP	204
C-A. thaliana	263	KIHYETTGPEIWDITKGVDFVAVIGTGGTITGVGRFIKERKPNKTPQVIG	312
3-C. reinhardtii	205	EIHRLTTGPEIWRDTAGTVDILVAGVGTGGTITGTEFLKSKKPSLQVIA	254
C-A. thaliana	313	VEPTESDILSSGGKPGPHKIQQIGAGFIPKNDLQKIMDEVIASSEEAET	362
3-C. reinhardtii	255	VFPSESFVLSGGKPGPHKIQQIGAGFVPAIINTVYDEVIKIPSDDAVRM	304
C-A. thaliana	363	AKQLALKEGLMVGISSGAAAAAIAKVAKRNAGKLIIVVFPFSFGERYLS	412
3-C. reinhardtii	305	ASRLAVEEGLFCGISSGAAVLAAVQVAARPENAGKLVAVVLPFSFGERYLS	354
C-A. thaliana	413	TPLFQSIREEVEKM-QPERV 431	
3-C. reinhardtii	355	SVLFNDRLECEQLKQDERV 374	
C1-A. thaliana	39	DLPKDFPSTNAKRDAELLIIGKTFPLVFLNKVTEGCEAYVAAKQEHFQPTCS	88
3-C. reinhardtii	45	DEPKYVKNKICKDVTEVIGNTPMVYLNVRTRGCVAKVAAKLEIMQPCSS	94
C1-A. thaliana	89	IKDRPAIAMIADAEEKKLIIFGKTTLIEPTSGNMGISLAFMAAMKGYRII	138
3-C. reinhardtii	95	VKDRIGRNMIEDAEKRGMIKPGVTTLVEPTSGNTGIGLAFTAAARGYKLI	144
C1-A. thaliana	139	MTMPSYTSLERRVTRMSFGAELVLTDPAKGMGGTVKKAAYDLDDSTPDAPM	188
3-C. reinhardtii	145	LTPASMSLERRVLLRAFGAELVLTDPAKGMRGAVEKCNETIAAKTPNSYI	194
C1-A. thaliana	189	CQQFANPANTQIHFDTTGPEIWEDETLGNVDIFVMGIGSGGTVSGVGRVLY	238
3-C. reinhardtii	195	LQQFENPANPEIHRLTTGPEIWRDTAGTVDILVAGVGTGGTITGTEFLK	244
C1-A. thaliana	239	SKNPNVKIYGVPAESNINLGGKPGPHAITGNVGVGFKEILDMDVMESVL	288
3-C. reinhardtii	245	SKKPSLQVIAVEPSESFVLSGGKPGPHKIQQIGAGFVPAIINTVYDEVI	294
C1-A. thaliana	289	EVSSDAIKMARELALKEGLMVGISSGANTVAAIRLAKMPENKGLIVTI	338
3-C. reinhardtii	295	KIPSDDAVRMASRLAVEEGLFCGISSGAAVLAAVQVAARPENAGKLVAVV	344
C1-A. thaliana	339	HASFGERYLSSVLFDELKAEEMK 363	
3-C. reinhardtii	345	LPSFGERYLSVLFNDRLECEQLK 369	

Fig. 7-36. Alignment of 3-C. reinhardtii OAS-TL amino acid with A. thaliana isoforms.

A-A. thaliana	5	I AKDVT ELIGN TPLVYLNNVAEGCVGRVAAKLEMMEPCCSVKDRIGFSMI	54
4-C. reinhardtii	40	IATDVT ELIGKTPMVYLNKVVATGTHAKIAAKLEIMEPCCSVKDRIGVSMI	89
A-A. thaliana	55	SDAEKKGLIKPGESVLI EPTSGNTGVGLAFTAAAKGYKLIITMPASMS TE	104
4-C. reinhardtii	90	SSAEKEGLITPGKTVLVEPTSGNTGIGLAFIAAARGYKLIITMPASMSLE	139
A-A. thaliana	105	RRILLAFGVELVLTDPAKGMKGAIKAEELAKTPNGYMLQQFENPANP	154
4-C. reinhardtii	140	RRILLRAFGAELVLTDPAKGMKGAVAKAEELASTPDAFMLQQFQNPNNP	189
A-A. thaliana	155	KIHYETTGP EIWKGTGGKIDG FVSGIGTGGTITGAGKYLKEQANVKLYG	204
4-C. reinhardtii	190	KVHYETTGP EIWSATDGGKVDILVSGVGTGGTITGTGRYLREKKS DVQLVA	239
A-A. thaliana	205	VEPVESAILSGGKPGPHKI QGIGAGFIPSVLNVLDLIDEVVQVSSDESIDM	254
4-C. reinhardtii	240	VEPAESPVLSSGGKPGPHKI QGIGAGFVPAVLDTALISEVVQVSSDDAIDM	289
A-A. thaliana	255	ARQLALKEGLLVGISSGAAAAA I KLAQRPENAGKLFVAIFPSFGERYLS	304
4-C. reinhardtii	290	ARRLAL E EGLMVGISSGAAVQAAIKVASRPENEGKLVVVVLP SFGERYLS	339
A-A. thaliana	305	TVLFDATRKEAEAMTFE 321	
4-C. reinhardtii	340	SVLFQQLRDEASKMTFE 356	
B-A. thaliana	51	RQSRSDVVC KAVSIKPEAGVEGLNIADNAAQLIGKTPMVYLN NVKGCVA	100
4-C. reinhardtii	17	RVSRVALVPKAVAAPEKAAVK-MNIATDVTE LIGKTPMVYLNKVVATGTHA	65
B-A. thaliana	101	SVAAKLEIMEPCCSVKDRIGYSMITDAEEKGLITPGKSVLVESTSGNTGI	150
4-C. reinhardtii	66	KIAAKLEIMEPCCSVKDRIGYSMISSAEKEGLITPGKTVLVEPTSGNTGI	115
B-A. thaliana	151	GLAFIAASKGYKLIITMPASMSLERRVLLRAFGAELVLT E PAKGMTGAIQ	200
4-C. reinhardtii	116	GLAFIAAARGYKLIITMPASMSLERRILLRAFGAELVLTDPAKGMKGAVA	165
B-A. thaliana	201	KAEELKKT P NSYMLQQFDN PANPKIHYETTGP EIWEDTRGKIDILVAGI	250
4-C. reinhardtii	166	KAEELASTPDAFMLQQFQNPNNPKVHYETTGP EIWSATDGGKVDILVSGV	215
B-A. thaliana	251	GTGGTITGVGRF IKERKPELVKIGVEPTESAILSGGKPGPHKI QGIGAGF	300
4-C. reinhardtii	216	GTGGTITGTGRYLREKKS DVQLVAVEPAESPVLSSGGKPGPHKI QGIGAGF	265
B-A. thaliana	301	VFNLDLAI VDEYIAISSEBAIETS KQLALQEGLLVGISSGAAAAA IQV	350
4-C. reinhardtii	266	VPAVLDTALISEVVQVSSDDAIDMARRLAL E EGLMVGISSGAAVQAAIKV	315
B-A. thaliana	351	AKRPENAGKLI AVVFP SFGERYLSTQLFQSIREECEQM 388	
4-C. reinhardtii	316	ASRPENEGKLVVVVLP SFGERYLSSVLFQQLRDEASKM 353	
C-A. thaliana	111	LNIADNVSQLIGKTPMVYLN SIAKGCVANIAAKLEIMEPCCSVKDRIGYS	160
4-C. reinhardtii	38	MNIATDVTE LIGKTPMVYLNKVVATGTHAKIAAKLEIMEPCCSVKDRIGYS	87
C-A. thaliana	161	MVTDAEQKGFISPGKSVLVEPTSGNTGIGLAFIAASRGYRLITMPASMS	210
4-C. reinhardtii	88	MISSAEKEGLITPGKTVLVEPTSGNTGIGLAFIAAARGYKLIITMPASMS	137
C-A. thaliana	211	MERRVLLKAFGAELVLTDPAKGMTGAVQKAEELKNTPDAYMLQQFDNPA	260
4-C. reinhardtii	138	LERRILLRAFGAELVLTDPAKGMKGAVAKAEELASTPDAFMLQQFQNPNN	187
C-A. thaliana	261	NPKIHYETTGP EIWDDTKGKVDIFVAGIGTGGTITGVGRF IKERNPKTQV	310
4-C. reinhardtii	188	NPKVHYETTGP EIWSATDGGKVDILVSGVGTGGTITGTGRYLREKKS DVQL	237
C-A. thaliana	311	IGVEPTESDILSGGKPGPHKI QGIGAGFIPKNLDQKIMDEYIAISSEEA I	360
4-C. reinhardtii	238	VAVEPAESPVLSSGGKPGPHKI QGIGAGFVPAVLDTALISEVVQVSSDDA I	287
C-A. thaliana	361	ETAKQLALKEGLMVGISSGAAAAA I KVAKR PENAGKLI AVVFP SFGERY	410
4-C. reinhardtii	288	DMARRLAL E EGLMVGISSGAAVQAAIKVASRPENEGKLVVVVLP SFGERY	337
C-A. thaliana	411	LSTPLFQSIREVEKM 426	
4-C. reinhardtii	338	LSSVLFQQLRDEASKM 353	
C1-A. thaliana	48	NAKRDA SLLIGKTPLVFLNKVTEGCEAYVAAKQEHFQPTCSIKDRPAIAM	97
4-C. reinhardtii	39	NIATDVTE LIGKTPMVYLNKVVATGTHAKIAAKLEIMEPCCSVKDRIGYSM	86
C1-A. thaliana	98	IADA EKKKLIIPGKTTLIEPTSGNMGISLAFMAAMKGYRIIMT P SYTSL	147
4-C. reinhardtii	89	ISSAEKEGLITPGKTVLVEPTSGNTGIGLAFIAAARGYKLIITMPASMSL	138
C1-A. thaliana	148	ERRVTMR SFGAELVLTDPAKGMGGTVKKAYDLDLSTPDAFMCQQFANPAN	197
4-C. reinhardtii	139	ERRILLRAFGAELVLTDPAKGMKGAVAKAEELASTPDAFMLQQFQNPNN	188
C1-A. thaliana	198	TQIHFDTTGP EIWEDTLGNVDIFVMGIGSGGTVSGVGRYLKSKNPNVKIY	247
4-C. reinhardtii	189	PKVHYETTGP EIWSATDGGKVDILVSGVGTGGTITGTGRYLREKKS DVQLV	238
C1-A. thaliana	248	GVEPAESN I LGGKPGPHAITGNVGFKPEILDMDVMESVLEVSSEDAIK	297
4-C. reinhardtii	239	AVEPAESPVLSSGGKPGPHKI QGIGAGFVPAVLDTALISEVVQVSSDDAID	288
C1-A. thaliana	298	MARELALKEGLMVGISSGANTVAAIRLAKMPENKGLIVTIHASFGERYL	347
4-C. reinhardtii	289	MARRLAL E EGLMVGISSGAAVQAAIKVASRPENEGKLVVVVLP SFGERYL	338
C1-A. thaliana	348	SSVLFDEL RKEAEM 362	
4-C. reinhardtii	339	SSVLFQQLRDEASKM 353	

Fig. 7-37. Alignment of 4-C. reinhardtii OAS-TL amino acid with A. thaliana isoforms.



A-A.thaliana	11	ELIGNTFLVYLNVAEGCVGRVAAKLEMMPECCSVKDRIGFSMISDAEKK	60
1-T.suecica	6	ELIGDTPLVDSLFLSAKPGVKIFGKAEFFNPSGSIKDRIANHIISCAEAE	55
A-A.thaliana	61	GLIKPGESVLIPTSGNTGVGLAFTAAAKGYKLIITMPASMTERRILL	110
1-T.suecica	56	GKLRPGGTV-VAATSGNTGSAIAMVCAMRGYKVIITNEKTSKEKRDMSA	104
A-A.thaliana	111	AFGVELVLT---PAKGMKGAIAKAEIILAKTPNGYMLQQFENPANPKIH	157
1-T.suecica	105	SYGGQVLVGGMPADHPLHYQNMVAVTLCKENPDYFDVDQYDNPRNPEAY	154
A-A.thaliana	158	YETTGPFIWKTGGKIDGFVSGIGTGGTITGAGKYLKEQNAVVKLYGVEP	207
1-T.suecica	155	YLTGPEIWEQTQGAVTHFVAGGSTGGTISGTGKYLKEMNPAVRVCMFDP	204
A-A.thaliana	208	VESAILSGGK-----PGPHKIQQIGAGFIPSVLNVLDLIDEVQVSS	248
1-T.suecica	205	KGSVFDWYKQVPEAEKLPSSYQVEGVGKDSIPTAMNFGVVDLQDLC	254
A-A.thaliana	249	DESIDMARQLALKEGLLVGISSGAAAAAIAKLAQRPENAGKLVVAIFPFSF	298
1-T.suecica	255	KQSFAMCRRVAEEDGMLLGGSSGLNLSAAAELSQTAPD-GSVIVAVLPDS	303
A-A.thaliana	299	GERYLSTVLFD 309	
1-T.suecica	304	GVKYLKIFND 314	
B-A.thaliana	81	QLIGKTFMVYLNVAEGCVGRVAAKLEIMEPCCSVKDRIGYSMTDAEEK	130
1-T.suecica	6	ELIGDTPLVDSLFLSAKPGVKIFGKAEFFNPSGSIKDRIANHIISCAEAE	55
B-A.thaliana	131	GLITPGKSVLVESTSGNTGIGLAFIAASKGYKLIITMPASMSLERRVLLR	180
1-T.suecica	56	GKLRPGGTV-VAATSGNTGSAIAMVCAMRGYKVIITNEKTSKEKRDMSA	104
B-A.thaliana	181	AFGAELVLTE---PAKGMTGAIQKAEIILKTPNSYMLQQFDNPNANPKIH	227
1-T.suecica	105	SYGGQVLVGGMPADHPLHYQNMVAVTLCKENPDYFDVDQYDNPRNPEAY	154
B-A.thaliana	228	YETTGPFIWEDTRGKIDILVAGIGTGGTITGVGRFIERKPELKVIGVEP	277
1-T.suecica	155	YLTGPEIWEQTQGAVTHFVAGGSTGGTISGTGKYLKEMNPAVRVCMFDP	204
B-A.thaliana	278	TESAILSGGK-----PGPHKIQQIGAGFIPKLNLDLAIVDEYIAISS	318
1-T.suecica	205	KGSVFDWYKQVPEAEKLPSSYQVEGVGKDSIPTAMNFGVVDLQDLC	254
B-A.thaliana	319	EEAIEETSKQLALQEGLLVGISSGAAAAAIAQVAKRFPENAGKLIIVFPFSF	368
1-T.suecica	255	KQSFAMCRRVAEEDGMLLGGSSGLNLSAAAELSQTAPD-GSVIVAVLPDS	303
B-A.thaliana	369	GERYLS 374	
1-T.suecica	304	GVKYLK 309	
C-A.thaliana	119	QLIGKTFMVYLNVAEGCVGRVAAKLEIMEPCCSVKDRIGYSMTDAEQK	168
1-T.suecica	6	ELIGDTPLVDSLFLSAKPGVKIFGKAEFFNPSGSIKDRIANHIISCAEAE	55
C-A.thaliana	169	GFISPGKSVLVEPTSGNTGIGLAFIAASRGYRLIITMPASMSMERRVLLK	218
1-T.suecica	56	GKLRPGGTV-VAATSGNTGSAIAMVCAMRGYKVIITNEKTSKEKRDMSA	104
C-A.thaliana	219	AFGAELVLTE---PAKGMTGAVQKAEIILKNTPDAYMLQQFDNPNANPKIH	265
1-T.suecica	105	SYGGQVLVGGMPADHPLHYQNMVAVTLCKENPDYFDVDQYDNPRNPEAY	154
C-A.thaliana	266	YETTGPFIWDDTKGKVDIFVAGIGTGGTITGVGRFIERKPELKVIGVEP	314
1-T.suecica	155	YLTGPEIWEQTQGAVTHFVAGGSTGGTISGTGKYLKEMNPAVRVCMFDP	204
C-A.thaliana	316	TES---DILSGG-----KPGPHKIQQIGAGFIPKLNLDQKIMDEYIAISS	356
1-T.suecica	205	KGSVFDWYKQVPEAEKLPSSYQVEGVGKDSIPTAMNFGVVDLQDLC	254
C-A.thaliana	357	EEAIEETAKQLALKEGLMVGISSGAAAAAIAKVAKRFENAGKLIIVFPFSF	406
1-T.suecica	255	KQSFAMCRRVAEEDGMLLGGSSGLNLSAAAELSQTAPD-GSVIVAVLPDS	303
C-A.thaliana	407	GERYLS 412	
1-T.suecica	304	GVKYLK 309	
Cl-A.thaliana	56	LIGKTFPLVFLNKVTEGCEAYVAAKQEHFQPTCSIKDRPAIAMIADAEEKK	105
1-T.suecica	7	LIGDTPLVDSLFLSAKPGVKIFGKAEFFNPSGSIKDRIANHIISCAEAE	56
Cl-A.thaliana	106	LIIPGKTTLIEPTSGNMGISLAFMAAMKGYRIITMPFSYTSLERRVTMRS	155
1-T.suecica	57	KLRPGGT-VVAATSGNTGSAIAMVCAMRGYKVIITNEKTSKEKRDMSAS	105
Cl-A.thaliana	156	FGAELVLT---PAKGMGTGKAYDLSDTDFDAFMQQFANPANTQIHF	202
1-T.suecica	106	YGGQVLVGGMPADHPLHYQNMVAVTLCKENPDYFDVDQYDNPRNPEAY	155
Cl-A.thaliana	203	DTTGPFIWEDTLGNVDIFVMGIGSGGTVSGVGRVLYKSKNPNVKIYGVPEA	252
1-T.suecica	156	LTLGPEIWEQTQGAVTHFVAGGSTGGTISGTGKYLKEMNPAVRVCMFDPK	205
Cl-A.thaliana	253	ES---NILNGG-----KPGPHAITGNGVGFKPEIILDMVMSVLEVSSE	293
1-T.suecica	206	GSVFDWYKQVPEAEKLPSSYQVEGVGKDSIPTAMNFGVVDLQDLC	255
Cl-A.thaliana	294	DAIKMARELALKEGLMVGISSGANTVAATRLAKMPENKGLIVTIHASF	343
1-T.suecica	256	QSFAMCRRVAEEDGMLLGGSSGLNLSAAAELSQTAPD-GSVIVAVLPDSG	304
Cl-A.thaliana	344	ERYLSSVLFDELK 358	
1-T.suecica	305	VKYLKIFNDEMME 319	

