2015

The Taurine Biosynthetic Pathway of Microalgae

Rahul Tevatia

University of Nebraska-Lincoln, rahultevatia_83@yahoo.co.in

James Allen

University of Nebraska-Lincoln, jallen7@unl.edu

Deepak Rudrappa

University of Nebraska-Lincoln

Derrick White

University of Nebraska-Lincoln, derrick.white@yahoo.com

Thomas E. Clemente

University of Nebraska-Lincoln, tclemente1@unl.edu

See next page for additional authors

Follow this and additional works at: <http://digitalcommons.unl.edu/plantscifacpub>

Part of the [Plant Biology Commons](#), [Plant Breeding and Genetics Commons](#), and the [Plant Pathology Commons](#)

Tevatia, Rahul; Allen, James; Rudrappa, Deepak; White, Derrick; Clemente, Thomas E.; Cerutti, Heriberto; Demirel, Yaşar; and Blum, Paul H., "The Taurine Biosynthetic Pathway of Microalgae" (2015). *Faculty Publications from the Center for Plant Science Innovation*. 166.

<http://digitalcommons.unl.edu/plantscifacpub/166>

This Article is brought to you for free and open access by the Plant Science Innovation, Center for at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Faculty Publications from the Center for Plant Science Innovation by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Authors

Rahul Tevatia, James Allen, Deepak Rudrappa, Derrick White, Thomas E. Clemente, Heriberto Cerutti, Yaşar Demirel, and Paul H. Blum

Published in *Algal Research* 9 (2015), pp. 21–26; doi: 10.1016/j.algal.2015.02.012

Copyright © 2015 Elsevier B.V. Used by permission.

Submitted October 2, 2014; revised December 31, 2014; accepted February 8, 2015; published online February 27, 2015.

Supporting information for this article is available following the references.

The Taurine Biosynthetic Pathway of Microalgae

Rahul Tevatia,^{1,2} James Allen,³ Deepak Rudrappa,¹ Derrick White,¹

Thomas E. Clemente,^{4,5} Heriberto Cerutti,^{1,5} Yaşar Demirel,²

and Paul Blum¹

1. School of Biological Sciences, University of Nebraska–Lincoln, Lincoln Nebraska, USA
2. Department of Chemical and Biomolecular Engineering, University of Nebraska–Lincoln, Lincoln, Nebraska, USA
3. Department of Biochemistry, University of Nebraska–Lincoln, Lincoln, Nebraska, USA
4. Department of Agronomy and Horticulture, University of Nebraska–Lincoln, Lincoln, Nebraska, USA
5. Center for Plant Science Innovation, University of Nebraska–Lincoln, Lincoln, Nebraska, USA

Corresponding author – Paul Blum, email pblum1@unl.edu

Abstract

Taurine (2-aminoethanesulfonic acid) is an amino acid-like compound widely distributed in animals and an essential nutrient in some species. Targeted metabolomics of marine and freshwater microalgae combined with medium supplementation identified biosynthetic pathway intermediates and necessary catalytic activities. Genomic analysis was then used to predict the first taurine biosynthetic pathway in these organisms. MRM-based electrospray ionization (ESI) LC–MS/MS analysis demonstrated that taurine is synthesized using a carbon backbone from L-serine combined with sulfur derived from sulfate. Metabolite analysis showed a nonuniform pattern in levels of pathway intermediates that were both species and supplement dependent. While increased culture salinity raised taurine levels modestly in marine alga, taurine levels were strongly induced in a freshwater species implicating taurine as an organic osmolyte. Conservation of the synthetic pathway in algae and metazoans together with a pattern of intermittent distribution in other lineages suggests that it arose early in

eukaryotic evolution. Elevated levels of cell-associated taurine in algae could offer a new and biorenewable source of this unusual bioactive compound.

Keywords: targeted metabolomics, microalgae, taurine, bioinformatics, osmolytes

1. Introduction

Taurine, 2-aminoethanesulfonic acid, is a unique sulfur-containing free amino acid because it does not form peptide bonds with other amino acids. Consequently, taurine can be an abundant small molecule in animal blood and tissues [1–3]. There are multiple biosynthetic pathways for taurine synthesis in animals (Fig. 1). Some species utilize cysteine and/or methionine to synthesize taurine referred to here as the CDO/CSAD pathway [1–3]. In this pathway, cysteine is first oxidized to cysteine sulfinic acid by cysteine dioxygenase (CDO) [4] that is then decarboxylated to form hypotaurine by cysteine sulfinic acid decarboxylase (CSAD) or glutamate decarboxylase (GAD) [5]. Hypotaurine undergoes oxidation to form taurine in what is thought to be a spontaneous process as no enzymes have been reported that catalyze this reaction [1,3]. A second animal pathway referred to here as the Serine/Sulfate pathway, occurs in chick livers and developing chick embryos and is instead repressed by cysteine [6]. This pathway utilizes inorganic sulfate to form sulfonate-2-aminocrylate followed by cysteine in a reaction coupled to the conversion of adenosine-3'-phosphate-5'-phosphosulfate (PAPS) to adenosine-3',5'-diphosphate (PAP). Cysteine is then decarboxylated to form taurine [7]. Bacterial taurine synthesis remains unclear though exogenous cysteine sulfinic acid supplementation enables accumulation of intracellular taurine indicating the presence of a CSAD homolog [8]. In many bacteria, taurine catabolism involves deamination of taurine to sulfoacetaldehyde followed by desulfonation producing acetyl-phosphate and sulfite [9]. Finally, acetyl-phosphate is either assimilated via the Krebs cycle or converted to acetate via substrate level phosphorylation [9]. Plants and fungi are currently not known to synthesize taurine, however a growth stimulatory response has been reported for plants [10].

Figure 1. Taurine metabolism in various biological systems (CDO = cysteine dioxygenase, CSAD = cysteine sulfinic acid decarboxylase, GAD = glutamate decarboxylase, TDH = taurine dehydrogenase, Xsc = sulfoacetaldehyde acetyltransferase, Ack = acetate kinase, Pta = phosphotransacetylase, ATP-sulfurylase = ATP:sulfate adenylyltransferase, APS kinase = adenylyl-sulfate kinase, PAPS-AS = 3'-phosphoadenylyl sulfate:2-aminoacrylate C-sulfotransferase). The CDO/CSAD pathway is found in animals [1–5]; the Serine/Sulfate pathway is found in developing chick embryos [6], chick liver [7], and microalgae (present study); the XDH/Xsc pathway is found in bacteria [8,9].