Fig. 7-38. Alignment of 1-*T. suecica* OAS-TL amino acid with *A. thaliana* isoforms.

A-A.thaliana	5	IAKDVT	ELIGNTPLVYLN	NVAEGCVGRVAAKLEMM	PCSSVKDRIGFSMI	54								
2-T.suecica	14	IAESIV	DLVGNTPLVYLN	NKVTAGSGARIAAKMES	MEPSCSVKDRIGKNI	63								
A-A.thaliana	55	SDAEK	KGLIKPGESV	LIEPTSGNTGVGLA	FTAAAKGYKLIITMPASMSTE	104								
2-T.suecica	64	EDAEK	KAGKITPGV	TLVEPTSGNTGIGLAF	VAAASKGYKLILTMPASMSLE	113								
A-A.thaliana	105	RRII	LAFVVELVLTDP	PAKGMKGAIKABE	ILAKTPNGYMLQQFENPANP	154								
2-T.suecica	114	RRVLL	QAFGATLVLTDP	PAKGMGGAVKABE	IAAATDSSYVLQQFENPANA	163								
A-A.thaliana	155	KIHV	ETGPEIWKGT	GGKIDGFSVSGIGT	GGTITGAGKYLKEQNAVVKLYG	204								
2-T.suecica	164	AVHRL	TGPEIFRDT	AGEVDILVAGIGT	GGTITGAGEYLVKSVKGGVQVVA	213								
A-A.thaliana	205	VEPV	SAIISGGK	PGPHKIQQIGAG	FIPSVLNVLDLDEVVQVSSDES	IDM 254								
2-T.suecica	214	VEPT	SPVLSGGQ	PGPHKIQQIGAG	FVPGVLNTHVYDEVVQISSDDA	ISM 263								
A-A.thaliana	255	ARQL	LALKEGLLVG	ISSGAAAAA	IKLAQRPENAGKLFVAIFP	SFGERYLS 304								
2-T.suecica	264	ARRL	AQEEGVL	CGISSGAAV	LAAIKVGRPENAGKLI	VVIIPSFGERYLS 313								
A-A.thaliana	305	TVL	FDA	TRKEAEM	318									
2-T.suecica	314	SAL	FAD	VQKECAGM	327									
B-A.thaliana	72	GLNI	ADNAAQLIG	KTPMVYLN	NVVRGCVASVAAKLEIME	PCSSVKDRIGY 121								
2-T.suecica	11	GTTI	AEISVDL	VGNTPLVYLN	NKVTAGSGARIAAKMES	MEPSCSVKDRIGK 60								
B-A.thaliana	122	SMIT	DAEBE	KLITPGKSV	LVEPTSGNTGIGLAF	IAASKGYKLIITMPASM 171								
2-T.suecica	61	NMIE	DAEKAG	KITPGV	TLVEPTSGNTGIGLAF	VAAASKGYKLILTMPASM 110								
B-A.thaliana	172	SLER	RVLRAF	GAEVLVTE	PAKGMTGAIQKABE	ILKKTNSYMLQQFDNF 221								
2-T.suecica	111	SLER	RVLQAF	GATLVLTDP	PAKGMGGAVKABE	IAAATDSSYVLQQFENP 160								
B-A.thaliana	222	ANPK	IHYETT	GPEI	WEDTRGKIDILVAG	IGTGGTITGVGRFIKERKPELK 271								
2-T.suecica	161	ANA	AVHRL	TGPEI	FRDTAGEVDILVAG	IGTGGTITGAGEYLVKSVKGGVQ 210								
B-A.thaliana	272	VIGV	PTESAIL	SGGKPGPH	KIQGIGAGFV	PKNLDLAI	VEYIAISSEEA 321							
2-T.suecica	211	VVA	VEPTES	PVLSGGQ	PGPHKIQQIGAG	FVPGVLNTHVYDEVVQISSDDA 260								
B-A.thaliana	322	IETSK	QALQEG	LLVGISSG	AAAAAAIQVAKR	PENAGKLI	AVVFPFGER 371							
2-T.suecica	261	ISM	ARRLAQ	EEGVL	CGISSGAAV	LAAIKVGRPENAGKLI	VVIIPSFGER 310							
B-A.thaliana	372	YLST	QLFQ	SIRE	EECEQM	388								
2-T.suecica	311	YLSS	ALFAD	VQKECAGM	327									
C-A.thaliana	100	EAVK	RETGPD	GLNIAD	NVSQLIGKTPMVYLN	IAKGCVANIAAKLEIMEP 149								
2-T.suecica	1	EPIA	KPARPT	GTTIAES	IVDLVGNTPLVYLN	NKVTAGSGARIAAKMESMEP 50								
C-A.thaliana	150	CCSV	KDRIGYS	MVTD	AEQKGFISPGKSV	LVEPTSGNTGIGLAF	IAASRGY 199							
2-T.suecica	51	SCSV	KDRIGK	NMIE	DAEKAGKITPGV	TLVEPTSGNTGIGLAF	VAAASKGY 100							
C-A.thaliana	200	RLIL	MPASMS	MERRV	LLKAFGAE	LVLTDP	PAKGMTGAVQKABE	ILKNTPD 249						
2-T.suecica	101	KLIL	MPASMS	LERRV	LQAFGATLVLTDP	PAKGMGGAVKABE	IAAATD 150							
C-A.thaliana	250	AYML	QQFDN	PANPKI	HYETT	GPEI	WDDTKGKVDIFVAG	IGTGGTITGVGR 299						
2-T.suecica	151	SYV	LQQFEN	PANA	AVHRL	TGPEI	FRDTAGEVDILVAG	IGTGGTITGAGE 200						
C-A.thaliana	300	FIKE	KPKTQ	VIGV	PTESDILSGGK	PGPHKIQQIGAG	FIPKNDQKIMD 349							
2-T.suecica	201	YLV	SVKGGV	QVVA	VEPTES	PVLSGGQ	PGPHKIQQIGAG	FVPGVLNTHVYD 250						
C-A.thaliana	350	EVIA	ISSEEA	IETAK	QLALKEGL	LMVGISSG	AAAAAAIKVAKR	PENAGKLI 399						
2-T.suecica	251	EVV	QISSDDA	ISMARR	LAQEEG	VLCGISSG	AAVLA	IKVGRPENAGKLI 300						
C-A.thaliana	400	AVV	FPFGER	YLST	PLFQ	SIRE	EEVEKM	426						
2-T.suecica	301	VVII	IPFGER	YLSS	ALFAD	VQKECAGM	327							
C1-A.thaliana	56	LIG	KTPLV	FLNKVTE	GCEAVVAAKQ	EHFQPTCS	IKDRPA	IAMIADA	AEKKK 105					
2-T.suecica	21	LVG	NTPLV	YLNKV	TAGSGARIAAK	MESMEP	SCSVKDRIG	KNMIED	AEKAG 70					
C1-A.thaliana	106	LII	PGKTT	LIEPT	SGNMGISLAF	MAAMKGYRI	IMT	MPYS	TSLERRV	TMS 155				
2-T.suecica	71	KIT	PGV	TLVEPT	SGNTGIGLAF	VAAASKGYKLI	IL	MPASMS	LERRV	LQA 120				
C1-A.thaliana	156	FGA	ELVLT	DP	PAKMG	GT	VVKAYD	LLD	STPD	AFMCQQFANPANTQIHFDFT 205				
2-T.suecica	121	FGA	TLVLT	DP	PAKMG	GGAVKABE	IAAATD	SSYVL	QQFENPANA	AVHRLTT 170				
C1-A.thaliana	206	GPEI	WED	TLGN	VDIFVM	GISGGT	VSGV	RYLKS	KNPNVKI	YGV	PAESN 255			
2-T.suecica	171	GPEI	FRD	TAGE	VDILVAG	IGTGGT	ITGAGE	YLVKSVKGGV	QVVA	VEPTESF 220				
C1-A.thaliana	256	ILNG	GKPG	PHAIT	GN	GVG	FKPEI	LD	M	VMESV	LEVSS	EDAIK	MAREL	LALK 305
2-T.suecica	221	VLS	GGQ	PGPHKI	QQIGAG	FVPGV	LNTHVY	DEVVQ	ISSDDA	ISMARR	LAQE 270			
C1-A.thaliana	306	EGL	MVGI	SSGANT	VAAIR	LAK	MPENK	GKLI	VTHAS	FGER	YLS	SVL	FDEL 355	
2-T.suecica	271	EGV	L	CGISSG	AAVLA	IKVGR	PENAGKLI	VVIIP	SFGER	YLSS	ALFADV 320			
C1-A.thaliana	356	RKEA	EEM	362										
2-T.suecica	321	QKECAGM	327											

Fig. 7-39. Alignment of 2-*T. suecica* OAS-TL amino acid with *A. thaliana* isoforms.



A-A.thaliana	4	RIAKDVTELGNTPLVYLNNVAEGCVGRVAAKLEMMPECCSSVKDRIGFSM	53
3-T.suecica	2	RVHRSVLDLAIAGNTPLIRINSLSDATGCEILGKAEFLNPGGSVKDRVALQI	51
A-A.thaliana	54	ISDAEKKGLIKPGESVLEIPTSNTGVGLAFTAAAKGYKLIITMPASMST	103
3-T.suecica	52	VTEALADGRKLPN-GLVTEGTVGSTGVSLAMVTAALGCRCHVMPDDAAI	100
A-A.thaliana	104	ERRIILLAFGVLELVTDPK--GMKGATAKAEIILAKTPNGYMLQQFENP	151
3-T.suecica	101	EKSQVLEALGATVQRVRFVSIHPDFVNIARRRAEEAGAIIFSDQFENL	150
A-A.thaliana	152	ANPKIHVETTGPFIWKGTTGGKIDGFVSGIGTGGTITGAGKYLKEQNAVVK	201
3-T.suecica	151	ANMRVHLKT-GQEIWDATAGRVDAFVSGAGTGGTIAGVSQLKARKKPSVR	199
A-A.thaliana	202	LYGVEPVESAI 212	
3-T.suecica	200	VFLADPPGSSL 210	
B-A.thaliana	83	IGKTFMVYLNNVKGCVASVAAKLEIMEPCCSVKDRIGYSMITDAEEKGL	132
3-T.suecica	11	IGNTPILIRINSLSDATGCEILGKAEFLNPGGSVKDRVALQIVTEALADGR	60
B-A.thaliana	133	ITPGKSVLVESTSGNTGIGLAFIAASKGYKLIITMPASMSLERRVLLRAF	182
3-T.suecica	61	LKPN-GLVTEGTVGSTGVSLAMVTAALGCRCHVMPDDAAIEKSQLLEAL	109
B-A.thaliana	183	GAELVLTPEK--GMTGAIQKABEILKKTTPNSYMLQQFDNPNPKIHYET	230
3-T.suecica	110	GATVQRVRFVSIHPDFVNIARRRAEEAGAIIFSDQFENLANMRVHLKT	159
B-A.thaliana	231	TGPEIWEEDTRGKIDILVAGIGTGGTITGVGRFIERKPELKVIGVEPTES	280
3-T.suecica	160	-GQEIWDATAGRVDAFVSGAGTGGTIAGVSQLKARKKPSVRVFLADPPGS	208
B-A.thaliana	281	AI 282	
3-T.suecica	209	SL 210	
C-A.thaliana	116	NVSQQLIGKTFMVYLNNSIAKGCVANIAAKLEIMEPCCSVKDRIGYSMTDA	165
3-T.suecica	6	SVLDAIGNTPLIRINSLSDATGCEILGKAEFLNPGGSVKDRVALQIVTEA	55
C-A.thaliana	166	EQKGFISPGKSVLVEPTSGNTGIGLAFIAASRGYRLIITMPASMSMERRY	215
3-T.suecica	56	LADGRKLPN-GLVTEGTVGSTGVSLAMVTAALGCRCHVMPDDAAIEKSQ	104
C-A.thaliana	216	LLKAFGAELVLTDPK--GMTGAVQKABEILKNTPDAYMLQQFDNPNPK	263
3-T.suecica	105	VLEALGATVQRVRFVSIHPDFVNIARRRAEEAGAIIFSDQFENLANMR	154
C-A.thaliana	264	IHYETTGPFIWDDTKGKVIDIFVAGIGTGGTITGVGRFIERKPKTKVIGV	313
3-T.suecica	155	VHLKT-GQEIWDATAGRVDAFVSGAGTGGTIAGVSQLKARKKPSVRVFLA	203
C-A.thaliana	314	EPTESDILSGGKPG-----PHK--IQGIGAGFIPKNDLQK	346
3-T.suecica	204	DPPGSSLYNKVQRGVLYTREEAEGKRLRNPYDTIVEGMGLNRLTANFGQA	253
C-A.thaliana	347	IMDEVIAISSEEAIEETAKQLALKEGLMVGISSGAAAAAIIKVAKRPENAG	396
3-T.suecica	254	RIDGAYKSSDRESVEMAHFLMREGLFLGSSACVNCVGAUKAAL-DLGGP	302
C-A.thaliana	397	KLIAVVFPSFGERVLS 412	
3-T.suecica	303	HTVVTVLDCSGQRHLS 318	
C1-A.thaliana	57	IGKTPLVFLNKVTEGCEAYVAAKQEHFQPTCSIKDRPAIAMIADAEEKKL	106
3-T.suecica	11	IGNTPILIRINSLSDATGCEILGKAEFLNPGGSVKDRVALQIVTEALADGR	60
C1-A.thaliana	107	IIPGKTTLIEPTSGNMGISLAFMAAMKGYRIIMTPSYTSLERRVTMRSF	156
3-T.suecica	61	LKPNGL-VTEGTVGSTGVSLAMVTAALGCRCHVMPDDAAIEKSQLLEAL	109
C1-A.thaliana	157	GAELVLTDPK--GMGGTVKKAYDLDDSTPDAFMQQFANPANTQIHFDT	204
3-T.suecica	110	GATVQRVRFVSIHPDFVNIARRRAEEAGAIIFSDQFENLANMRVHLKT	159
C1-A.thaliana	205	TGPEIWEEDTLGNVDIFVMGIGSGGTVSGVGRYLKSKNPNVKIYGVPEAES	254
3-T.suecica	160	-GQEIWDATAGRVDAFVSGAGTGGTIAGVSQLKARKKPSVRVFLADPPGS	208
C1-A.thaliana	255	NILNGGKPG-----PHAITGNGVGFKPEILDMDV--MESV	287
3-T.suecica	209	SLYNKVQRGVLYTREEAEGKRLRNPYDTIVEGMGLNRLTANFGQARIDGA	258
C1-A.thaliana	288	LEVSSDAIKMARELALKEGLMVGISSGANIVAAIRLAKMPENKGLIVT	337
3-T.suecica	259	YKSSDRESVEMAHFLMREGLFLGSSACVNCVGAUKAALDLGGPHTVVT	307
C1-A.thaliana	338	IHASFGERVLS 348	
3-T.suecica	308	VLCDSGQRHLS 318	

Fig. 7-40. Alignment of 3-*T. suecica* OAS-TL amino acid with *A. thaliana* isoforms.