In contrast to animals, plants, and bacteria, algal taurine metabolism is understudied. The presence of taurine has been confirmed in selected dinoflagellates, prasinophytes, rhodophytes, and diatoms but not in prymnesiophytes or chlorophytes [11–14]. However in most cases taurine identification was based purely on migration with standards during HPLC and not more on definitive and sensitive methods such as mass spectrometry. Analysis of heterotrophic dinoflagellates suggests that taurine can be correlated with chlorophyll *a* [15,16] in some as yet unknown manner. However, the metabolic pathway(s) resulting in taurine formation in algae has not been reported. In the present study, algal species were selected based on the following criteria: (i) prior reports regarding production of taurine by the species; (ii) the availability of a genome sequence; and (iii) the potential applications of the algal species. Here a combination of metabolomics combined with chemical supplementation and genomic analyses enabled the identification of the first taurine biosynthetic pathway in algae. These results may encourage the use of algae as a biological source of taurine.

2. Materials and methods

2.1. Microalgae strains and culturing

Chlamydomonas reinhardtii CC124 (laboratory collection), *Tetraselmis* sp. (PLY 429, Reed Mariculture Inc., USA), and *Ostreococcus tauri* (RCC 745, Roscoff Culture Collection, France) were used for experimental studies, and were grown in TAP [17], f/2 modified [18], or K medium [19], respectively. All cultures were grown in 25 mL of their respective media in 125 mL shake flasks. The conditions were maintained at 25°C under continuous illumination (light intensity $\sim 50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and agitation (180 rpm). The growth curves were prepared by plotting respective OD₅₅₀ values with time. All the experiments were performed under aseptic conditions in triplicate. Samples were taken for dry weight analysis and centrifuged at $12,000 \times g$ for 15 min. Pellets were dried in a SpeedVac (Savant, USA) and then used for dry weight analysis.

2.2. Sulfur compound or serine supplementation

Ten different sulfur compounds were investigated for their effect on growth physiology and taurine levels in *O. tauri*. These compounds were chemical grade quality purchased from Sigma-Aldrich, USA. The stock solutions of filter sterilized sulfur compounds were aseptically added to sterile K-medium at 6 mM final concentration.

2.3. Salinity effect

Sea salt (Sigma-Aldrich, USA) consisted of: chloride (19.29 g/L), sodium (10.78 g/L), sulfate (2.66 g/L), potassium (420 mg/L), calcium (400 mg/L), bicarbonate (200 mg/L), strontium (8.8 mg/L), boron (5.6 mg/L), and bromide (56 mg/L). TAP (freshwater algal medium) contained 0% sea salts, whereas f/2 modified media (marine microalgal medium) had 1.6% (w/v) sea salts (both of these media were used as controls). The sea salts were added in 1.6% (w/v) increments to the salts present in the respective media. All solutions were autoclaved prior to use. Cell pellets were stored at -20°C until taurine analysis was performed.

2.4. Extraction of intracellular amino acids

Total intracellular amino acid pools were extracted from selected microalgae grown in their respective media. The cells were centrifuged at $12,000 \times g$ and then suspended in 1 mL HPLC grade water (VWR). Samples were sonicated five successive times for 30 s followed each time by a 30 s interval on ice. Sonicated samples were then incubated at 65°C for 1 h and then centrifuged at $15,600 \times g$ at 4°C for 20 min. The supernatant was then used for LC-MS/MS analysis. All algal samples were processed in triplicate.

2.5. Amino acid analysis using LC-MS/MS

Sulfur containing amino acids and related compounds was quantified from algal extracts with a Shimadzu UFLC-XR (Shimadzu Scientific Instruments, Inc., Columbia, MD) and an AB SCIEX Q-Trap 4000 LC-MS/MS (AB SCIEX, Framingham, MA) electron spray ionization detector. Isocratic chromatographic separations were achieved within 8 min using

a Zorbax Eclipse plus 3.5 μm , 150 $\times$ 4.6 mm reverse phase C18 column (Agilent Technologies, Santa Clara, CA) and a 10 mM aqueous ammonium acetate solution (pH 9.3) [20] at a constant flow rate of 0.3 mL min⁻¹ and 40°C column temperature. The column was washed for 2 min with methanol between sample runs and allowed to reequilibrate for 3 min prior to the next injection to remove photosynthetic pigments. Taurine, hypotaurine, cysteic acid, methionine, cysteine, and norvaline were analyzed in positive ionization mode. Cysteine sulfinic acid was analyzed in negative mode (SI_01 Figs. S1–S9). Multiple reaction monitoring (MRM) was used to obtain peak areas. Optimal depolarization potentials, collision energies, precursor and product ions were obtained experimentally through direct MS infusion experiments (Table 1), and the respective values for standard cysteine, taurine, norvaline, and methionine corresponded with published values [21]. MRM peak areas were compared with calibration graphs of known standard concentrations measured before and after each run. Quantities were extrapolated using norvaline to calculate the internal response factor of amino acids during sample processing.

Table 1. Results of optimal multiple reaction monitoring (MRM), depolarization potentials (DP), collision energies (CE), precursor and product ions (transition) empirically derived from MS infusion experiments (CYS: cysteine; CSA: cysteine sulfinic acid; CA: cysteic acid; HYP: hypotaurine; TAU: taurine; NVA: norvaline).

	MRM transition	DP	CE
HPT	110 > 92	10	18
NVA	118 > 72	10	18
CYS	122 > 76	15	20
TAU	126 > 108	15	8
MET	150 > 104	10	14
CA	170 > 124	50	5
CSA	172 > 155	-100	-20

2.6. Bioinformatic analysis

A phylogenetic tree was constructed using 18s rRNA sequences of microalgae obtained from various taxonomic divisions available in the NCBI database. Sequence alignments and phylogenetic analysis were performed using the neighbor-joining method implemented in MEGA 6.0 software [22] with 1000 bootstrap replicates. The Domain Enhanced Lookup Time Accelerated BLAST (DELTA-BLAST, NCBI) [23] was used to identify enzymes involved in all possible taurine biosynthetic pathways in known microalgal genome sequences using respective conserved domains as query sequences (SI_01 Tables S1–S12).