A- <i>A. thaliana</i>	11	ELIGNTPLVLYLNNVAEGCV---GRVAAKLEMMPEPCSSVKDRIGFSMISD	56
4- <i>T. suecica</i>	1	ETIGNTPLVRLERLPEEVRANGATILCKMEMQNPFGGSIKDRIAKSMIET	50
A- <i>A. thaliana</i>	57	AEKKGLIKPGESVLEIPTSNGTGVGLAFTAAAKGYKLIITMPASMS-TER	105
4- <i>T. suecica</i>	51	AEAEGLKPKGGTV-VEYTSNGTIGLAMVCAAKGYKCIIMPQLPFPQER	99
A- <i>A. thaliana</i>	106	RIILLAFGVLEVLTPAKGMKGAIAKAEELAKTPNGYMLQQFENPANPK	155
4- <i>T. suecica</i>	100	YTICRQFGAEVHLTAPAKGFPGLRAYTESLMAANPDYFLANQFYNQANPD	149
A- <i>A. thaliana</i>	156	IHYETTGPFIWKGTGGKIDGFVSGIGTGGTITGAGKYLKEQANVKLYGV	205
4- <i>T. suecica</i>	150	IHYATTGPEIWEQTGGKMDYFIAGVGTGGTVAGAGRFLTEKNPDIKVMVA	199
A- <i>A. thaliana</i>	206	EPVESAILSGGKPGPHKIQQIGAG---FIPSVL-NVDLID-----EV	243
4- <i>T. suecica</i>	200	EPTESRVHVGAQHAPHTILGIGPGVATHFLES LAPGAPLVEGPRGHVSEF	249
A- <i>A. thaliana</i>	244	VQVSSDESIDMARQLALKEGLLVGISSGAAAAAATKLAQRPENAGKLFVA	293
4- <i>T. suecica</i>	250	LHTNSSQAI EWAQRMAQMEGMMVGPSSGAVISAAAMAVAAPESAGKTFV	299
A- <i>A. thaliana</i>	294	IFPSFGERYLSTVLFDAATRKEA 315	
4- <i>T. suecica</i>	300	MCASHGIRYTAHPLWAEKDEA 321	
B- <i>A. thaliana</i>	81	QLIGKTPMVLNNV---VKGCVASVAAKLEIMEPCSSVKDRIGYSMID	126
4- <i>T. suecica</i>	1	ETIGNTPLVRLERLPEEVRANGATILCKMEMQNPFGGSIKDRIAKSMIET	50
B- <i>A. thaliana</i>	127	AEEKGLITPCKSVLVESTSGNTGIGLAFIAASKGYKLIITMPASMSL-ER	175
4- <i>T. suecica</i>	51	AEAEGLKPKGGTV-VEYTSNGTIGLAMVCAAKGYKCIIMPQLPFPQER	99
B- <i>A. thaliana</i>	176	RVLRAFAGAEVLVTEPAKGMTGAIQKAEILKKTFNYSYMLQQFDN PANPK	225
4- <i>T. suecica</i>	100	YTICRQFGAEVHLTAPAKGFPGLRAYTESLMAANPDYFLANQFYNQANPD	149
B- <i>A. thaliana</i>	226	IHYETTGPFIWEDTRGKIDILVAGIGTGGTITGVRFIKERKPELVKIVG	275
4- <i>T. suecica</i>	150	IHYATTGPEIWEQTGGKMDYFIAGVGTGGTVAGAGRFLTEKNPDIKVMVA	199
B- <i>A. thaliana</i>	276	EPTESAILS GGKPGPHKIQQIGAGFVFNKLDLAI-----VDEY	313
4- <i>T. suecica</i>	200	EPTESRVHVGAQHAPHTILGIGPGVATHFLES LAPGAPLVEGPRGHVSEF	249
B- <i>A. thaliana</i>	314	IAISSEEA IETSKQLALQEGLLVGISSGAAAAA IQVAKR PENAGKLI AV	363
4- <i>T. suecica</i>	250	LHTNSSQAI EWAQRMAQMEGMMVGPSSGAVISAAAMAVAAPESAGKTFV	299
B- <i>A. thaliana</i>	364	VFPSSFGERYLSTQLFQSIREECCQMFP 390	
4- <i>T. suecica</i>	300	MCASHGIRYTAHPLWAEKDEACRALP 326	
C- <i>A. thaliana</i>	138	ANIAAKLEIMEPCSSVKDRIGYSMVTDAEQKGFISPGKSVLVEPTSNGTG	187
4- <i>T. suecica</i>	24	ATILCKMEMQNPFGGSIKDRIAKSMIETAEAEGLKPKGGTV-VEYTSNGTG	72
C- <i>A. thaliana</i>	188	IGLAFIAASRGYRLIITMPASMSM-ERRVLLKAFGAEVLVTPAKGMTGA	236
4- <i>T. suecica</i>	73	IGLAMVCAAKGYKCIIMPQLPFPQERYTICRQFGAEVHLTAPAKGFPGL	122
C- <i>A. thaliana</i>	237	VQKAEIILKNTPDAYMLQQFDN PANPKIHYETTGPFIWDDTKGKVDIFVA	286
4- <i>T. suecica</i>	123	RAYTESLMAANPDYFLANQFYNQANPDIHYATTGPEIWEQTGGKMDYFIA	172
C- <i>A. thaliana</i>	287	GIGTGGTITGVRGFIKEKNPKTQVIGVEPTESDILSGGKPGPHKIQQIGA	336
4- <i>T. suecica</i>	173	GVGTGGTVAGAGRFLTEKNPDIKVMVAEPTESRVHVGAQHAPHTILGIGP	222
C- <i>A. thaliana</i>	337	GFIPKNDQKI-----MDEVIAISSEEA IETAKQLALKEGLMV	374
4- <i>T. suecica</i>	223	GVATHFLES LAPGAPLVEGPRGHVSEFLHTNSSQAI EWAQRMAQMEGMMV	272
C- <i>A. thaliana</i>	375	GISSGAAAAA IKVAKR PENAGKLI AVFPSSFGERYLSTPLFQSIREEVE	424
4- <i>T. suecica</i>	273	GPSSGAVISAAAMAVAAPESAGKTFVVMCASHGIRYTAHPLWAEKDEAC	322
C- <i>A. thaliana</i>	425	KMQPER 430	
4- <i>T. suecica</i>	323	RALPSQ 328	
Cl- <i>A. thaliana</i>	57	IGKTPLVFLNKV---TEGCEAYVAAKQEHFQPTCSIKDRPAIAMIADAE	102
4- <i>T. suecica</i>	3	IGNTPLVRLERLPEEVRANGATILCKMEMQNPFGGSIKDRIAKSMIETAE	52
Cl- <i>A. thaliana</i>	103	KKKLII PGKTTLEIPTSNGMGISLAFMAAMKGYRIIMTMSYTSLERRVT	152
4- <i>T. suecica</i>	53	AEGKLPKGGT-VVEYTSNGTIGLAMVCAAKGYKCIIMPQLPFPQERYT	101
Cl- <i>A. thaliana</i>	153	M-RSFGAEVLVTPAKGMGGTVKKAYDLLDSTPD AFMCQQFANPANTQIH	201
4- <i>T. suecica</i>	102	ICRQFGAEVHLTAPAKGFPGLRAYTESLMAANPDYFLANQFYNQANPDIH	151
Cl- <i>A. thaliana</i>	202	FDDTGPFIWEDTLGNVDIFVMGIGSGGTVSGVGRYLSKKNPNVKIYGVPE	251
4- <i>T. suecica</i>	152	YATTGPEIWEQTGGKMDYFIAGVGTGGTVAGAGRFLTEKNPDIKVMVAPE	201
Cl- <i>A. thaliana</i>	252	AESNII LGGKPGPHAITGNVGVFKPEILDMDV-----MESVLE	289
4- <i>T. suecica</i>	202	TESRVHVGAQHAPHTILGIGPGVATHFLES LAPGAPLVEGPRGHVSEFLH	251
Cl- <i>A. thaliana</i>	290	VSSEDA IAKMARELALKEGLMVGISSGANTVAAIRLAKMPENKGLIIVTII	339
4- <i>T. suecica</i>	252	TNSSQAI EWAQRMAQMEGMMVGPSSGAVISAAAMAVAAPESAGKTFVVMC	301
Cl- <i>A. thaliana</i>	340	ASFGERYLSVLFDELKREA 359	
4- <i>T. suecica</i>	302	ASHGIRYTAHPLWAEKDEA 321	

Fig. 7-41. Alignment of 4-*T. suecica* OAS-TL amino acid with *A. thaliana* isoforms.

### **7.3.6. Alignment of the OAS-TL sequence of red and red-lineage algae with *A. thaliana* OAS-TL isoforms**

A-A.thaliana	5	IADVTELGNTPLVYLNNVAE--GCVGRVAAKLEMEPCSSVKDRIGFS	52
1-C.merolae	67	LARDVSDLVGNTPIVELKKIPEEEGVQAHILCKLESMEPCSSVKDRIGKY	116
A-A.thaliana	53	MISDAEKKGLIKPGESVLIPTSGNTGVGLAFTAAAKGYKLIITMPASMS	102
1-C.merolae	117	MIVEAEKRGDIQPGKTVLIEPTSGNTGIALAYLAAARGVRLILTMPDSMS	166
A-A.thaliana	103	TERRIILLAFGVVELVLTDPAGMKGAIAKABEILAKTPNGYMLQQFENPA	152
1-C.merolae	167	IERRMVLRAFGEVVLTPAAKGMKGAVAKAEQLFHTTPNAYMLQQFNNPD	216
A-A.thaliana	153	NPKIHVETTGPEIWKGTGGKIDGFSVIGTGGTITGAGRYLKEQNANVKL	202
1-C.merolae	217	NPKAHVETTGPEIWAATGGKVDAFVAGVGTGGTITGAGRYLKEQNPHVYI	266
A-A.thaliana	203	YGVPEVESAILSGGKPGPHKIQQIGAGFIPSVLNVLDLDEVVQVSSDESI	252
1-C.merolae	267	MAVEPAESFVLSGGRPGPHKIQQIGAGFVPGILDTKIYNEVKQVTSMSDI	316
A-A.thaliana	253	DMARQLALKEGLLVGISSGAAAAAIAKLAQRPENAGKLFVAIFPSFGERY	302
1-C.merolae	317	EMARRLAVEEGLLGGISSGAAVVALELGRRPENMGKKNIVVVIIPSFGERY	366
A-A.thaliana	303	LSTVLFDATRKEAEM 318	
1-C.merolae	367	LTSALFDKQREEAYNM 382	
B-A.thaliana	75	IADNAAQLIGKTPMVYLNNVV--GCVASVAAKLEIMEPCSSVKDRIGFS	122
1-C.merolae	67	LARDVSDLVGNTPIVELKKIPEEEGVQAHILCKLESMEPCSSVKDRIGKY	116
B-A.thaliana	123	MITDAEKKGLITPGKSVLVESTSGNTGIGLAFIAASKGYKLIITMPASMS	172
1-C.merolae	117	MIVEAEKRGDIQPGKTVLIEPTSGNTGIALAYLAAARGVRLILTMPDSMS	166
B-A.thaliana	173	LERRVLLRAFGEVVLTEPAKGMTGAIQKABEILKKTTPNSYMLQQFDNPA	222
1-C.merolae	167	IERRMVLRAFGEVVLTPAAKGMKGAVAKAEQLFHTTPNAYMLQQFNNPD	216
B-A.thaliana	223	NPKIHVETTGPEIWEIDTRGKIDILVAGIGTGGTITGVGRFIERKPELKV	272
1-C.merolae	217	NPKAHVETTGPEIWAATGGKVDAFVAGVGTGGTITGAGRYLKEQNPHVYI	266
B-A.thaliana	273	IGVEPTESAILSGGKPGPHKIQQIGAGFVFNKLDLAIIVDEVIAISSEBAI	322
1-C.merolae	267	MAVEPAESFVLSGGRPGPHKIQQIGAGFVPGILDTKIYNEVKQVTSMSDI	316
B-A.thaliana	323	ETSKQLALQEGLLVGISSGAAAAAIAQVAKRPENAGKLIIVVFPFSGERY	372
1-C.merolae	317	EMARRLAVEEGLLGGISSGAAVVALELGRRPENMGKKNIVVVIIPSFGERY	366
B-A.thaliana	373	LSTQLFQSIREECEQM 388	
1-C.merolae	367	LTSALFDKQREEAYNM 382	
C-A.thaliana	106	TGPDGLNIADNVSQLIGKTPMVYLNNSIAK--GCVANIAAKLEIMEPCSSV	153
1-C.merolae	60	TAPFVALARDVSDLVGNTPIVELKKIPEEEGVQAHILCKLESMEPCSSV	109
C-A.thaliana	154	KDRIGYSMTDAEQKGFISPGKSVLVEPTSGNTGIGLAFIAASRGYRLIL	203
1-C.merolae	110	KDRIGKYMIVEAEKRGDIQPGKTVLIEPTSGNTGIALAYLAAARGVRLIL	159
C-A.thaliana	204	TMPASMSMERRVLLKAFGAEVLTDPAGMTGAVQKABEILKNTPDAYML	253
1-C.merolae	160	TMPDSMSIERRMVLRAFGEVVLTPAAKGMKGAVAKAEQLFHTTPNAYML	209
C-A.thaliana	254	QQFDNPNPKIHVETTGPEIWDITGKVDIFVAGIGTGGTITGVGRFIEKE	303
1-C.merolae	210	QQFNNPDNPKAHVETTGPEIWAATGGKVDAFVAGVGTGGTITGAGRYLRE	259
C-A.thaliana	304	KNPKTQVIGVEPTESDILSGGKPGPHKIQQIGAGFIPKNDLQKIMDEVIA	353
1-C.merolae	260	QNPVHYIMAVEPAESFVLSGGRPGPHKIQQIGAGFVPGILDTKIYNEVKQ	309
C-A.thaliana	354	ISSEEAIAETAKQLALKEGLMVGISSGAAAAAIAKVAKRPENAGKLIIVVF	403
1-C.merolae	310	VTSMSDIEMARRLAVEEGLLGGISSGAAVVALELGRRPENMGKKNIVVVI	359
C-A.thaliana	404	PSFGERYLSTPLFQSIREEVEKM 426	
1-C.merolae	360	PSFGERYLTSALFDKQREEAYNM 382	
Cl-A.thaliana	30	SFSFAQLRDLPKDFPSTNAKRDASLLIGKTPLVFLNKVTE--GCEAYVA	77
1-C.merolae	48	SFRARQVQLALTAPPVALARDVSDLVGNTPIVELKKIPEEEGVQAHIL	97
Cl-A.thaliana	78	AKQEHFQPTCSIKDRPAIAMIADAEEKKLIIPGKTTLIEPTSGNMGISLA	127
1-C.merolae	98	CKLESMEPCSSVKDRIGKYMIVEAEKRGDIQPGKTVLIEPTSGNTGIALA	147
Cl-A.thaliana	128	FMAAMKGYRIIMTPSYTSLERRVMTMSFGAELVLTDPAGMGGTVKKAY	177
1-C.merolae	148	YLAAARGVRLILTMPDSMSIERRMVLRAFGEVVLTPAAKGMKGAVAKAE	197
Cl-A.thaliana	178	DLDDSTPDAFMCQQFANPANTQIHFDTTGPEIWEIDTLGNVDIFVMGIGSG	227
1-C.merolae	198	QLFHTTPNAYMLQQFNNPDNPKAHVETTGPEIWAATGGKVDAFVAGVGTG	247
Cl-A.thaliana	228	GTVSGVGRYLKSNPNVKIYGVPEAESNILNGGKPGPHAITGNGVGFKPE	277
1-C.merolae	248	GTVTGTGAGRYLKEQNPHVYIMAVEPAESFVLSGGRPGPHKIQQIGAGFVPG	297
Cl-A.thaliana	278	ILDMDVMESVLEVSSEDAIKMARELALKEGLMVGISSGANVAAIRLAKM	327
1-C.merolae	298	ILDTKIYNEVKQVTSMSDIEMARRLAVEEGLLGGISSGAAVVALELGRR	347
Cl-A.thaliana	328	PENKGLIVTIIHASFGERYLSVLFDELKAEEMKPVSD 368	
1-C.merolae	348	FEMKGNIVVVIIPSGERYLTSALFDKQREEAYNMVAVEVE 388	

Fig. 7-42. Alignment of 1-C. merolae OAS-TL amino acid with A. thaliana isoforms.