3. Results

3.1. Effect of sulfur supplementation

As taurine is a sulfonic acid, it seemed plausible that taurine abundance might be influenced by sulfur supplementation. Ten sulfur compounds were therefore tested at a concentration of 6 mM as algal medium supplements using the genome sequenced marina alga and chlorophyte, *O. tauri* (Fig. 2a). Growth was unaffected by methionine, sodium

thiosulfate, sodium sulfite, sodium sulfate, or elemental sulfur but inhibited by potassium tetrathionate or sodium sulfide. In contrast, the addition of sodium metabisulfite, cysteine, or cysteine and methionine in combination had cytotoxic effects as culture absorbance values decreased during incubation (Fig. 2a). LC-MS/MS-based metabolic profiling was performed on samples from cultures where growth was normal in the presence of added sulfur compounds to identify their respective intracellular taurine levels. Taurine was evident in the untreated control (0.5 mg/g-DW) demonstrating chlorophytes can synthesize this compound, while the addition of methionine, sodium thiosulfate, sodium sulfite, and elemental sulfur did not alter basal levels. However, upon supplementation with sodium sulfate, levels of taurine increased three-fold to 1.5 mg/g-DW (Fig. 2b). This indicates that sodium sulfate addition had a positive effect on taurine biosynthesis in this organism. Further, a linear relationship was observed between taurine production in *O. tauri* and sodium sulfate supplementation in K-medium with an $R^2 = 0.968$ (Fig. 2c). The intercept represents the amount of taurine produced when the cells are not supplemented with additional sulfate and was calculated to be 0.597mg/g-DW, consistent with the control. This positive linear correlation suggests that the initial concentration of sulfate has a direct influence on taurine production in *O. tauri*.

Figure 2. Effect of various sulfur compounds on the growth and taurine concentration in *Ostreococcus tauri*: (a) growth curve of *O. tauri* grown in K medium supplemented with various sulfur compounds, (b) taurine concentration of *O. tauri* grown in various sulfur compounds, (c) taurine levels in *O. tauri* grown in various concentration of sodium sulfate.

3.2. Effect of increased salinity

As taurine was evident in *O. tauri*, it seemed reasonable to examine other chlorophytes for this compound. Since prior studies have demonstrated that the exogenous addition of taurine to growth media improves salt tolerance in algae [24,25] suggesting some interplay in osmoregulation, the effect of salinity on sulfur-containing intracellular amino acids, taurine and its precursor, hypotaurine was examined. Both freshwater (*C. reinhardtii*) and marine (*Tetraselmis* sp.) microalgae were grown to high cell density (OD₅₅₀ of 1.2) and resuspended in their respective media with sea salt exceeding the control media concentrations by 1.6%. Table 2 lists the changes in sulfur amino acids in both microalgae. Taurine levels increased in response to elevated salinity in both species. Taurine in *C. reinhardtii* increased from < 0.01 $\mu\text{g/g-DW}$ to 150.7 ± 8.89 $\mu\text{g/g-DW}$, whereas in *Tetraselmis* sp. taurine increased from

687.0 ± 140.1 µg/g-DW to 1635.3 ± 332.6 µg/g-DW (2.4 fold increase). These data indicated that taurine synthesis was highly inducible in freshwater algae and was largely constitutive in marine algae, consistent with the hypothesis that this compound is involved in osmoregulation.

Table 2. Free intracellular amino acid pool analysis by targeted metabolomics using LC–MS/MS of freshwater (*Chlamydomonas reinhardtii*) and marine microalgae (*Tetraselmis* sp.) supplemented with sulfur compounds, serine, or sea salts (MET: methionine; CYS: cysteine; CSA: cysteine sulfinic acid; CA: cysteic acid; HYP: hypotaurine; TAU: taurine).

Free amino acid conc. (µg/g-DW)	<i>Chlamydomonas reinhardtii</i>				<i>Tetraselmis</i> sp.			
	Supplementation in TAP medium				Supplementation in f/2 medium			
	Control (0% salt)	Na ₂ SO ₄ (6 mM)	Serine (6 mM)	Salt (increased by 1.6%)	Control (1.6% salt)	Na ₂ SO ₄ (6 mM)	Serine (6 mM)	Salt (increased by 1.6%)
MET	3.5 ± 0.25	8.0 ± 0.10	68.0 ± 3.14	1.50 ± 0.27	6.87 ± 3.01	89.9 ± 3.51	130.0 ± 6.53	18.7 ± 10.4
CYS	1.19 ± 0.14	16.7 ± 0.84	82.1 ± 4.92	0.19 ± 0.12	12.0 ± 1.4	7.3 ± 0.34	7.5 ± 0.33	0.44 ± 0.21
CSA	0.14 ± 0.01	4.4 ± 0.11	4.6 ± 0.18	0.51 ± 0.09	2.5 ± 0.65	4.4 ± 0.22	6.6 ± 0.27	1.99 ± 0.96
CA	0.15 ± 0.03	2.6 ± 0.02	30.7 ± 0.91	1.65 ± 0.42	6.8 ± 2.01	31.6 ± 1.58	45.2 ± 3.16	34.6 ± 5.42
HYP	< 0.01	1.9 ± 0.05	0.33 ± 0.01	0.06 ± 0.02	0.25 ± 0.03	1.5 ± 0.19	1.2 ± 0.04	0.22 ± 0.10
TAU	< 0.01	127.0 ± 6.35	155.0 ± 10.86	150.7 ± 8.89	687.0 ± 140.1	1480.0 ± 141.3	1280.0 ± 192.4	1635.3 ± 332.6

3.3. Bioinformatic analysis

Based on the metabolic and physiologic findings that taurine did indeed occur in algae thought previously not to synthesize this compound, a comprehensive examination of algal genomes was conducted for the possible taurine synthetic pathways. A neighbor joining phylogenetic tree was constructed using 18S rRNA sequences of algae from different taxonomic classes using MEGA 6.0 software [22] with bootstrap values of 1000 replicates. Algae used for building the phylogenetic tree had either sequenced genomes or showed the presence of taurine (based on empirically determined results or literature values). Bioinformatic analysis by domain based protein search used DELTA-BLAST [23] was performed with enzyme sequences associated with CDO/CSAD [26], Serine/Sulfate taurine biosynthetic pathways and TDH/Xsc taurine catabolism (Fig. 1). In addition, an examination of plant, fungi, human, and chicken genomes was included. In microalgal genomes of *C. reinhardtii*, *Coccomyxa subellipsoidea*, *O. tauri*, *Thalassiosira pseudonana*, *Aureococcus anophagefferens*, *Bigelowiella natans*, *Chlorella variabilis*, *Cyanidioschyzon merolae*, *Fragilariopsis cylindrus*, *Guillardia theta*, *Micromonas pusilla*, *Phaeodactylum tricornutum*, *Pseudo-nitzschia multiseries*, and *Volvox carteri* (SI_01 Tables S1–S12) bioinformatic analysis showed that homologous enzymes involved in the CDO/CSAD biosynthetic pathway or TDH/Xsc (bacteria) taurine catabolism were absent. However, in all of the microalgae tested homologous sequences were present for all enzymes of the Serine/Sulfate pathway with the exceptions of *F. cylindrus* and *P. multiseries* where none of the enzymes were apparent. A summary of the bioinformatic analysis is presented (Fig. 3) and suggests broad though not complete conservation of the “Serine/Sulfate” pathway in microalgae. Additional analysis of 60 higher plant genomes indicated variable distribution of the Serine/Sulfate pathway with only one third of species containing all necessary genes (SI_02). In contrast, analysis of 102 fungal genomes indicated the complete absence of the Serine/Sulfate pathway (SI_02).

Figure 3. Phylogenetic tree and presence/absence of various enzymes involved in taurine pathway using domain based bioinformatics analysis (18s rRNA phylogenetic tree of algae. The tree was constructed using MEGA 6.0. The bootstrap values were based on 1000 replicates. *Mus musculus*, *Homo sapiens*, and *Gallus gallus* were used as an out-group. Bootstrap values greater than 50% are shown).

3.4. Confirmation of the Serine/Sulfate pathway

The presence of the Serine/Sulfate pathway in selected algal species was tested using cells that were grown in media supplemented with either sulfate, serine, or cysteine and methionine combined, and pathway intermediates were quantified using multiple reaction monitoring (MRM) LC-MS/MS (Table 2, SI_01 Figs. S1.1–S1.9). Sulfate supplementation increased cysteic acid levels in *C. reinhardtii* by 22.7 fold and 4.6 fold in *Tetraselmis* sp. PLY429. Taurine quantities also increased in *C. reinhardtii* from below the limit of detection ($< 0.01 \mu\text{g/g-DW}$) to $127.0 \pm 6.35 \mu\text{g/g-DW}$. In contrast, levels increased only 2.2 fold in *Tetraselmis* sp. PLY429 above initial concentrations. Serine addition had similar stimulatory effects in *C. reinhardtii* as cysteic acid levels increased 60-fold while taurine increased over 1000-fold. When *Tetraselmis* sp. PLY429 was provided with exogenous serine both cysteic acid and taurine levels increased but modestly and comparably to sulfate addition at 6.6 and 1.9 fold, respectively. In addition, serine and sulfate supplementation increased cysteine sulfinic acid and hypotaurine pools (intermediates in CDO/CSAD pathway) in *C. reinhardtii*, whereas in *Tetraselmis* sp. PLY429 these pools were unaffected. Since the effect of serine and sulfate supplementation on cysteine, methionine, cysteine sulfinic acid, and hypotaurine pools (CDO/CSAD path-

way) was variable across tested species there is a lack of conservation in associated metabolic pathways. In contrast, serine and sulfate addition consistently increased cysteic acid and taurine levels (Serine/Sulfate pathway), supporting the hypothesis that the Serine/Sulfate pathway is conserved in these algal lineages.

4. Discussion

An MRM-based electrospray ionization (ESI) LC-MS/MS method was developed for the detection of taurine, hypotaurine, and taurine pathway intermediates (i.e., cysteic acid and cysteine sulfinic acid) in microalgae. Combined with physiologic manipulations including nutrient supplementation and osmotic challenge together with bioinformatic analysis, the Serine/Sulfate taurine biosynthetic pathway was identified as a broadly conserved feature of the algal lineage. The MRM method does not require metabolite alteration and is therefore advantageous compared to more traditional HPLC-detection methods which normally require ortho-phthaldialdehyde derivatization [14–16, 27–29]. Analytical methods utilizing NMR spectroscopy [30] or electrochemical detection (ECD) LC-MS/MS [31] have also been reported but lack the sensitivity required for measuring taurine biosynthetic pathway intermediates at the trace quantities evident in this work. The use of the ESI-LC-MS/MS method enabled a metabolite-based determination of the presence of specific pathways and complemented the use of bioinformatic analyses.

The CDO/CSAD pathway is thought to be the taurine biosynthetic pathway in animals and involves cysteine dioxygenase (CDO) and cysteine sulfinic acid decarboxylase (CSAD)/glutamate decarboxylase (GAD) that convert cysteine to taurine [1–3]. In contrast, the Serine/Sulfate pathway was first reported using certain developmental stages of chickens [6,7] and involves the PAPS (adenosine-3'-phosphate-5'-phosphosulfate) pathway to convert sulfate to cysteate and then to taurine. In this pathway, taurine is produced as a result of the use of sulfur from sulfate in the carbon backbone of L-serine. Based on the bioinformatic studies reported here, the Serine/Sulfate pathway is also present in animals including humans, mice and chickens and therefore may represent a previously under recognized route for taurine production in these organisms.

In plants and algae, sulfur assimilation is chloroplast-associated where photosynthetic reductant and ATP reduce sulfur compounds (such as sulfate) for biosynthesis of sulfur-containing amino acids like methionine and cysteine [32,33]. Since taurine contains sulfur, the effect of sulfur supplementation on taurine synthesis was investigated. *O. tauri*, marine microalgae, was used for the study of effects of various sulfur compounds on growth and taurine synthesis because it is a broadly distributed marine species [34,35] that might encounter environments containing high sulfur compounds. For example, the presence of large amounts of hypotaurine and thiotaurine (a derivative of taurine, which serves as an organic osmolyte) has been shown in invertebrates growing in sulfide rich environments near hydrothermal vents and cold seeps of the deep oceans [36]. *O. tauri* tolerated five of ten sulfur compounds but only one of them, sulfate increased taurine levels. In addition, similar results were obtained with fresh and marine algal species indicating conservation of this response.