A-A.thaliana	11	ELIGNTPLVYLNVAEGCVGRVAAKLEMMEPCSSVKDRIGFMSISDAEKK	60
2-C.merolae	55	DAIGNTFLIKLRRASERTGNCIYGKAEFMEPGGSVKDRAALYLLTDAEKR	104
A-A.thaliana	61	GLIKPGESVLIPTSGNTGVGLAFTAARKGYKLIITMPASMTERRIILL	110
2-C.merolae	105	GTLKLG-SIVVEGTAGNTGIGLTLGNSRGYRTVIVIPETQSEEKKEFLR	153
A-A.thaliana	111	AFGVELVLTDPKGMK-GAIKAEELAKTPNGYMLQQFNPANPKIHYE	159
2-C.merolae	154	SGAELVQVPAAPYRNPNVRLSERLAKELGAFWANQFDNPNRAHEE	203
A-A.thaliana	160	TTGPEIWKGTGGKIDGFVSGIGTGGTITGAGKYLKEQANVLYGVEPVE	209
2-C.merolae	204	TTGPEIWDQLDGHI DAFNCAVGTGGTLAGVSAFLRAKNPGIKIALTDPQG	253
A-A.thaliana	210	SAILSGGK-----PGPHKIQIGGAGFIPSVLNVLDLIDEVVQVSSDESID	253
2-C.merolae	254	AALVRYQCQGELVSVGDSITEGIGQSRITGNLEGFVPMDSFEISDAEALQ	303
A-A.thaliana	254	MARQLALKEGLLVGISSGAAAAAIKLAQRPNAGKLFVAIFPSFGERY	302
2-C.merolae	304	AAYDVVRHEGLHIGLSSGINIAGAIRVAESL-GPGHTIVTILCDGGSRV	351
B-A.thaliana	74	NIADNAQLIGKTPMVYLNVAEGCVGRVAAKLEIMEPCSSVKDRIGYSM	123
2-C.merolae	48	NVYDSFEDAIGNTFLIKLRRASERTGNCIYGKAEFMEPGGSVKDRAALYL	97
B-A.thaliana	124	ITDAEEKGLITPGKSVLVESTSGNTGIGLAFIAASKGYKLIITMPASMSL	173
2-C.merolae	98	LTAEKRGTLKLG-SIVVEGTAGNTGIGLTLGNSRGYRTVIVIPETQSE	146
B-A.thaliana	174	ERRVLLRAFGAELVLTPEKAGMT-GAIQKAEELKKTNPNSYMLQQFDNPA	222
2-C.merolae	147	EKKEFLRSCGAEVLVQVPAAPYRNPNVRLSERLAKELGAFWANQFDNPA	196
B-A.thaliana	223	NPKIHYETTGPDIWEDTRGKIDILVAGIGTGGTITGVGRFIKERKPELV	272
2-C.merolae	197	NRAHEETTGPDIWQLDGHI DAFNCAVGTGGTLAGVSAFLRAKNPGIKI	246
B-A.thaliana	273	IGVEPTESAILSGGK-----PGPHKIQIGGAGFIPKNDLAIIVDEYIAI	316
2-C.merolae	247	ALTDPPQGAALVRYQCQGELVSVGDSITEGIGQSRITGNLEGFVPMDSFEI	296
B-A.thaliana	317	SSEEALETAKQLALQEGLLVGISSGAAAAAIQVAKRPNAGKLIIVVFP	366
2-C.merolae	297	SDAEALQAAYDVVRHEGLHIGLSSGINIAGAIRVAESL-GPGHTIVTILC	345
B-A.thaliana	367	SFGERYLSTQLFQSIREE 384	
2-C.merolae	346	DGGSRYQKKMFQPSFLEQ 363	
C-A.thaliana	112	NIADNVSQLIGKTPMVYLNVAEGCVGRVAAKLEIMEPCSSVKDRIGYSM	161
2-C.merolae	48	NVYDSFEDAIGNTFLIKLRRASERTGNCIYGKAEFMEPGGSVKDRAALYL	97
C-A.thaliana	162	VTDAEQKGFISPGKSVLVEPTSGNTGIGLAFIAASRGYRLIITMPASMSM	211
2-C.merolae	98	LTAEKRGTLKLG-SIVVEGTAGNTGIGLTLGNSRGYRTVIVIPETQSE	146
C-A.thaliana	212	ERRVLLKAFGAELVLTDPKGMK-GAIKAEELKKTNPNSYMLQQFDNPA	259
2-C.merolae	147	EKKEFLRSCGAEVLVQVPAAPYRNPNVRLSERLAKEL-GAFWANQFDNPA	196
C-A.thaliana	260	ANPKIHYETTGPDIWDDTKGKVIDFVAGIGTGGTITGVGRFIKEKNPKTQ	309
2-C.merolae	196	ANRAHEETTGPDIWQLDGHI DAFNCAVGTGGTLAGVSAFLRAKNPGIKI	245
C-A.thaliana	310	VIGVEPTESDILSGGK-----PGPHKIQIGGAGFIPKNDLQKIMDEVIA	353
2-C.merolae	246	IALTDPQGAALVRYQCQGELVSVGDSITEGIGQSRITGNLEGFVPMDSFEI	295
C-A.thaliana	354	ISSEEALETAKQLALKEGLVMVGISSGAAAAAIKVAKRPNAGKLIIVVFP	403
2-C.merolae	296	SDAEALQAAYDVVRHEGLHIGLSSGINIAGAIRVAESL-GPGHTIVTIL	344
C-A.thaliana	404	PSFGERY 410	
2-C.merolae	345	CDGGSRY 351	
C1-A.thaliana	57	IGKTPLVFLNKVTEGCEAYVAAKQEHFQPTCSIKDRPAIAMIADAEEKKL	106
2-C.merolae	57	IGNTFLIKLRRASERTGNCIYGKAEFMEPGGSVKDRAALYLLTDAEKRT	106
C1-A.thaliana	107	IIPGKTTLIEPTSGNMGISLAFMAAMKGYRIIMTMSVYSLERRVIMRSF	156
2-C.merolae	107	LKLG-SIVVEGTAGNTGIGLTLGNSRGYRTVIVIPETQSEEKKEFLRSC	155
C1-A.thaliana	157	GAEVLVLTDPKGMK-GTVKAYDLDLSTPDAFMQQFANPANTQIHFDIT	205
2-C.merolae	156	GAEVLVQVPAAPYRNPNVRLSERLAKELGAFWANQFDNPNRAHEETT	205
C1-A.thaliana	206	GPEIWDTLGNVDIFVMGIGSGGTIVSGVGRYLSKSNPNVKIYGVFAESN	255
2-C.merolae	206	GPEIWDQLDGHI DAFNCAVGTGGTLAGVSAFLRAKNPGIKIALTDPQGAA	255
C1-A.thaliana	256	ILNGGKPGPHAITGN---GVGFKPEILDMD--VMESVLEVSSEDAIKMA	299
2-C.merolae	256	LVRYYQCQGELVSVGDSITEGIGQSRITGNLEGFVPMDSFEISDAEALQAA	305
C1-A.thaliana	300	RELALKEGLVMVGISSGANTVAATR LAKMPENKGLIIVTIIHASFGERY	346
2-C.merolae	306	YDVVRHEGLHIGLSSGINIAGAIRVAESL-GPGHTIVTILCDGGSRV	351

Fig. 7-43. Alignment of 2-C. merolae OAS-TL amino acid with A. thaliana isoforms.

A-A. thaliana	8	DVTELGNTPLVYLNVAEGCVGRVAAKLEMMEPCSSVKDRIGFSMISDA	57
1-T. pseudonana	14	NISEAVGNTPLVKISDRLCFAGRTIYAKCEFFNPLSSVKDRLLALSIIETA	63
A-A. thaliana	58	EKKGLIKPGESVLIIEPTSGNTGVGLAFTAAAKGVKLIITMPASMSTERRI	107
1-T. pseudonana	64	EKDGLKPGQTV-VEATSGNTGIAVAMMCAQRGYPCVITMAEPPFSIERRK	112
A-A. thaliana	108	ILLAFGVVELVLTDPKAGMKGAIKAEIBLAKTPNGYMLQQFENPANPKIH	157
1-T. pseudonana	113	IMRMLGAKVIVTPKAGKGTGMVEKARE-LADKNGWFLCHQFETDANWKFH	161
A-A. thaliana	158	YETTGPFIWKGTTGG-KIDGFVSGIGTGGTITGAGKYLKEQANVVKLYGVE	206
1-T. pseudonana	162	YETTGPFIENDLKGTKLDYVVTGYGTGGTFHGTAKYLKEQSPDTKIIILAE	211
A-A. thaliana	207	PVESAILSGG-----KPG-----PHKIQQIGAGFIPSVLN--VD	238
1-T. pseudonana	212	PGAANLIGSGIKTERNADGSPAGSHPAFAPHPIQGWTDFIPLVLEKGLD	261
A-A. thaliana	239	LIDEVVQVSSDESIDMARQLALKEGLLVGISSGAAAAAAIKLAQRPENAG	288
1-T. pseudonana	262	IPHEMFDIPDGAAVETSQALARNEGILTGISGGATMYAAIEIAKKAPE-G	310
A-A. thaliana	289	KLFVAIFPSFGERVLSVLF 308	
1-T. pseudonana	311	SVIVTMLPDTGERVLSPLF 330	
B-A. thaliana	77	DNAQLIGKTPMVYLNVAEGCVGRVAAKLEIMEPCSSVKDRIGYSMTID	126
1-T. pseudonana	13	NNISEAVGNTPLVKISDRLCFAGRTIYAKCEFFNPLSSVKDRLLALSIIET	62
B-A. thaliana	127	AEEKGLITPGKSVLVESTSGNTGIGLAFIAASKGYKLIITMPASMSLERR	176
1-T. pseudonana	63	AEKDGLKPGQTV-VEATSGNTGIAVAMMCAQRGYPCVITMAEPPFSIERR	111
B-A. thaliana	177	VLLRAFGAELVLTPEAKGMTGAIQKAEIILKKTNSYMLQQFDNPNANPKI	226
1-T. pseudonana	112	KIMRMLGAKVIVTPKAGKGTGMVEKARELADKN-GWFLCHQFETDANWKF	160
B-A. thaliana	227	HYETTGPFIWEDTRG-KIDILVAGIGTGGTITGVGRFIFERKPELKVIGV	275
1-T. pseudonana	161	HYETTGPFIENDLKGTKLDYVVTGYGTGGTFHGTAKYLKEQSPDTKIIILA	210
B-A. thaliana	276	EPTESAILSGG-----KPG-----PHKIQQIGAGFVFNKLD--L	307
1-T. pseudonana	211	EPGAANLIGSGIKTERNADGSPAGSHPAFAPHPIQGWTDFIPLVLEKGL	260
B-A. thaliana	308	AIVDEYIAISSEEAIEISKQLALQEGLLVGISSGAAAAAAIQVAKRPENA	357
1-T. pseudonana	261	DIPHEMFDIPDGAAVETSQALARNEGILTGISGGATMYAAIEIAKKAPE-	309
B-A. thaliana	358	GKLIIVVFPFSGERYLSTQLFQSIREECEQMQLPEL 392	
1-T. pseudonana	310	GSVIVTMLPDTGERVLSPLFAGIAEAMNEEELEI 344	
C-A. thaliana	115	DNVSQLIGKTPMVYLNVAEGCVGRVAAKLEIMEPCSSVKDRIGYSMTID	164
1-T. pseudonana	13	NNISEAVGNTPLVKISDRLCFAGRTIYAKCEFFNPLSSVKDRLLALSIIET	62
C-A. thaliana	165	AEQKGFISPGKSVLVEPTSGNTGIGLAFIAASRGYRLIITMPASMSMERR	214
1-T. pseudonana	63	AEKDGLKPGQTV-VEATSGNTGIAVAMMCAQRGYPCVITMAEPPFSIERR	111
C-A. thaliana	215	VLLKAFGAELVLTDPKAGMTGAVQKAEIILKNTPDYMLQQFDNPNANPKI	264
1-T. pseudonana	112	KIMRMLGAKVIVTPKAGKGTGMVEKARE-LADKNGWFLCHQFETDANWKF	160
C-A. thaliana	265	HYETTGPFIWDDTKG-KVDIFVAGIGTGGTITGVGRFIEKKNPKTQVIGV	313
1-T. pseudonana	161	HYETTGPFIENDLKGTKLDYVVTGYGTGGTFHGTAKYLKEQSPDTKIIILA	210
C-A. thaliana	314	EPTESDILSGG-----KPG-----PHKIQQIGAGFIPKNLDQ--	345
1-T. pseudonana	211	EPGAANLIGSGIKTERNADGSPAGSHPAFAPHPIQGWTDFIPLVLEKGL	260
C-A. thaliana	346	KIMDEVIAISSEEAIEITAKQLALKEGLMVGISSGAAAAAAIKVAKRPENA	395
1-T. pseudonana	261	DIPHEMFDIPDGAAVETSQALARNEGILTGISGGATMYAAIEIAKKAPE-	309
C-A. thaliana	396	GKLIIVVFPFSGERYLSTPLFQSIREEVEKMQPEL 429	
1-T. pseudonana	310	GSVIVTMLPDTGERVLSPLFAGIAEAMNEEELEI 343	
C1-A. thaliana	57	IGKTPLVFLNKVTEGCEAYVAAKQEHFQPTCSIKDRPAIAMIADAEEKKL	106
1-T. pseudonana	19	VGNTPLVKISDRLCFAGRTIYAKCEFFNPLSSVKDRLLALSIIETAEKDGS	68
C1-A. thaliana	107	IIPGKTTLIEPTSGNMGISLAFMAAMKGVRIIMTSPSYTSLERRVTMRSF	156
1-T. pseudonana	69	LKPGQT-VVEATSGNTGIAVAMMCAQRGYPCVITMAEPPFSIERRKIMRML	117
C1-A. thaliana	157	GAEVLVLTDPKAGMGGTVKKAYDLLDSTPDAFMCCQFANPANTQIHFDTTG	206
1-T. pseudonana	118	GAKVIVTPKAGKGTGMVEKARELADKN-GWFLCHQFETDANWKFHYETTG	166
C1-A. thaliana	207	PEIWEIDLGN-VDIFVMGIGSGGTVSGVGRYLSKSNPNVKIYGVPEAESN	255
1-T. pseudonana	167	PEICNDLKGTKLDYVVTGYGTGGTFHGTAKYLKEQSPDTKIIILAEPGAAN	216
C1-A. thaliana	256	ILNGG-----KPG-----PHAITGNGVGFKPEILD--MDVMESV	287
1-T. pseudonana	217	LIGSGIKTERNADGSPAGSHPAFAPHPIQGWTDFIPLVLEKGLDIPHEM	266
C1-A. thaliana	288	LEVSEDAIKMARELALKEGLMVGISSGANTVAAIRLAKMPENKGLIVT	337
1-T. pseudonana	267	FDIPDGAAVETSQALARNEGILTGISGGATMYAAIEIAKKAP-EGSVIVT	315
C1-A. thaliana	338	IHFVSGERYLSSVLFDELKAEIE 361	
1-T. pseudonana	316	MLPDTGERVLSPLFAGIAEAMNE 339	

Fig. 7-44. Alignment of 1-T. pseudonana OAS-TL amino acid with A. thaliana isoforms.