Since taurine levels increased in response to osmotic stress in both marine and freshwater algal species, taurine appears to function as an organic osmolyte in these organisms. The external addition of taurine in hypersaline conditions has been shown to improve microalgae growth [24]. However, in these studies, the amount of intracellular amino acids was not investigated. Taurine has been reported to have a role as an organic osmolyte in diatoms, animals, and mammals [25,37]. Therefore, based on the results presented here, taurine production during high saline conditions may be a result of salt stress and acting as an osmolyte confer protection on both freshwater and marine algae.

Sulfate and serine supplementation in *C. reinhardtii* improved the levels of intermediates of both CDO/CSAD and Serine/Sulfate pathways. This indicated that either or both pathways might be present in these freshwater microalgae. In the marine microalgae, *Tetraselmis* sp., both sulfate and serine supplementation increased the levels of methionine, cysteic acid (Serine/Sulfate pathway intermediate), and taurine. However, the levels of cysteine (precursor of CDO/CSAD pathway), cysteine sulfinic acid, and hypotaurine (intermediates of CDO/CSAD pathway) did not change. Genomic analyses clarified these results by identifying genes sufficient for the “Serine/Sulfate” pathway but not those for alternative routes of taurine synthesis or catabolism.

5. Conclusion

Taurine has been reported in a broad range of algal species. Here, LC-MS/MS-based metabolite profiling combined with bioinformatic analysis was performed on selected freshwater and marine algal species to determine the identity of the taurine biosynthetic pathway. Specific nutrient supplementation showed a direct involvement of serine and sulfate in taurine synthesis. The data were consistent with the presence of the “Serine/Sulfate pathway” and the exclusion of alternate taurine biosynthetic pathways and indicated that taurine likely functions as an osmolyte in these organisms.

Conflict of interest – The authors declare no competing financial interest.

Acknowledgments – This research was supported by a grant from the United soybean Board and Nebraska Soybean Board (grant number 26-1215-0065-001) and the UNL Cell Development Facility (CDF).

References

- [1] R.J. Huxtable, Physiological actions of taurine, *Physiol. Rev.* 72 (1992) 101–163.
- [2] M.H. Stipanuk, Sulfur amino acid metabolism: pathways for production and removal of homocysteine and cysteine, *Annu. Rev. Nutr.* 24 (2004) 539–577.
- [3] J.G. Jacobsen, L.H. Smith, Biochemistry and physiology of taurine and taurine derivatives, *Physiol. Rev.* 48 (1968) 424–511.
- [4] C.A. Joseph, M.J. Maroney, Cysteine dioxygenase: structure and mechanism, *Chem. Commun.* (2007) 3338–3349.

- [5] A.A. Jerkins, R.D. Steele, Quantification of cysteine sulfinic acid decarboxylase in male and female rats: effect of adrenalectomy and methionine, *Arch. Biochem. Biophys.* 294 (1992) 534–538.
- [6] L.J. Machlin, P.B. Pearson, C.A. Denton, The utilization of sulfate sulfur for the synthesis of taurine in the developing chick embryo, *J. Biol. Chem.* 212 (1955) 469–475.
- [7] N.L. Sass, W.G. Martin, The synthesis of taurine from sulfate III. Further evidence for the enzymatic pathway in chick liver, *Exp. Biol. Med.* 139 (1972) 755–761.
- [8] G. Agnello, L.L. Chang, C.M. Lamb, G. Georgiou, E.M. Stone, Discovery of a substrate selectivity motif in amino acid decarboxylases unveils a taurine biosynthesis pathway in prokaryotes, *ACS Chem. Biol.* 8 (2013) 2264–2271.
- [9] A.M. Cook, K. Denger, Metabolism of taurine in microorganisms: a primer in molecular biodiversity? *Adv. Exp. Med. Biol.* 583 (2006) 3–13.
- [10] L.H. Hao, P.Q. He, C.Y. Liu, K.S. Chen, G.Y. Li, Physiological effects of taurine on the growth of wheat (*Triticum aestivum* L.) seedlings, *Zhi Wu Sheng Li Yu Fen Zi Sheng Wu Xue Bao* 30 (2004) 595–598.
- [11] H. Kataoka, N. Ohnishi, Occurrence of taurine in plants, *Agric. Biol. Chem.* 50 (1986) 1887–1888.
- [12] B. Lindberg, Methylated taurines and choline sulphate in red algae, *Acta Chem. Scand.* 9 (1955) 1323–1326.
- [13] F.P.C. Leclerc, Product containing an extract of red algae of the genus *Porphyra* and methods for protecting cells, Patent US7128914, (2004).
- [14] K.J. Flynn, K. Flynn, Non-protein free amines in microalgae: consequences for the measurement of intracellular amino acids and of the glutamine/glutamate ratio, *Mar. Ecol. Prog. Ser.* 89 (1992) 73–79.
- [15] K. Flynn, K.J. Flynn, K. Jones, Changes in dinoflagellate intracellular amino-acids in response to diurnal changes in light and n-supply, *Mar. Ecol. Prog. Ser.* 100 (1993) 245–252.
- [16] K.J. Flynn, R. Raine, J. Richard, K. Flynn, Use of intracellular amino acid analysis as an indicator of the physiological status of natural dinoflagellate populations, *Mar. Ecol. Prog. Ser.* 103 (1994) 175–186.
- [17] D.S. Gorman, R.P. Levine, Cytochrome f and plastocyanin: their sequence in the photosynthetic electron transport chain of *Chlamydomonas reinhardtii*, *Proc. Natl. Acad. Sci. U.S.A.* 54 (1965) 1665–1669.
- [18] R.L. Guillard, Culture of phytoplankton for feeding marine invertebrates, in: W. Smith, M. Chanley (Eds.) *Culture of Marine Invertebrate Animals*, Springer US1975, pp. 29–60.
- [19] M.D. Keller, R.C. Selvin, W. Claus, R.R.L. Guillard, Media for the culture of oceanic ultraphytoplankton, *J. Phycol.* 23 (1987) 633–638.
- [20] P. Chaimbault, P. Alberic, C. Elfakir, M. Lafosse, Development of an LC–MS–MS method for the quantification of taurine derivatives in marine invertebrates, *Anal. Biochem.* 332 (2004) 215–225.
- [21] M. Piraud, C. Vianey-Saban, K. Petritis, C. Elfakir, J.P. Steghens, A. Morla, D. Bouchu, ESI-MS/MS analysis of underivatized amino acids: a new tool for the diagnosis of inherited disorders of amino acid metabolism. Fragmentation study of 79 molecules of biological interest in positive and negative ionization mode, *Rapid Commun. Mass Spectrom.* 17 (2003) 1297–1311.
- [22] K. Tamura, G. Stecher, D. Peterson, A. Filipski, S. Kumar, MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0, *Mol. Biol. Evol.* 30 (2013) 2725–2729.
- [23] G.M. Boratyn, A.A. Schaffer, R. Agarwala, S.F. Altschul, D.J. Lipman, T.L. Madden, Domain enhanced lookup time accelerated BLAST, *Biol. Direct* 7 (2012) 1745–6150.

- [24] G.T. Reynoso, B.A. DeGamba, Salt tolerance in the freshwater algae *Chlamydomonas reinhardtii*: effect of proline and taurine, *Comp. Biochem. Physiol. Part A* 73 (1982) 95–99.
- [25] A.E. Jackson, S.W. Ayer, M.V. Laycock, The effect of salinity on growth and amino acid composition in the marine diatom *Nitzschia pungens*, *Can. J. Bot.* 70 (1992) 2198–2201.
- [26] J.E. Dominy Jr., C.R. Simmons, P.A. Karplus, A.M. Gehring, M.H. Stipanuk, Identification and characterization of bacterial cysteine dioxygenases: a new route of cysteine degradation for eubacteria, *J. Bacteriol.* 188 (2006) 5561–5569.
- [27] G.P. McMahon, R. O’Kennedy, M.T. Kelly, High-performance liquid chromatographic determination of taurine in human plasma using pre-column extraction and derivatization, *J. Pharm. Biomed. Anal.* 14 (1996) 1287–1294.
- [28] H. Inoue, K. Fukunaga, Y. Tsuruta, Determination of taurine in plasma by high-performance liquid chromatography using 4-(5,6-dimethoxy-2-phthalimidinyl)-2-methoxyphenylsulfonyl chloride as a fluorescent labeling reagent, *Anal. Biochem.* 319 (2003) 138–142.
- [29] S. Dupre, A. Spirito, K. Sugahara, H. Kodama, Hypotaurine oxidation: an HPLC-mass approach, *Adv. Exp. Med. Biol.* 442 (1998) 3–8.
- [30] S.J. Lillard, D.T. Chiu, R.H. Scheller, R.N. Zare, S.E. Rodriguez-Cruz, E.R. Williams, O. Orwar, M. Sandberg, J.A. Lundqvist, Separation and characterization of amines from individual atrial gland vesicles of *Aplysia californica*, *Anal. Chem.* 70 (1998) 3517–3524.
- [31] T. Togawa, A. Ohsawa, K. Kawanabe, S. Tanabe, Simultaneous determination of cysteine sulfinic acid and hypotaurine in rat tissues by column-switching high-performance liquid chromatography with electrochemical detection, *J. Chromatogr. B Biomed. Sci. Appl.* 704 (1997) 83–88.
- [32] A. Melis, H.C. Chen, Chloroplast sulfate transport in green algae—genes, proteins and effects, *Photosynth. Res.* 86 (2005) 299–307.
- [33] H. Takahashi, S. Kopriva, M. Giordano, K. Saito, R. Hell, Sulfur assimilation in photosynthetic organisms: molecular functions and regulations of transporters and assimilatory enzymes, *Annu. Rev. Plant Biol.* 62 (2011) 157–184.
- [34] C. Courties, A. Vaquer, M. Troussellier, J. Lautier, M.J. Chretiennot-Dinet, J. Neveux, C. Machado, H. Claustre, Smallest eukaryotic organism, *Nature* 370 (1994) 255.
- [35] E. Derelle, C. Ferraz, S. Rombauts, P. Rouze, A.Z. Worden, S. Robbens, F. Partensky, S. Degroeve, S. Echeynie, R. Cooke, Y. Saeys, J. Wuyts, K. Jabbari, C. Bowler, O. Panaud, B. Piegu, S.G. Ball, J.P. Ral, F.Y. Bouget, G. Piganeau, B. De Baets, A. Picard, M. Delseny, J. Demaille, Y. Van de Peer, H. Moreau, Genome analysis of the smallest free-living eukaryote *Ostreococcus tauri* unveils many unique features, *Proc. Natl. Acad. Sci. U.S.A.* 103 (2006) 11647–11652.
- [36] P.H. Yancey, J. Ishikawa, B. Meyer, P.R. Girguis, R.W. Lee, Thiotaurine and hypotaurine contents in hydrothermal-vent polychaetes without thiotrophic endosymbionts: correlation with sulfide exposure, *J. Exp. Zool. A Ecol. Genet. Physiol.* 311 (2009) 439–447.
- [37] H. Ripps, W. Shen, Review: taurine: a “very essential” amino acid, *Mol. Vis.* 18 (2012) 2673–2686.

The taurine biosynthetic pathway of microalgae

Rahul Tevatia^{1,2}, James Allen³, Deepak Rudrappa¹, Derrick White¹, Thomas E. Clemente^{4,5},
Heriberto Cerutti^{1,5}, Yaşar Demirel², Paul Blum^{1,*}

Supporting Information

S1. This section presents the chromatograms for sulfur containing amino acids and taurine pathway intermediates run in optimized LC conditions coupled with both positive and negative electrospray ionization.

S1.1. Standards

a)

b)

Figure S1.1. LC-MS/MS analysis of standard solution of undervitized sulfur containing amino acids and intermediates in (a) positive ion mode: CA – Cysteic acid, TAU – Taurine, HPO – Hypotaurine, CYS –Cysteine, NVA – Norvaline, MET – Methionine, (b) Negative ion mode: cysteine sulfinic acid (CSA).