A-A. thaliana	4	RIAKDVTELIQNTPLVYLNNVA-EGCVGRVAAKLEMMFPCSSVKDRIGFS	52
2-T. pseudonan	46	KIAENVLELIGHTPLVKITKVTGSPSCVAEIVVAKLESSNPANSVKDRIALS	95
A-A. thaliana	53	MISDAEKKGLIKPGESVLIEPTSGNTGVGLAFTAAAKGYKLIITMPASMS	102
2-T. pseudonan	96	MIQEAARGDISPGKSTLVEPTSGNTGIGLAMVAASKGYKLIITMPESMS	145
A-A. thaliana	103	TERRILLAFGVLEVLTPAKGMKGAIKAAEELAKT-PNGYMLQQFENP	151
2-T. pseudonan	146	MERRVLLKAFGADVKLTPAAKMGGAIAKAAEIVDSLGPDPGYLLQQFNNP	195
A-A. thaliana	152	ANPKIHVETTGPDIWKGTGGKIDGFVSGIGTGGTITGAGKYLKEQANVVK	201
2-T. pseudonan	196	DNPVHRETTGPEIWEEDTDGKIDILLGGVGTGGTITGCGQYLKPRNPDMDK	245
A-A. thaliana	202	LYGVEPVESAILSGGKPGPHKIQQIGAGFIPSVLNVLDLIDEVQVSSDES	251
2-T. pseudonan	246	IVAVEPAESAVLSGGKPGPHKIQQIGAGFIPGNADTSLIDEVQVIGSEDA	295
A-A. thaliana	252	IDMARQLALKEGLLVGISSGAAAAAIAKLAQRPENAGKLFVAIFPSPFGER	301
2-T. pseudonan	296	MAMARKLATDEGIFCGISSGAAILAAKEVGSRPENADKRIVVILPSPFGER	345
A-A. thaliana	302	YLSTVLFDAATKAEAMTFE	321
2-T. pseudonan	346	YLSTALFQNLWDEASALKAE	365
B-A. thaliana	75	IADNAQLIGKTPMVYLNVA-KGCVASVAAKLEIMEPCCSVKDRIGYSM	123
2-T. pseudonan	47	IAENVLELIGHTPLVKITKVTGSPSCVAEIVVAKLESSNPANSVKDRIALSM	96
B-A. thaliana	124	ITDAEEKGLITPGKSVLVESTSGNTGIGLAFIAASKGYKLIITMPASMSL	173
2-T. pseudonan	97	IQEAARGDISPGKSTLVEPTSGNTGIGLAMVAASKGYKLIITMPESMSM	146
B-A. thaliana	174	ERRVLLRAFAGAEVLVTEPAKGMTGAIQKAAEILKKT-PNSYMLQQFDNPA	222
2-T. pseudonan	147	ERRVLLKAFGADVKLTPAAKMGGAIAKAAEIVDSLGPDPGYLLQQFNNPD	196
B-A. thaliana	223	NPKIHVETTGPDIWEEDTRGKIDILVAGIGTGGTITGVGRFIKPKRPELVK	272
2-T. pseudonan	197	NPKVHRETTGPEIWEEDTDGKIDILLGGVGTGGTITGCGQYLKPRNPDMDKI	246
B-A. thaliana	273	IGVEPTESAILSGGKPGPHKIQQIGAGFIPKNDLDAIVDEYIAISSEEA	322
2-T. pseudonan	247	VAVEPAESAVLSGGKPGPHKIQQIGAGFIPGNADTSLIDEVQVIGSEDA	296
B-A. thaliana	323	ETSKQLALQEGLLVGISSGAAAAAIAQVAKRPENAGKLIIVVFPSPFGER	372
2-T. pseudonan	297	AMARKLATDEGIFCGISSGAAILAAKEVGSRPENADKRIVVILPSPFGER	346
B-A. thaliana	373	LSTQLFQSIREECEQMPE	391
2-T. pseudonan	347	LSTALFQNLWDEASALKAE	365
C-A. thaliana	113	IADNVSQLIGKTPMVYLNVA-KGCVANIAAKLEIMEPCCSVKDRIGYSM	161
2-T. pseudonan	47	IAENVLELIGHTPLVKITKVTGSPSCVAEIVVAKLESSNPANSVKDRIALSM	96
C-A. thaliana	162	VTDAEQKGFISPGKSVLVEPTSGNTGIGLAFIAASRGYRILITMPASMSM	211
2-T. pseudonan	97	IQEAARGDISPGKSTLVEPTSGNTGIGLAMVAASKGYKLIITMPESMSM	146
C-A. thaliana	212	ERRVLLKAFGAEVLVTEPAKGMTGAVQKAAEILKNT-PDAYMLQQFDNPA	260
2-T. pseudonan	147	ERRVLLKAFGADVKLTPAAKMGGAIAKAAEIVDSLGPDPGYLLQQFNNPD	196
C-A. thaliana	261	NPKIHVETTGPDIWDDTKGKVIDFVAGIGTGGTITGVGRFIKPKRPELVK	310
2-T. pseudonan	197	NPKVHRETTGPEIWEEDTDGKIDILLGGVGTGGTITGCGQYLKPRNPDMDKI	246
C-A. thaliana	311	IGVEPTESAILSGGKPGPHKIQQIGAGFIPKNDLQKIMDEYIAISSEEA	360
2-T. pseudonan	247	VAVEPAESAVLSGGKPGPHKIQQIGAGFIPGNADTSLIDEVQVIGSEDA	296
C-A. thaliana	361	ETAKQLALKEGLMVGISSGAAAAAIAKVAKRPENAGKLIIVVFPSPFGER	410
2-T. pseudonan	297	AMARKLATDEGIFCGISSGAAILAAKEVGSRPENADKRIVVILPSPFGER	346
C-A. thaliana	411	LSTPLFQSIREEVEKMQPE	429
2-T. pseudonan	347	LSTALFQNLWDEASALKAE	365
C1-A. thaliana	56	LIGKTPVFLNKVT-EGCEAYVAAKQEHQPTCSIKDRPAIAMIADAEEK	104
2-T. pseudonan	54	LIGHTPLVKITKVTGSPSCVAEIVVAKLESSNPANSVKDRIALSMIQEAAR	103
C1-A. thaliana	105	KLIIPGKTTLIEPTSGNMGISLAFMAAMKGYRIIMTMSYTSLERRVIMR	154
2-T. pseudonan	104	GDISPFGKSTLVEPTSGNTGIGLAMVAASKGYKLIITMPESMSMERRVLLK	153
C1-A. thaliana	155	SFGAEVLVTEPAKMGMTVKKAYDILLDST-PDAFMQQFANPANTQIHFD	203
2-T. pseudonan	154	AFGADVKLTPAAKMGGAIAKAAEIVDSLGPDPGYLLQQFNNPDNPKVHRE	203
C1-A. thaliana	204	TTGPEIWEEDTLGNVDIFVMGIGSGGTVSGVGRYLSKKNPNVKIYGVPAE	253
2-T. pseudonan	204	TTGPEIWEEDTDGKIDILLGGVGTGGTITGCGQYLKPRNPDMDKIVAVEPAE	253
C1-A. thaliana	254	SNILNGGKPGPHAITGNGVGFKPEILDMDVMESVLEVSSEDAIKMARELA	303
2-T. pseudonan	254	SAVLSGGKPGPHKIQQIGAGFIPGNADTSLIDEVQVIGSEDAAMARKLA	303
C1-A. thaliana	304	LKEGLMVGISSGANTVAAIRLAKMPENKGLIVTIHASFGERYLSVSLFD	353
2-T. pseudonan	304	TDEGIFCGISSGAAILAAKEVGSRPENADKRIVVILPSPFGERYLSLALFQ	353
C1-A. thaliana	354	ELRKEAEEMK	363
2-T. pseudonan	354	NLWDEASALK	363

Fig. 7-45. Alignment of 2-T. pseudonana OAS-TL amino acid with A. thaliana isoforms.

A-A. thaliana	2	ASRIAKDVTELGNTPLVYLNNV--AEGCVG--RVAAKLEMPECCSSVKD	47
1-P. tricornutum	4	ALNVAARPSDLTGNTPLLDLGRILQAHGIDNGSRLFGKMSLGPCCSSVKD	53
A-A. thaliana	48	RIGFSMISDAEKKGLIKPGESVLEPTSGNTGVGLAFTAAAKGYKLIITM	97
1-P. tricornutum	54	RIGRSMIDQAEQAGLITPGRTTLVEPTSGNTGIALAFIARERGYRCILTM	103
A-A. thaliana	98	PASMSTERRIILLAFGVLEVLTPAKGMKGAIAKAEIILAK-TPNGYMLQ	146
1-P. tricornutum	104	PEQMSTERRMMLLALGAQVVLTPKETAVFGALAKAHEIVESLNGDGFMLQ	153
A-A. thaliana	147	QFENPANPKIHYETTGPFIWKGTGGKIDGFVSGIGTGGTITGAGKYLKEQ	196
1-P. tricornutum	154	QFENPHNPKAHRETTGPEIWRDTDGDIIDIFVAGVGTGGTITGVSYLXKS	203
A-A. thaliana	197	NA-----NVKLYGVEFVE---SAILSGGK---PGPHKIQQIGAGFI	231
1-P. tricornutum	204	PAHGLPPLRPNLQTVAVEPMEQMLITAALGGAKIGPQGPHKIQGMGAGLV	253
A-A. thaliana	232	FSVLNVDLIDEVQVSSDESIDMARQLALKEGLLVGISSGAAAAAAIKLA	281
1-P. tricornutum	254	FQVLDLTLLEDEVVPHSDQAIDMAHELWMT-GLPVGASAGAIVHAAVQVL	302
A-A. thaliana	282	QRPENAGKLFVAIFPSFGERVLTSTVLFDAATRKEAE 316	
1-P. tricornutum	303	QRPASAHKMAVCVIPSFGERYVTFHPFAEIKEKAQ 337	
B-A. thaliana	69	GVEGLNIADNAQLIGKTPMVYLNNVVKGCV---ASVAAKLEIMEPCCS	114
1-P. tricornutum	1	GTRALNVAARPSDLTGNTPLLDLGRILQAHGIDNGSRLFGKMSLGPCCSS	50
B-A. thaliana	115	VKDRIQYSMTDAEKKGLITPGKSVLVESTSGNTGIGLAFIAASKGYKLI	164
1-P. tricornutum	51	VKDRIQYSMTDAEKKGLITPGRTTLVEPTSGNTGIALAFIARERGYRCI	100
B-A. thaliana	165	LTMPASMSLERRVLLRFAEGLVLETPAKMTGAIQKAEIILKK-TPNSY	213
1-P. tricornutum	101	LTMPEQMSTERRMMLLALGAQVVLTPKETAVFGALAKAHEIVESLNGDGF	150
B-A. thaliana	214	MLQQFDNPANPKIHYETTGPFIWEDTRGKIDILVAGIGTGGTITGVGRFI	263
1-P. tricornutum	151	MLQQFENPHNPKAHRETTGPEIWRDTDGDIIDIFVAGVGTGGTITGVSYL	200
B-A. thaliana	264	KER-----KPELKVIGVEPTESAILSG-----GKPGPHKIQQIGA	298
1-P. tricornutum	201	KGSPAHLPLRPNLQTVAVEPMEQMLITAALGGAKIGPQGPHKIQGMGA	250
B-A. thaliana	299	GFVFNLDLAIVDEYTAISSEEAETSKQLALQEGLLVGISSGAAAAAAI	348
1-P. tricornutum	251	GLVPQVLDLTLLEDEVVPHSDQAIDMAHELWMT-GLPVGASAGAIVHAAV	299
B-A. thaliana	349	QVAKRPENAGKLIIVVPSFGERVLTSTVLFQSIREECEQMQPE 391	
1-P. tricornutum	300	QVLRQPASAHKMAVCVIPSFGERYVTFHPFAEIKEKAQNLQKQ 342	
C-A. thaliana	107	PGDGLNIADNVSQLIGKTPMVYLNVI--AKGCV--ANIAAKLEIMEPCCS	152
1-P. tricornutum	1	GTRALNVAARPSDLTGNTPLLDLGRILQAHGIDNGSRLFGKMSLGPCCSS	50
C-A. thaliana	153	VKDRIQYSMTDAEQKGFISPGKSVLVEPTSGNTGIGLAFIAASRGYRLI	202
1-P. tricornutum	51	VKDRIQYSMTDAEQAGLITPGRTTLVEPTSGNTGIALAFIARERGYRCI	100
C-A. thaliana	203	LTMPASMSMERRVLLKAFGAELVLTDPAKMTGAVQKAEIILKN-TPDAY	251
1-P. tricornutum	101	LTMPEQMSTERRMMLLALGAQVVLTPKETAVFGALAKAHEIVESLNGDGF	150
C-A. thaliana	252	MLQQFDNPANPKIHYETTGPFIWDDTKGKVDIFVAGIGTGGTITGVGRFI	301
1-P. tricornutum	151	MLQQFENPHNPKAHRETTGPEIWRDTDGDIIDIFVAGVGTGGTITGVSYL	200
C-A. thaliana	302	KEK-----NPKTQVIGVEPTESDILSG-----GKPGPHKIQQIGA	336
1-P. tricornutum	201	KGSPAHLPLRPNLQTVAVEPMEQMLITAALGGAKIGPQGPHKIQGMGA	250
C-A. thaliana	337	GFIPKNDQKIMDEVIAISSEEAETAKQLALKEGLMVGISSGAAAAAAI	386
1-P. tricornutum	251	GLVPQVLDLTLLEDEVVPHSDQAIDMAHELWMT-GLPVGASAGAIVHAAV	299
C-A. thaliana	387	KVAKRPENAGKLIIVVPSFGERVLTSTVLFQSIREEVEKMQPE 429	
1-P. tricornutum	300	QVLRQPASAHKMAVCVIPSFGERYVTFHPFAEIKEKAQNLQKQ 342	
Cl-A. thaliana	48	NAKRDAALLIGKTPVFLNKVTE--GCE--AYVAAKQEHFQPTCSIKDRP	93
1-P. tricornutum	6	NVAARPSDLTGNTPLLDLGRILQAHGIDNGSRLFGKMSLGPCCSSVKDRI	55
Cl-A. thaliana	94	ATAMIADAEEKKLIIPGKTTLEPTSGNMGISLAFMAAMKGYRIIMTMS	143
1-P. tricornutum	56	GRSMIDQAEQAGLITPGRTTLVEPTSGNTGIALAFIARERGYRCIILTMPE	105
Cl-A. thaliana	144	YTSLEERVMTMSFGAELVLTDPAKMGGTVKKAYDLLDS-TPDAFMCQQF	192
1-P. tricornutum	106	QMSTERRMMLLALGAQVVLTPKETAVFGALAKAHEIVESLNGDGFMLQQF	155
Cl-A. thaliana	193	ANPANTQIHFDTTGPEIWEEDTLGNVDIFVMGIGSGGTVSGVGRVLSK--	240
1-P. tricornutum	156	ENPHNPKAHRETTGPEIWRDTDGDIIDIFVAGVGTGGTITGVSYLXGSPA	205
Cl-A. thaliana	241	-----NPNVKIYGVPEAESNILNG-----GKPGPHAITGNVGFKPE	277
1-P. tricornutum	206	HGLPPLRPNLQTVAVEPMEQMLITAALGGAKIGPQGPHKIQGMGAGLVQ	255
Cl-A. thaliana	278	ILDMDVMSVLEVSSDAIKMARELALKEGLMVGISSGANTVAAATRLAKM	327
1-P. tricornutum	256	VLDLTLLEDEVVPHSDQAIDMAHELWMT-GLPVGASAGAIVHAAVQVLR	304
Cl-A. thaliana	328	FENKGLIVTIHASFGERVLSVLFDELKAEEMK 363	
1-P. tricornutum	305	PASAHKMAVCVIPSFGERYVTFHPFAEIKEKAQNLQ 340	

Fig. 7-46. Alignment of 1-*P. tricornutum* OAS-TL amino acid with *A. thaliana* isoforms.



A-A. thaliana	3	SRIAKDVTLELIGNTPLVVLNNVAEGCVGRVAAKLEMMEPCSSVKDRIGFS	52
2-P. tricornut	13	AKIAENVLGLIGQTPLVQLNRVTEGCVQIVAKLESSNFANSVKDRIALS	62
A-A. thaliana	53	MISDAEKKGLIKPGESVLIPTSGNTGVGLAFTAAAKGYKLIITMPFASMS	102
2-P. tricornut	63	MITEAEKRGDIKPGKTIIVEPTSGNTGIGLAMVAAAKGYKLIITMPFASMS	112
A-A. thaliana	103	TERRIILLAFGVLELVDPAKGMKGATAKAEIILAKTFNGVYML-QQFENP	151
2-P. tricornut	113	MERRVLLKAFGADVLTFAAKGMGGATAKAEIIVNSLGS DAMLLQQFNNP	162
A-A. thaliana	152	ANPKIHVETTGPETWKGTTGGKIDGFEVSGITGGTITGAGKYLKEQMANVK	201
2-P. tricornut	163	DNPKVHRETTGPETWSDTDGEVDIIVGGIGTGGTITGCAQYLKPLNPKLQ	212
A-A. thaliana	202	LYGVEPVESAILSGGKPGPHKIQQIGAGFIPSVLNVLDLDEVVQVSSDES	251
2-P. tricornut	213	VVAVEPTESAVLSGGKPGPHKIQQIGAGFIPGNADTSLLEVVQISGEDS	262
A-A. thaliana	252	IDMARQLALKEGLLVGISSGAAAAAIAKLAQRPENAGKLVVAIFPSFGER	301
2-P. tricornut	263	MAMARKMATEEGIFCGISSGAAVLAIIQIGKRPENADKRIVVIPSFGER	312
A-A. thaliana	302	YLSTVLFDAATKAEAMTFFEA 322	
2-P. tricornut	313	YLSTALFQDLWDEMAALKPES 333	
B-A. thaliana	75	IADNAALQIGKTPMVYLNNVKGCVASAAKLEIMEPCSSVKDRIGYSMI	124
2-P. tricornut	15	IAENVLGLIGQTPLVQLNRVTEGCVQIVAKLESSNFANSVKDRIALSMI	64
B-A. thaliana	125	TDAAEKGLITPGKSVLVESTSGNTGIGLAFIAASKGYKLIITMPASMSLE	174
2-P. tricornut	65	TEAEKRGDIKPGKTIIVEPTSGNTGIGLAMVAAAKGYKLIITMPASMSME	114
B-A. thaliana	175	RRVLLRAFGAELVLTDPKGMGTGAIQKABEILKKTFSNYSML-QQFDNPAN	223
2-P. tricornut	115	RRVLLKAFGADVLTFAAKGMGGATAKAEIIVNSLGS DAMLLQQFNNP DN	164
B-A. thaliana	224	PKIHVETTGPETWEDTRGKIDILVAGITGGTITGVGRFIKERKPELKVI	273
2-P. tricornut	165	PKVHRETTGPETWSDTDGEVDIIVGGIGTGGTITGCAQYLKPLNPKLQV	214
B-A. thaliana	274	GVEPTESAILSGGKPGPHKIQQIGAGFVFNKLDLIVDEVIATISSEAEIE	323
2-P. tricornut	215	AVEPTESAVLSGGKPGPHKIQQIGAGFIPGNADTSLLEVVQISGEDSMA	264
B-A. thaliana	324	TSKQLALQEGLLVGISSGAAAAAIAQVAKRPENAGKLIIVVFFSFGERYL	373
2-P. tricornut	265	MARKMATEEGIFCGISSGAAVLAIIQIGKRPENADKRIVVIPSFGERYL	314
B-A. thaliana	374	STQLFQSIREECEQMQPE 391	
2-P. tricornut	315	STALFQDLWDEMAALKPE 332	
C-A. thaliana	113	IADNVSQLIGKTPMVYLNNSIAKGCVANIAAKLEIMEPCSSVKDRIGYSMV	162
2-P. tricornut	15	IAENVLGLIGQTPLVQLNRVTEGCVQIVAKLESSNFANSVKDRIALSMI	64
C-A. thaliana	163	TDAAEQKGFISPGKSVLVEPTSGNTGIGLAFIAASRGYRILITMPASMSME	212
2-P. tricornut	65	TEAEKRGDIKPGKTIIVEPTSGNTGIGLAMVAAAKGYKLIITMPASMSME	114
C-A. thaliana	213	RRVLLKAFGAELVLTDPKGMGTGAVQKABEILKNT-PDAYMLQQFDNPAN	261
2-P. tricornut	115	RRVLLKAFGADVLTFAAKGMGGATAKAEIIVNSLGS DAMLLQQFNNP DN	164
C-A. thaliana	262	PKIHVETTGPETWDDTKGKVDIFVAGITGGTITGVGRFIKENPKTQVI	311
2-P. tricornut	165	PKVHRETTGPETWSDTDGEVDIIVGGIGTGGTITGCAQYLKPLNPKLQV	214
C-A. thaliana	312	GVEPTESDILSGGKPGPHKIQQIGAGFIPKMLDQIMDEVIATISSEAEIE	361
2-P. tricornut	215	AVEPTESAVLSGGKPGPHKIQQIGAGFIPGNADTSLLEVVQISGEDSMA	264
C-A. thaliana	362	TAKQLALKEGLMVGISSGAAAAAIAKVAKRPENAGKLIIVVFFSFGERYL	411
2-P. tricornut	265	MARKMATEEGIFCGISSGAAVLAIIQIGKRPENADKRIVVIPSFGERYL	314
C-A. thaliana	412	STPLFQSIREEVEKMQPERV 431	
2-P. tricornut	315	STALFQDLWDEMAALKPESV 334	
C1-A. thaliana	56	LIGKTPLVFLNKNVTEGCEAYVAAKQENHFQPTCSIKDRPAIAMIADAEEKK	105
2-P. tricornut	22	LIGQTPLVQLNRVTEGCVQIVAKLESSNFANSVKDRIALSMITEAEKRG	71
C1-A. thaliana	106	LIIPGKTTLIEPTSGNMGISLAFMAAMKGYRIIMTSPSYTSLERRVMTMS	155
2-P. tricornut	72	DIKPGKTIIVEPTSGNTGIGLAMVAAAKGYKLIITMPASMSMERRVLLKA	121
C1-A. thaliana	156	FGAELVLTDPKGMGGTVKKAVDLLDST-PDAFMCQFANPANTQIHFDT	204
2-P. tricornut	122	FGADVLTFAAKGMGGATAKAEIIVNSLGS DAMLLQQFNNP DNPKVHRET	171
C1-A. thaliana	205	TGPEIMEDTLGNVDIFVMGIGSGGTVSGVGRYLSKNPNVVIYGVPEAES	254
2-P. tricornut	172	TGPEIWSDTDGEVDIIVGGIGTGGTITGCAQYLKPLNPKLQVVAVEPTES	221
C1-A. thaliana	255	NILNGGKPGPHAITGNVGVKPEILDMVMESVLEVSSEDAIKMARELAL	304
2-P. tricornut	222	AVLSGGKPGPHKIQQIGAGFIPGNADTSLLEVVQISGEDSMAMARKMAT	271
C1-A. thaliana	305	KEGLMVGISSGANTVAATRLAKMPENKGLIVTIHASFGERYLSSVLFDE	354
2-P. tricornut	272	EEGIFCGISSGAAVLAIIQIGKRPENADKRIVVIPSFGERYLSTALFQD	321
C1-A. thaliana	355	LRKEAEMKPVSV 367	
2-P. tricornut	322	LWDEMAALKPESV 334	

Fig. 7- 47. Alignment of 2-*P. tricornutum* OAS-TL amino acid with *A. thaliana* isoforms.



A-A.thaliana	7	KDVTEIIGNTPLVYLNVAEGCVGRVAAKLEMMPECCSSVKDRIGFSMISD	56
E.huxleyi	29	EDVTEIIGNTPCVKLEKLCPPGTTVFAKCEFLNPLSSVKDRDLALAVIEE	78
A-A.thaliana	57	AEKGLIKPGESVLEIPTSNGTGVGLAFTAAAKGYKLIITMPASMTERR	106
E.huxleyi	79	AEASGKLPKGDIV-IEATSGNTGIAMVAVCAQRGYKVCVIMAEQFSVERR	127
A-A.thaliana	107	IILLAFGVVELVLTDPKAGMKGAIKAEIILAKTPNGYMLQQFENPANPKI	156
E.huxleyi	128	RLMRMLGAKVVLTPKAGKGFVGMVKKAEI-LAEKHGWFLCHQFETEANWKF	176
A-A.thaliana	157	HYETTGPFIWKGTTGGK-IDGFVSGIGTGGTITGAGKYLKEQNAVVKLYGV	205
E.huxleyi	177	HNVTGPEILADFEKGRLDYVVTGYGTGGTFHGAGKAIKAARPDVKIVLA	226
A-A.thaliana	206	EPVESAILSGGKPGPHK-----IQQIGAGFIPSVLN---	236
E.huxleyi	227	EPEDAGLLASGVPTENKPDGSPSASHPAFSAHPIQGWTPDFIPKVLHDAP	276
A-A.thaliana	237	VD-LIDEVVQVSSDESIDMARQLALKEGLLVGISSGAAAAAAIKLAQRPE	285
E.huxleyi	277	MDMLLHELVPVPGAGAIATAQSLAAKEGLLTGISGGGTMWAALETAKKAP	326
A-A.thaliana	286	NAGKLFVAIFPFSFGERYLSTVLF 308	
E.huxleyi	327	E-GSVILAMLPDTGERYLSTPLF 348	
B-A.thaliana	77	DNAQLIGKTPMYYLNVAEGCVASVAAKLEIMEPCSSVKDRIGYSMTID	126
E.huxleyi	29	EDVTEIIGNTPCVKLEKLCPPGTTVFAKCEFLNPLSSVKDRDLALAVIEE	78
B-A.thaliana	127	AEEKGLITPGKSVLVESTSGNTGIGLAFIAASKGYKLIITMPASMSLERR	176
E.huxleyi	79	AEASGKLPKGDIV-IEATSGNTGIAMVAVCAQRGYKVCVIMAEQFSVERR	127
B-A.thaliana	177	VLLRAFGAELVLTPEAKGMTGAIQKAEIILKKTSPNSYMLQQFDNPANPKI	226
E.huxleyi	128	RLMRMLGAKVVLTPKAGKGFVGMVKKAEELAEKH-GWFLCHQFETEANWKF	176
B-A.thaliana	227	HYETTGPFIWEDTRGK-IDILVAGIGTGGTITGVGRFIERKPELKVIGV	275
E.huxleyi	177	HNVTGPEILADFEKGRLDYVVTGYGTGGTFHGAGKAIKAARPDVKIVLA	226
B-A.thaliana	276	EPTESAILSGGKPGPHK-----IQQIGAGFVFNKLDLAI	309
E.huxleyi	227	EPEDAGLLASGVPTENKPDGSPSASHPAFSAHPIQGWTPDFIPKVLHDAP	276
B-A.thaliana	310	VD----EYIAISSEEAIEITKQLALQEGLLVGISSGAAAAAAIQVAKRPE	355
E.huxleyi	277	MDMLLHELVPVPGAGAIATAQSLAAKEGLLTGISGGGTMWAALETAKKAP	326
B-A.thaliana	356	NAGKLIIVFPFSFGERYLSTQLFQSI 381	
E.huxleyi	327	E-GSVILAMLPDTGERYLSTPLFSDI 351	
C-A.thaliana	115	DNVSQLIGKTPMYYLNVAEGCVANIAAKLEIMEPCSSVKDRIGYSMTVD	164
E.huxleyi	29	EDVTEIIGNTPCVKLEKLCPPGTTVFAKCEFLNPLSSVKDRDLALAVIEE	78
C-A.thaliana	165	AEQKGFISPGKSVLVEPTSNGTIGLAFIAASRGVRLIITMPASMSMERR	214
E.huxleyi	79	AEASGKLPKGDIV-IEATSGNTGIAMVAVCAQRGYKVCVIMAEQFSVERR	127
C-A.thaliana	215	VLLKAFGAELVLTDPKAGMTGAVQKAEIILKNTPDAYMLQQFDNPANPKI	264
E.huxleyi	128	RLMRMLGAKVVLTPKAGKGFVGMVKKAEI-LAEKHGWFLCHQFETEANWKF	176
C-A.thaliana	265	HYETTGPFIWDDTKGK-VDIFVAGIGTGGTITGVGRFIERKPNKPTQVIGV	313
E.huxleyi	177	HNVTGPEILADFEKGRLDYVVTGYGTGGTFHGAGKAIKAARPDVKIVLA	226
C-A.thaliana	314	EPTESDILSGGKPGPHK-----IQQIGAGFIPKNDLQKI	347
E.huxleyi	227	EPEDAGLLASGVPTENKPDGSPSASHPAFSAHPIQGWTPDFIPKVLHDAP	276
C-A.thaliana	348	MD----EYIAISSEEAIEITAKQLALKEGLLVGISSGAAAAAAIKVAKRPE	393
E.huxleyi	277	MDMLLHELVPVPGAGAIATAQSLAAKEGLLTGISGGGTMWAALETAKKAP	326
C-A.thaliana	394	NAGKLIIVFPFSFGERYLSTPLFQSIREEVEK 425	
E.huxleyi	327	E-GSVILAMLPDTGERYLSTPLFSDIPADMSE 357	
Cl-A.thaliana	52	DASLLIGKTPVFLNKVTEGCEAYVAAKQEHFQPTCSIKDRPAIAMIADA	101
E.huxleyi	30	DVTEIIGNTPCVKLEKLCPPGTTVFAKCEFLNPLSSVKDRDLALAVIEEA	79
Cl-A.thaliana	102	EKKKLIIPGKTTLEIPTSNGMGISLAFMAAMKGVRIIMTSPSYTSLERRV	151
E.huxleyi	80	EASGKLPKGDIV-IEATSGNTGIAMVAVCAQRGYKVCVIMAEQFSVERR	128
Cl-A.thaliana	152	TMRSFGAELVLTDPKAGMGGTVKKAYDLLDSTPDAFMQQFANFANTQIH	201
E.huxleyi	129	LMRMLGAKVVLTPKAGKGFVGMVKKAEELAEKH-GWFLCHQFETEANWKFH	177
Cl-A.thaliana	202	FDDTGPFIWEDTDLGN-VDIFVMGIGSGGTVSGVGRYLSKSNPNVKIYGV	250
E.huxleyi	178	NVTGPEILADFEKGRLDYVVTGYGTGGTFHGAGKAIKAARPDVKIVLAE	227
Cl-A.thaliana	251	PAESNILNGGKPF-----GPHAITNGVGFKEPFIELD---M	281
E.huxleyi	228	PEDAGLLASGVPTENKPDGSPSASHPAFSAHPIQGWTPDFIPKVLHDAPM	277
Cl-A.thaliana	282	DVM-ESVLEVSSEDAIKMARELALKEGLLVGISSGANTVAAIRLAKMPEN	330
E.huxleyi	278	MDMLLHELVPVPGAGAIATAQSLAAKEGLLTGISGGGTMWAALETAKKAP-	326
Cl-A.thaliana	331	KGKLIIVTHASFGERYLSSVLFDELKAEI 361	
E.huxleyi	327	EGSVILAMLPDTGERYLSTPLFSDIPADMSE 357	

Fig. 7-48. Alignment of *E. huxleyi* OAS-TL amino acid with *A. thaliana* isoforms.