S1.2. *Ostreococcus tauri* (grown in K-medium supplemented with sodium sulfate)

a)

b)

Figure S1.2. LC-MS/MS analysis of undervitized free amino acid pool from *Ostreococcus tauri* grown in sulfate (100 mM) in (a) positive ion mode: CA – Cysteic acid, TAU – Taurine, HPO – Hypotaurine, CYS –Cysteine, NVA – Norvaline, MET – Methionine, (b) Negative ion mode: cysteine sulfinic acid (CSA).

S1.3. *Chlamydomonas reinhardtii* (Control – grown in TAP medium)

a)

b)

Figure S1.3. LC-MS/MS analysis of undervitized free amino acid pool from *Chlamydomonas reinhardtii* grown in TAP media as control in (a) positive ion mode: CA – Cysteic acid, TAU – Taurine, HPO – Hypotaurine, CYS –Cysteine, NVA – Norvaline, MET – Methionine, (b) Negative ion mode: cysteine sulfinic acid (CSA).

S1.4. *Chlamydomonas reinhardtii* (Serine supplementation)

a)

b)

Figure S1.4. LC-MS/MS analysis of undervitirized free amino acid pool from *Chlamydomonas reinhardtii* grown in TAP media supplemented with 6 mM serine in (a) positive ion mode: CA – Cysteic acid, TAU – Taurine, HPO – Hypotaurine, CYS –Cysteine, NVA – Norvaline, MET – Methionine, (b) Negative ion mode: cysteine sulfinic acid (CSA).

S1.5. *Chlamydomonas reinhardtii* (Sulfate supplementation)

a)

b)

Figure S1.5. LC-MS/MS analysis of undervitized free amino acid pool from *Chlamydomonas reinhardtii* grown in TAP media supplemented with 6 mM sulfate in (a) positive ion mode: CA – Cysteic acid, TAU – Taurine, HPO – Hypotaurine, CYS –Cysteine, NVA – Norvaline, MET – Methionine, (b) Negative ion mode: cysteine sulfinic acid (CSA).

S1.6. *Tetraselmis* sp. (Control – grown in f/2 media)

a)

b)

Figure S1.6. LC-MS/MS analysis of undervitized free amino acid pool from *Tetraselmis* sp. grown in f/2 media as control in (a) positive ion mode: CA – Cysteic acid, TAU – Taurine, HPO – Hypotaurine, CYS –Cysteine, NVA – Norvaline, MET – Methionine, (b) Negative ion mode: cysteine sulfinic acid (CSA).

S1.7. *Tetraselmis* sp. (Serine)

a)

b)

Figure S1.7. LC-MS/MS analysis of undervitirized free amino acid pool from *Tetraselmis* sp. grown in f/2 media supplemented with 6 mM serine in (a) positive ion mode: CA – Cysteic acid, TAU – Taurine, HPO – Hypotaurine, CYS –Cysteine, NVA – Norvaline, MET – Methionine, (b) Negative ion mode: cysteine sulfinic acid (CSA).

S1.8. *Tetraselmis* sp. (Sulfate supplementation)

a)

b)

Figure S1.8. LC-MS/MS analysis of undervitimized free amino acid pool from *Tetraselmis* sp. grown in f/2 media supplemented with 6 mM sulfate in (a) positive ion mode: CA – Cysteic acid, TAU – Taurine, HPO – Hypotaurine, CYS –Cysteine, NVA – Norvaline, MET – Methionine, (b) Negative ion mode: cysteine sulfinic acid (CSA).

S1.9. *Tetraselmis* sp. (Cysteine + Methionine supplementation)

a)

b)

Figure S1.9. LC-MS/MS analysis of undervitimized free amino acid pool from *Tetraselmis* sp. grown in f/2 media supplemented with 6 mM each cysteine and methionine in (a) positive ion mode: CA – Cystic acid, TAU – Taurine, HPO – Hypotaurine, CYS –Cysteine, NVA – Norvaline, MET – Methionine, (b) Negative ion mode: cysteine sulfinic acid (CSA).

S2. This section shows detailed Domain Enhanced Lookup Time Accelerated BLAST (DELTA-BLAST, NCBI) analysis to identified key enzymes involved in algal taurine biosynthesis.

Table S1. Cysteine dioxygenase (*Cyprinus carpio* – Accession: BAE73112)

Organisms		Tax id	Result	Max score	Total score	Query cover	E value	Max iden.
<i>Aureococcus</i>	<i>anophagefferens</i>	44056	HP	105	105	69%	2e-28	34%
<i>Bigelowiella</i>	<i>natans</i>	227086	N	-	-	-	-	-
<i>Coccomyxa</i>	<i>subellipsoidea</i>	574566	HP	38.1	38.1	45%	7.00E-04	16%
<i>Chlorella</i>	<i>variabilis</i>	554065	HP	94.7	94.7	63%	1.00E-23	27%
<i>Chlamydomonas</i>	<i>reinhardtii</i>	3055	Y	95.1	95.1	85%	1.00E-23	26%
<i>Ostreococcus</i>	<i>tauri</i>	70448	N	-	-	-	-	-
<i>Cyanidioschyzon</i>	<i>merolae</i>	45157	N	-	-	-	-	-
<i>Thalassiosira</i>	<i>pseudonana</i>	35128	N	-	-	-	-	-
<i>Fragilariopsis</i>	<i>cylindrus</i>	186039	N	-	-	-	-	-
<i>Guillardia</i>	<i>theta</i>	905079	HP	84.7	84.7	83%	2e-19	26%
<i>Micromonas</i>	<i>pusilla</i>	564608	N	-	-	-	-	-
<i>Phaeodactylum</i>	<i>tricornutum</i>	2850	N	-	-	-	-	-
<i>Pseudo-nitzschia</i>	<i>multiseries</i>	37319	N	-	-	-	-	-
<i>Dictyostelium</i>	<i>discoideum</i>	44689	Y	103	103	83%	7.00E-30	29%
<i>Volvox</i>	<i>carteri</i>	3067	Y	84.7	84.7	79%	8.00E-20	25%
<i>Galdieria</i>	<i>sulphuraria</i>	130081	N	-	-	-	-	-
<i>Homo</i>	<i>sapiens</i>	9606	Y	261	261	100%	4.00E-87	76%
<i>Mus</i>	<i>musculus</i>	10090	Y	256	256	100%	2.00E-85	77%
<i>Gallus</i>	<i>gallus</i>	9031	Y	243	243	99%	2.00E-80	73%