A-A. thaliana	5	IAKDVTLEIGNTFLVYLNVAEGCVGRVAAKLEMPEPCSSVKDRIGFSMI	54
1-A. klebsii	1	IYEDITKTIGDTPIVKINKLAP*AGVELYVKIEYFNPLSSVKDRALALAI	50
A-A. thaliana	55	SDAEKKGLIKPGESVLIIEPTSGNTGVGLAFTAAAKGYKLIITPASMSTE	104
1-A. klebsii	51	EDAESGGLKPG*GTVIEATSGNTGIALAMVCAQRGYNFVSTMAASFVSE	100
A-A. thaliana	105	RRIIILAFGVLELVLTDPAKGMKGAIKAEIILAKTFNGVYMLQQFENPANP	154
1-A. klebsii	101	RRKVMRMLGAKVIVTPAPLGGTGMVKKAEELAEKH*GWYLARQFENPANP	150
A-A. thaliana	155	KIHVETTGPEIWKGTGGK-IDGFVSGIGTGGTITGAGKYLKEQNANVKLY	203
1-A. klebsii	151	EFHYKTTGQEILKDFNGKKLDYVWVTGYGTGGTFSGAGKALKEARPDVKIV	200
A-A. thaliana	204	GVEPVESAILSGG-----KPGF-----HKIQGIGAGFIPS	233
1-A. klebsii	201	LSEPKPAPLLTSGIKQTRKEVMGKFGAPAEGHSAMTAHPIQGWTNFIPL	250
A-A. thaliana	234	V---LNVDLIDEVQVSSDESIDMARQLALKEGLLVGISSGAAAAAAIK	279
1-A. klebsii	251	VTEQGVDAKYHEKVMLEPKVAMETSHKLARQEGIFCGVSGGATVATALD	300
A-A. thaliana	280	LAQRPENAGKLFVAIFPSFGERYLSTVLF 308	
1-A. klebsii	301	VCAEAPT*GSVVLAMIPDTAERYLSTPLF 329	
B-A. thaliana	75	IADNAAQLIGKTPMVVYLNVAEGCVGRVAAKLEIMEPCSSVKDRIGYSMI	124
1-A. klebsii	1	IYEDITKTIGDTPIVKINKLAP*AGVELYVKIEYFNPLSSVKDRALALAI	50
B-A. thaliana	125	TDABEKGLITPGKSVLVESTSGNTGIGLAFIAASKGYKLIITPASMSTE	174
1-A. klebsii	51	EDAESGGLKPG*GTVIEATSGNTGIALAMVCAQRGYNFVSTMAASFVSE	100
B-A. thaliana	175	RRVLLRAFGAELVLTDPAKGMTGAIQKAEIILKKTTPNSYMLQQFDNPNP	224
1-A. klebsii	101	RRKVMRMLGAKVIVTPAPLGGTGMVKKAEELAEKH*GWYLARQFENPANP	150
B-A. thaliana	225	KIHVETTGPEIWEEDTRGK-IDILVAGIGTGGTITGVGRFIERKPELKI	273
1-A. klebsii	151	EFHYKTTGQEILKDFNGKKLDYVWVTGYGTGGTFSGAGKALKEARPDVKIV	200
B-A. thaliana	274	GVEPTESAILSGG-----KPGF-----HKIQGIGAGFVP-	302
1-A. klebsii	201	LSEPKPAPLLTSGIKQTRKEVMGKFGAPAEGHSAMTAHPIQGWTNFIPL	250
B-A. thaliana	303	---KNLDLAIVDEYIAISSEEAETSKQLALQEGLLVGISSGAAAAAAIQ	349
1-A. klebsii	251	VTEQGVDAKYHEKVMLEPKVAMETSHKLARQEGIFCGVSGGATVATALD	300
B-A. thaliana	350	VAKRPENAGKLIIVVFPFSGERYLSTQLFQSIREECEQ 387	
1-A. klebsii	301	VCAEAPT*GSVVLAMIPDTAERYLSTPLFAEIDAEMDQ 338	
C-A. thaliana	113	IADNVSQIGKTPMVVYLNVAEGCVGRVAAKLEIMEPCSSVKDRIGYSMV	162
1-A. klebsii	1	IYEDITKTIGDTPIVKINKLAP*AGVELYVKIEYFNPLSSVKDRALALAI	50
C-A. thaliana	163	TDABEKGLITPGKSVLVEPTSGNTGIGLAFIAASRGYRILITPASMSTE	212
1-A. klebsii	51	EDAESGGLKPG*GTVIEATSGNTGIALAMVCAQRGYNFVSTMAASFVSE	100
C-A. thaliana	213	RRVLLKAFGAELVLTDPAKGMTGAVQKAEIILKNTPDAYMLQQFDNPNP	262
1-A. klebsii	101	RRKVMRMLGAKVIVTPAPLGGTGMVKKAEELAEKH*GWYLARQFENPANP	150
C-A. thaliana	263	KIHVETTGPEIWDITGK-VDIFVAGIGTGGTITGVGRFIERKPNKTVI	311
1-A. klebsii	151	EFHYKTTGQEILKDFNGKKLDYVWVTGYGTGGTFSGAGKALKEARPDVKIV	200
C-A. thaliana	312	GVEPTESAILSGG-----KPGF-----HKIQGIGAGFIP-	340
1-A. klebsii	201	LSEPKPAPLLTSGIKQTRKEVMGKFGAPAEGHSAMTAHPIQGWTNFIPL	250
C-A. thaliana	341	---KNLDQKIMDEVIAISSEEAETAKQLALKEGLVMGVISSGAAAAAAIK	387
1-A. klebsii	251	VTEQGVDAKYHEKVMLEPKVAMETSHKLARQEGIFCGVSGGATVATALD	300
C-A. thaliana	368	VAKRPENAGKLIIVVFPFSGERYLSTPLFQSIREEVEK 425	
1-A. klebsii	301	VCAEAPT*GSVVLAMIPDTAERYLSTPLFAEIDAEMDQ 338	
Cl-A. thaliana	52	DASLLIGKTPFLVFLNKVTEGCEAYVAAKQEHFQPTCSIKDRPAIAMIADA	101
1-A. klebsii	4	DITKTIGDTPIVKINKLAP*AGVELYVKIEYFNPLSSVKDRALALAI	53
Cl-A. thaliana	102	EKKKLIIPGKTTLEPTSGNMGISLAFMAAMKGYRIIMTSPSYTSLERRV	151
1-A. klebsii	54	EKSGELKPG*GTVIEATSGNTGIALAMVCAQRGYNFVSTMAASFVERRK	103
Cl-A. thaliana	152	TMRSFGAELVLTDPAKMGGTGKAYDLDLSTPDAFMCQQFANPANTQIH	201
1-A. klebsii	104	VMRMLGAKVIVTPAPLGGTGMVKKAEELAEKH*GWYLARQFENPANPEFH	153
Cl-A. thaliana	202	PDITGPEIWEEDTLGN-VDIFVMGIGSGGTVSVGVGRYLSKSNPNVKIYGV	250
1-A. klebsii	154	YKTTGQEILKDFNGKKLDYVWVTGYGTGGTFSGAGKALKEARPDVKIVLSE	203
Cl-A. thaliana	251	PAESNIILNGG-----KPGF-----HAITGNGVGFKPEILD	280
1-A. klebsii	204	PKPAPLLTSGIKQTRKEVMGKFGAPAEGHSAMTAHPIQGWTNFIPLVTE	253
Cl-A. thaliana	281	MDV---MESVLEVSSDAIKMARELALKEGLVMGVISSGANTVAAIRLAK	326
1-A. klebsii	254	QGVDAKYHEKVMLEPKVAMETSHKLARQEGIFCGVSGGATVATALDVCA	303
Cl-A. thaliana	327	MPENKGLIIVTIIASFGERYLSSVLFDELKAEAE 361	
1-A. klebsii	304	EAPT*GSVVLAMIPDTAERYLSTPLFAEIDAEMDQ 338	

Fig. 7-49. Alignment of 1-*A. klebsii* OAS-TL amino acid with *A. thaliana* isoforms.



A-A.thaliana	12	LIGNTPLVYLNNVAEGCVGRVAAKLEMMEPCCSSVKDRIGFSMISDAEKKG	61
2-A.klebsii	5	LVGNTPLIELRALSAATGGRVVGKAEFLSPGGCQKDRVAVSILAEAEATG	54
A-A.thaliana	62	LIKPGESVLIIEPTSGNTGVGLAFTAAAKGYKLIITMPASMSTERRIILLA	111
2-A.klebsii	55	RLQPG-STIVEGTSGSTGISLTLAARSRGYKVLIVMPDDQAEKVKQLLR	103
A-A.thaliana	112	FGVELVLTDPKGMKGA----IAKAEELAKTPNGYMLQQFENPANPKIH	157
2-A.klebsii	104	LGAEVELVLRPASIVSPDHVNVARRRAHELDATGGLFADQFENLANYKAH	153
A-A.thaliana	158	YETTGPDIWKGTGGKIDGFVSGIGTGGTITGAGKYLKEQNANVKLYGVEP	207
2-A.klebsii	154	FEGTGPPELWEQCCHRLDAFVMSAGTGGTIVGTGSFLKQQAPEIGVYLADV	203
A-A.thaliana	208	VESAIL 213	
2-A.klebsii	204	PGSSLL 209	
B-A.thaliana	80	AQLIGKTPMVYLNNVKGCVASVAAKLEIMEPCCSVKDRIGYSMITDAEE	129
2-A.klebsii	3	AGLVGNTPLIELRALSAATGGRVVGKAEFLSPGGCQKDRVAVSILAEAEA	52
B-A.thaliana	130	KGLITPGKSVLVESTSGNTGIGLAFIAASKGYKLIITMPASMSLERRVLL	179
2-A.klebsii	53	TGRLQPG-STIVEGTSGSTGISLTLAARSRGYKVLIVMPDDQAEKVKQLL	101
B-A.thaliana	180	RAFGAELVLTEPAKGMTGA----IQKAEELKKTTPNSYMLQQFDNPANPK	225
2-A.klebsii	102	RRLGAEVELVLRPASIVSPDHVNVARRRAHELDATGGLFADQFENLANYK	151
B-A.thaliana	226	IHYETTGPDIWEDTRGKIDILVAGIGTGGTITGVGRFIKERKPELVIGV	275
2-A.klebsii	152	AHFEGTGPPELWEQCCHRLDAFVMSAGTGGTIVGTGSFLKQQAPEIGVYLA	201
B-A.thaliana	276	EPTESAIL 283	
2-A.klebsii	202	DVPGSSLL 209	
C-A.thaliana	120	LIGKTPMVYLNLSIAKGCVANIAAKLEIMEPCCSVKDRIGYSMTVDAEQKG	169
2-A.klebsii	5	LVGNTPLIELRALSAATGGRVVGKAEFLSPGGCQKDRVAVSILAEAEATG	54
C-A.thaliana	170	FISPGKSVLVEPTSGNTGIGLAFIAASRGYRLIITMPASMSMERVLLKA	219
2-A.klebsii	55	RLQPG-STIVEGTSGSTGISLTLAARSRGYKVLIVMPDDQAEKVKQLLR	103
C-A.thaliana	220	FGAELVLTDPKAGMTGA----VQKAEELKNTPDAYMLQQFDNPANPKIH	265
2-A.klebsii	104	LGAEVELVLRPASIVSPDHVNVARRRAHELDATGGLFADQFENLANYKAH	153
C-A.thaliana	266	YETTGPDIWDDTKGKVDIFVAGIGTGGTITGVGRFIKEKNPKTQVIGVEP	315
2-A.klebsii	154	FEGTGPPELWEQCCHRLDAFVMSAGTGGTIVGTGSFLKQQAPEIGVYLADV	203
C-A.thaliana	316	TESDIL 321	
2-A.klebsii	204	PGSSLL 209	
C1-A.thaliana	56	LIGKTPLVFLNKVTEGCEAYVAAKQEHFQPTCSIKDRPAIAMIADAEEKK	105
2-A.klebsii	5	LVGNTPLIELRALSAATGGRVVGKAEFLSPGGCQKDRVAVSILAEAEATG	54
C1-A.thaliana	106	LIIPGKTTLIEPTSGNMGISLAFMAAMKGYRIIMTMPSYTSLERRVTMRS	155
2-A.klebsii	55	RLQPG-STIVEGTSGSTGISLTLAARSRGYKVLIVMPDDQAEKVKQLLR	103
C1-A.thaliana	156	FGAELVLTDPKAGMG-----GTVKKAYDLLDSTPDAFMCQQFANPANTQI	200
2-A.klebsii	104	LGAEVELVLRPASIVSPDHVNVARRRAHELDATGGLF-ADQFENLANYKA	152
C1-A.thaliana	201	HFDTTGPDIWEDTLGNVDIFVMGIGSGGTIVSGVGRYLKSKNPNVKIYGVE	250
2-A.klebsii	153	HFEGTGPPELWEQCCHRLDAFVMSAGTGGTIVGTGSFLKQQAPEIGVYLAD	202
C1-A.thaliana	251	PAESNIL 257	
2-A.klebsii	203	VPGSSLL 209	

Fig. 7-50. Alignment of 2-*A. klebsii* OAS-TL amino acid with *A. thaliana* isoforms.

A-A. thaliana	5	IADVTELGNTPLVYLNVAE--GCVGRVAAKLEMEPCSSVKDRIGFS	52
3-A. klebsii	10	ICESALDLVGFPMVMSRLQKHLDVECELVAKCEFFNAGGSSVKDRIGKR	59
A-A. thaliana	53	MISDAEKKGLIKPGESVLIPTSGNTGVGLPFAAAGYKLIITMPASMS	102
3-A. klebsii	60	MVEEAESGRKIPGD-LIIEPTSGNTGIGLCMTAAIKGYKMIICLPQKMS	108
A-A. thaliana	103	TERRIILLAFGVELVLT--DPAKMGKAIKAEIILAKTNPVYMLQQFEN	150
3-A. klebsii	109	GEKVNTMKCLGAEILRTPTEAAWDKDSHIFLSQRLAKDLGGHVLDDQYKN	158
A-A. thaliana	151	PANPKIHYETTGPPEIWKGTGGKIDGFSVGGITGGTITGAGYKLEQNAV	200
3-A. klebsii	159	PGNPLAHVEGTAEIYEQTEGKLDYVMSAGTGGTVGTALKLKEKIPGI	208
A-A. thaliana	201	KLVGVPEVSAI-----LGGKPGF-HK-----IQGIGAGFIPS	233
3-A. klebsii	209	KIVAVDPYGSILGK**PDNVNDASPRTGKRLQAYH***VEGIGYDFVPT	258
A-A. thaliana	234	VLNVDLIDEVQVSSDESIDMARQLALKEGLLVGISSGAAAAAIK-LAQ	282
3-A. klebsii	259	VLDQDVVDYWKTDDESFMGRNVVRHEGLLIGGSCGATMAGAYKFIKQ	308
A-A. thaliana	283	RFENAGKLFVAIFPSFGERYLSTVLF 309	
3-A. klebsii	309	NNIGAGKRVGVLFDSSRNYSKFMDD 335	
B-A. thaliana	75	IADNAQLIGKTPMVLNNVVK--GCVASVAAKLEIMEPCSSVKDRIGYS	122
3-A. klebsii	10	ICESALDLVGFPMVMSRLQKHLDVECELVAKCEFFNAGGSSVKDRIGKR	59
B-A. thaliana	123	MITDAEEKGLITPGKSVLVESTSGNTGIGLPIAASKGYKLIITMPASMS	172
3-A. klebsii	60	MVEEAESGRKIPG-DILIEPTSGNTGIGLCMTAAIKGYKMIICLPQKMS	108
B-A. thaliana	173	LERRVLLRAFGAELVLT--EPAKGMTGAIKAEIILKKTNPVYMLQQFDN	220
3-A. klebsii	109	GEKVNTMKCLGAEILRTPTEAAWDKDSHIFLSQRLAKDLGGHVLDDQYKN	158
B-A. thaliana	221	PANPKIHYETTGPPEIWDTRGKIDILVAGIGTGGTITGVGRFKEKRPFL	270
3-A. klebsii	159	PGNPLAHVEGTAEIYEQTEGKLDYVMSAGTGGTVGTALKLKEKIPGI	208
B-A. thaliana	271	KVIGVEPTSAI-----LGGKPGF-HK-----IQGIGAGFVFK	303
3-A. klebsii	209	KIVAVDPYGSILGK**PDNVNDASPRTGKRLQAYH***VEGIGYDFVPT	258
B-A. thaliana	304	NLDLAIVDEYIAISSEBAIETSQQLALQEGLLVGISSGAAAAAIQVAKR	353
3-A. klebsii	259	VLDQDVVDYWKTDDESFMGRNVVRHEGLLIGGSCGATMAGAYKFIKQ	308
B-A. thaliana	354	FE--NAGKLIIVFPFSGERYLS 374	
3-A. klebsii	309	NNIGAGKRVGVLFDSSRNYS 330	
C-A. thaliana	113	IADNVSLIGKTPMVLNSIAK--GCVANTAAKLEIMEPCSSVKDRIGYS	160
3-A. klebsii	10	ICESALDLVGFPMVMSRLQKHLDVECELVAKCEFFNAGGSSVKDRIGKR	59
C-A. thaliana	161	MVTDAEQKGFISPGKSVLVEPTSGNTGIGLPIAASRGYRLIITMPASMS	210
3-A. klebsii	60	MVEEAESGRKIPG-DILIEPTSGNTGIGLCMTAAIKGYKMIICLPQKMS	108
C-A. thaliana	211	MERRVLLKAFGAELVLT--DPAKGMTGAVQKAEIILKNTFPDYMLQQFDN	258
3-A. klebsii	109	GEKVNTMKCLGAEILRTPTEAAWDKDSHIFLSQRLAKDLGGHVLDDQYKN	158
C-A. thaliana	259	PANPKIHYETTGPPEIWDTRKGVDFVAGIGTGGTITGVGRFKEKNPKT	308
3-A. klebsii	159	PGNPLAHVEGTAEIYEQTEGKLDYVMSAGTGGTVGTALKLKEKIPGI	208
C-A. thaliana	309	QVIGVEPTSAI-----DILSGKPGF-HK-----IQGIGAGFIPK	341
3-A. klebsii	209	KIVAVDPYGSILGK**PDNVNDASPRTGKRLQAYH***VEGIGYDFVPT	258
C-A. thaliana	342	NLDQKIMDEYIAISSEBAIETAKQLALKEGLMVGISSGAAAAAIKVAKR	391
3-A. klebsii	259	VLDQDVVDYWKTDDESFMGRNVVRHEGLLIGGSCGATMAGAYKFIKQ	308
C-A. thaliana	392	FE--NAGKLIIVFPFSGERYLS 412	
3-A. klebsii	309	NNIGAGKRVGVLFDSSRNYS 330	
C1-A. thaliana	56	LIGKTPVFLNKVTE--GCEAVAAKQEHFPQPTCSIKDRPALMIADAER	103
3-A. klebsii	17	LVGFTPMVMSRLQKHLDVECELVAKCEFFNAGGSSVKDRIGKRVVEAEK	66
C1-A. thaliana	104	KKLIIPGKTTLIEPTSGNMGISLAFMAAMKGYRIIMTNPVSTLERRVTM	153
3-A. klebsii	67	SGRIKPGDI-LIEPTSGNTGIGLCMTAAIKGYKMIICLPQKMSGEKVNTM	115
C1-A. thaliana	154	RSFGAELVLTDPKMGMGTVKKAY--DLLDSTPDAFMCCQFANPANTQIH	201
3-A. klebsii	116	KCLGAEILRTPTEAAWDKDSHIFLSQRLAKDLGGHVLDDQYKNGNPLAH	165
C1-A. thaliana	202	FDTTGPPEIWDTRGNVDIFVMSGIGSGGTVSGVGRYLSKNFNKIVGVPE	251
3-A. klebsii	166	YEGTAEIYEQTEGKLDYVMSAGTGGTVGTALKLKEKIPGIKIVAVDF	215
C1-A. thaliana	252	AES-----NILNGGKPGPHA-----ITGNVGVFKPEILDMVDM	284
3-A. klebsii	216	YGSILGK**PDNVNDASPRTGKRLQAYH***VEGIGYDFVPTVLDQVV	265
C1-A. thaliana	285	ESVLEVSSEDAIKMARELALKEGLMVGISSGANTVAAIRLAKMPE-NKGK	333
3-A. klebsii	266	DYVKTDDDESFMGRNVVRHEGLLIGGSCGATMAGAYKFIKQNNIGAGK	315
C1-A. thaliana	334	LIVTHASFGERYLSSVLFDE 354	
3-A. klebsii	316	RVGVLFDSSRNYSKFMDD 336	

Fig. 7-51. Alignment of 3-*A. klebsii* OAS-TL amino acid with *A. thaliana* isoforms.



A- <i>A. thaliana</i>	3	SRIAKDVTELGNTPLVYLVNVAEGCVGRVAAKLEMMPECCSVKDRIGFS	52
4- <i>A. klebsii</i>	3	SDITQLLSDEFNPTPLVVLNHTVGFKAELVAKLEWCNPFVGSVKDRIAN	52
A- <i>A. thaliana</i>	53	MISDAEKKGLIKPGESVLEIPTSNTGVGLAFTAAAKGYKLIITMPASMS	102
4- <i>A. klebsii</i>	53	LVLAABEAGHLTKDTESMVEPTSNTGGLGIMMANTRRVPLTVPISTRVP	102
A- <i>A. thaliana</i>	103	TERRIILLAFGVVELVLTD---PAKGMK-GAIAKAEIILAKTFNGYMLQQ	147
4- <i>A. klebsii</i>	103	QEKRNALKLMGAKLIELDDEL*PKPGAREGATAVAEQ*MAKRNWYGPDQ	152
A- <i>A. thaliana</i>	148	FENFANPKIHVETTGPEIWKGTGGKIDGFVSGIGTGGTITGAGKYLKEQN	197
4- <i>A. klebsii</i>	153	YRNLANPEAHFRRTGPEIWKQTEGKVTFFASLGTGCTISGTGKFLK HMS	202
A- <i>A. thaliana</i>	198	AN-VKLYGVPEVESAISLGGKPGP--HKIQGIGAGFIPSVLNVLDIDEVV	244
4- <i>A. klebsii</i>	203	SGKVKVCQHPTAQHDIPGVRSLPQLHATQH*****YNTGIHDELCE	252
B- <i>A. thaliana</i>	86	TPMVYLVNVAEGCVASVAAKLEIMPECCSVKDRIGYSMITDAEKGKLIPT	135
4- <i>A. klebsii</i>	16	TPLVKLNHTVGFKAELVAKLEWCNPFVGSVKDRIANLVLAABEAGHLTK	65
B- <i>A. thaliana</i>	136	GKSVLVESTSGNTGIGLAFIAASKGYKLIITMPASMSLERRVLLRAFGE	185
4- <i>A. klebsii</i>	66	DTESMVEPTSNTGGLGIMMANTRRVPLTVPISTRVPQEKRNALKLMGAK	115
B- <i>A. thaliana</i>	186	LVLTE---PAKGMT-GAIQKAEIILKKTNPNSYMLQQFDNPNPKIHVET	230
4- <i>A. klebsii</i>	116	LIELDDEL*PKPGAREGATAVAEQ*MAKRNWYGPDQVRNLANPEAHFRT	165
B- <i>A. thaliana</i>	231	TGPEIWEEDTRGKIDILVAGIGTGGTITGVGRFIKERKP-ELKVI GVEPTE	279
4- <i>A. klebsii</i>	166	TGPEIWKQTEGKVTFFASLGTGCTISGTGKFLKHMSSGKVKVCQHPTA	215
B- <i>A. thaliana</i>	280	SAILSGGKPGP--HKIQGIGAGFVFNKLDLAVDEYIAISSEEA IETSKQ	327
4- <i>A. klebsii</i>	216	QHDIPGVRSLPQLHATQH*****YNTGIHDELCEVTNEEAFQMCLR	265
B- <i>A. thaliana</i>	328	LALQEGLLVGISSGAAAAAIIQVAK-RPENAGKLI	361
4- <i>A. klebsii</i>	266	LNREESLIAGPSSGLQVVGAMKLMEDKPGNVGVII	300
C- <i>A. thaliana</i>	112	NIADNVSQLIGKTPMVVLSIAKGCVANIAAKLEIMPECCSVKDRIGYSM	161
4- <i>A. klebsii</i>	4	DITQLLSDEFNPTPLVVLNHTVGFKAELVAKLEWCNPFVGSVKDRIANL	53
C- <i>A. thaliana</i>	162	VTDAEQKGFISPGKSVLVEPTSNTGIGLAFIAASRGYRLIITMPASMSM	211
4- <i>A. klebsii</i>	54	VLAABEAGHLTKDTESMVEPTSNTGGLGIMMANTRRVPLTVPISTRVPQ	103
C- <i>A. thaliana</i>	212	ERRVLLKAFGAELVLTD---PAKGMT-GAVQKAEIILKNTPDAYMLQQF	256
4- <i>A. klebsii</i>	104	EKRNALKLMGAKLIELDDEL*PKPGAREGATAVAEQ*MAKRNWYGPDQY	153
C- <i>A. thaliana</i>	257	DNPNPKIHVETTGPEIWDITKGVDFVAGIGTGGTITGVGRFIKERKP	306
4- <i>A. klebsii</i>	154	RNLANPEAHFRRTGPEIWKQTEGKVTFFASLGTGCTISGTGKFLKHMSS	203
C- <i>A. thaliana</i>	307	-KTQVIGVEPTESDILSGGKPGP--HKIQGIGAGFIPKNDQKIMDEVIA	353
4- <i>A. klebsii</i>	204	GKVKVCQHPTAQHDIPGVRSLPQLHATQH*****YNTGIHDELCE	253
C- <i>A. thaliana</i>	354	ISSEEA IETAKLALKEGLMVGISSGAAAAAIIKQVAK-RPENAGKLI	399
4- <i>A. klebsii</i>	254	VTNEEAFQMCLR LNREESLIAGPSSGLQVVGAMKLMEDKPGNVGVII	300
C1- <i>A. thaliana</i>	60	TPLVFLNKVTEGCEAYVAAKQEHFQPTCSIKDRPAIAMIADA EKKKLIIP	109
4- <i>A. klebsii</i>	16	TPLVKLNHTVGFKAELVAKLEWCNPFVGSVKDRIANLVLAABEAGHLTK	65
C1- <i>A. thaliana</i>	110	GKTTLIEPTSGNMGISLAFMAAMKGYRIIMTMSVYTSLERRVTMRSFGAE	159
4- <i>A. klebsii</i>	66	DTESMVEPTSNTGGLGIMMANTRRVPLTVPISTRVPQEKRNALKLMGAK	115
C1- <i>A. thaliana</i>	160	LVLTD---PAKGMG-GTVKKAYDLLDSTPD AFMCQQFANPANTQIHFDT	204
4- <i>A. klebsii</i>	116	LIELDDEL*PKPGAREGATAVAEQ*MAKRNWYGPDQVRNLANPEAHFRT	165
C1- <i>A. thaliana</i>	205	TGPEIWEEDTLGNVDIFVMGIGSGGTVSGVGRVYLSKNPN-VKIYGVPEAE	253
4- <i>A. klebsii</i>	166	TGPEIWKQTEGKVTFFASLGTGCTISGTGKFLKHMSSGKVKVCQHPTA	215
C1- <i>A. thaliana</i>	254	SNILNGGKPGP--HAITGNVGFKPEILDMDVMSVLEVSEDAIKMARE	301
4- <i>A. klebsii</i>	216	QHDIPGVRSLPQLHATQH*****YNTGIHDELCEVTNEEAFQMCLR	265
C1- <i>A. thaliana</i>	302	LALKEGLMVGISSGANTVAAIRLAK-MPENK GKLI	335
4- <i>A. klebsii</i>	266	LNREESLIAGPSSGLQVVGAMKLMEDKPGNVGVII	300

Fig. 7-52. Alignment of 4-*A. klebsii* OAS-TL amino acid with *A. thaliana* isoforms.

## 7.4. The amino acid code

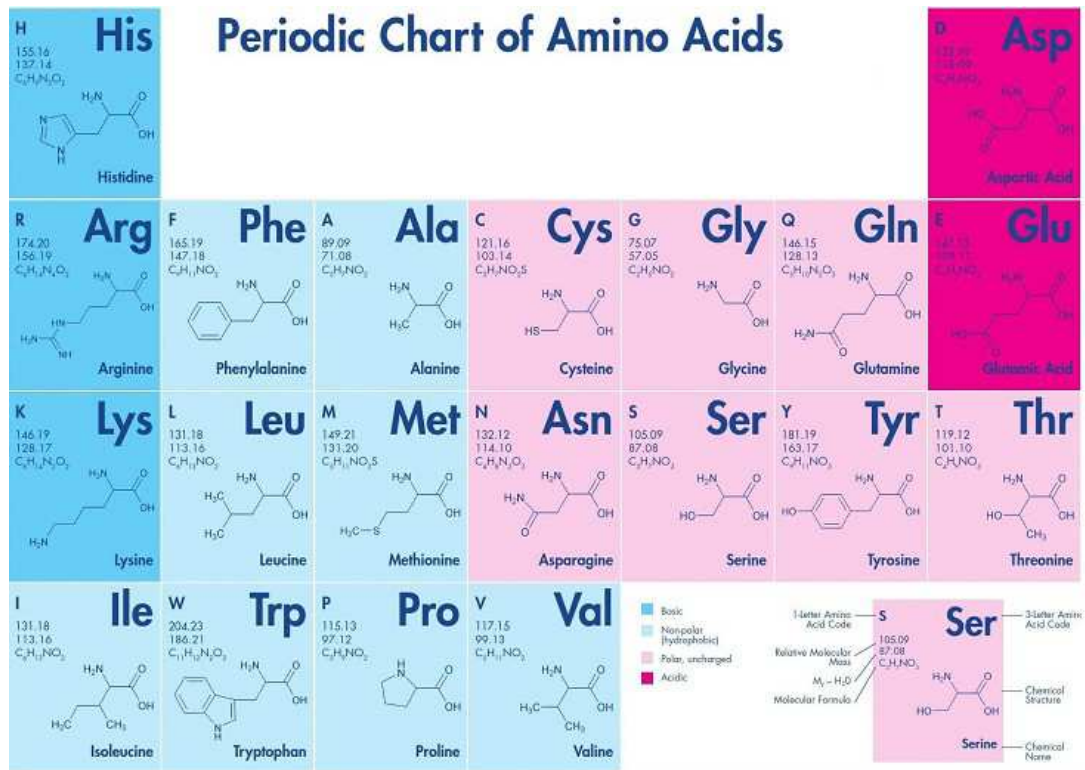


Fig. 7-53. Amino acid code and relative character.



# ACKNOWLEDGEMENTS

First of all, I would like to say thanks to my supervisor Mario Giordano for all useful suggestions he gave me during my whole project. Just after, I must express my deeply and sincerely thanks, Alessandra Norici: when I arrived in Italy my life seems linked with her. She trained me in algal culturing techniques and in the comprehension of lab protocols.

I'm particularly grateful to the whole research group of Prof. Rüdiger Hell Centre for Organismal Studies (COS), Heidelberg Plant Molecular Biology, where I spent an unforgettable period. Not only because the cooperation time, but especially for the people I met. Thanks, guys! I'll never forget you. Especially, Shanshan Wang and Eric Linster!

Deeply thanks for the Masbic of Prof Tiziana cacciamani research group. Thanks for your whole group share the time with my long time working schedule. Dr. Valentina Pozzi and Dr. Enrico Junior Baldassarri, two of these young talent researchers are good research cooperators friends.

It's my great honor to meet the following lab mates: Larisa, Marianna Venuleo, Laura, Andrea Pessina, Lucia Gastoldi, Angela Anxhela, Givonna Simonetti. All these guys are very nice :)

Thanks to my neighbor lab research groups: Prof. Carnevali, Prof. Beolchini, Prof. Cecelia, Prof. Totti and Prof. Danovaro. All of these research groups in our department composed a big research family, I was lucky that I could join them.

Luca Lambertucci and Simone Bellagamba you always smile.

Monica Ferraioli thanks for you pay a lot of attention to me. Even you are so busy for dealing with a lot of document, while every time you send the document keeps two languages (English and Italiano) notice.

My big present in Italy I can meet two sisters family Viviana Fonti and Laura Rocchetti. Thanks you bring a lot of unforgettable memory. I will miss you no matter where I will be in the future.



During the past three years study, even far from my homeland, while all of these nice people in my life are all the big surprise from the God. Thanks so much all of you give me the permission I can share these three year with all of you :)

My heart will always be with you mum, dad, and all my family. Thank you always stand on my back. Waiting for my long time hug and big kiss:)

Last but not least, thanks the reviewers.

Anyway, all these nice people I mentioned above rise me up :)