HP – Hypothetical Protein

N – No significant hits

Y – Significant hits

Table S2. Cysteine sulfinic acid decarboxylase (*Cyprinus carpio* – Accession: BAE73113)

Organisms	Taxid	Result	Max score	Total score	Query cover	E value	Max iden.
<i>Aureococcus</i>	<i>anophagefferens</i> 44056	HP	172	172	40%	2e-50	41%
<i>Bigelowiella</i>	<i>natans</i> 227086	GSA	41.2	41.2	60%	2e-05	12%
<i>Coccomyxa</i>	<i>subellipsoidea</i> 574566	AAD	373	373	89%	3.00E-123	22%
<i>Chlorella</i>	<i>variabilis</i> 554065	HP	246	246	85%	2.00E-76	15%
<i>Chlamydomonas</i>	<i>reinhardtii</i> 3055	AAD	296	296	86%	4.00E-94	22%
<i>Ostreococcus</i>	<i>tauri</i> 70448	GD	305	305	76%	1.00E-98	42%
<i>Cyanidioschyzon</i>	<i>merolae</i> 45157	GD	251	251	89%	3.00E-76	16%
<i>Thalassiosira</i>	<i>pseudonana</i> 35128	HD	283	283	68%	2.00E-90	38%
<i>Fragilariopsis</i>	<i>cylindrus</i> 186039	N	-	-	-	-	-
<i>Guillardia</i>	<i>theta</i> 905079	HP	369	369	97%	4e-121	39%
<i>Micromonas</i>	<i>pusilla</i> 564608	PDC	334	334	87%	1e-103	42%
<i>Phaeodactylum</i>	<i>tricornutum</i> 2850	HP	219	219	79%	3e-66	19%
<i>Pseudo-nitzschia</i>	<i>multiseries</i> 37319	N	-	-	-	-	-
<i>Dictyostelium</i>	<i>discoideum</i> 44689	GD	254	254	86%	2.00E-77	17%
<i>Volvox</i>	<i>carteri</i> 3067	SD	199	199	76%	7.00E-58	16%
<i>Galdieria</i>	<i>sulphuraria</i> 130081	GD	258	258	88%	1.00E-79	14%
<i>Homo</i>	<i>sapiens</i> 9606	Y	501	501	94%	3.00E-172	65%
<i>Mus</i>	<i>musculus</i> 10090	Y	496	496	94%	2.00E-173	64%
<i>Gallus</i>	<i>gallus</i> 9031	Y	489	489	97%	3.00E-168	65%

AAD – Aromatic aminoacid decarboxylase

GD – Glutamate decarboxylase

GSA - Glutamate 1-semialdehyde 2,1-aminomutase

HD – Histidine decarboxylase

N – No significant hits

PDC – Pyridoxal dependent decarboxylase

SD – Serine decarboxylase

Y – Significant hits

Table S3. Cysteamine dioxygenase (*Galdieria sulphuraria* – Accession: XP_005707183)

Organisms	Taxid	Result	Max score	Total score	Query cover	E value	Max iden.
<i>Aureococcus</i>	<i>anophagefferens</i> 44056	HP	129	129	56%	5e-37	34%
<i>Bigelowiella</i>	<i>natans</i> 227086	N	-	-	-	-	-
<i>Coccomyxa</i>	<i>subellipsoidea</i> 574566	HP	152	152	53%	5.00E-47	32%
<i>Chlorella</i>	<i>variabilis</i> 554065	HP	167	167	72%	5.00E-50	30%
<i>Chlamydomonas</i>	<i>reinhardtii</i> 3055	N	-	-	-	-	-
<i>Ostreococcus</i>	<i>tauri</i> 70448	HP	155	155	69%	6.00E-47	24%
<i>Cyanidioschyzon</i>	<i>merolae</i> 45157	HP	108	108	68%	2.00E-28	30%
<i>Thalassiosira</i>	<i>pseudonana</i> 35128	N	-	-	-	-	-
<i>Fragilariopsis</i>	<i>cylindrus</i> 186039	N	-	-	-	-	-
<i>Guillardia</i>	<i>theta</i> 905079	HP	155	155	89%	7e-46	27%
<i>Micromonas</i>	<i>pusilla</i> 564608	HP	153	153	73%	6e-45	27%
<i>Phaeodactylum</i>	<i>tricornutum</i> 2850	HP	137	137	74%	1e-38	22%
<i>Pseudo-nitzschia</i>	<i>multiseries</i> 37319	N	-	-	-	-	-
<i>Dictyostelium</i>	<i>discoideum</i> 44689	HP	145	145	68%	2.00E-42	25%
<i>Volvox</i>	<i>carteri</i> 3067	HP	136	136	97%	9.00E-39	21%
<i>Galdieria</i>	<i>sulphuraria</i> 130081	Y	311	311	100%	9.00E-108	100%
<i>Homo</i>	<i>sapiens</i> 9606	Y	184	184	88%	4.00E-56	23%
<i>Mus</i>	<i>musculus</i> 10090	Y	182	182	73%	1.00E-55	23%
<i>Gallus</i>	<i>gallus</i> 9031	N	-	-	-	-	-

HP – Hypothetical protein

N – No significant hit

Y – Significant hit

Table S4. Taurine dioxygenase (*Bradyrhizobium* sp. STM 3843 – Accession: WP_008971131)

Organisms	Taxid	Result	Max score	Total score	Query cover	E value	Max iden.
<i>Aureococcus</i>	<i>anophagefferens</i> 44056	HP	202	202	91%	4e-63	33%
<i>Bigelowiella</i>	<i>natans</i> 227086	N	-	-	-	-	-
<i>Coccomyxa</i>	<i>subellipsoidea</i> 574566	HP	117	117	89%	9.00E-31	18%
<i>Chlorella</i>	<i>variabilis</i> 554065	HP	110	110	92%	3.00E-28	20%
<i>Chlamydomonas</i>	<i>reinhardtii</i> 3055	HP	64.7	64.7	67%	2.00E-12	11%
<i>Ostreococcus</i>	<i>tauri</i> 70448	HP	65.5	65.5	87%	7.00E-13	13%
<i>Cyanidioschyzon</i>	<i>merolae</i> 45157	N	-	-	-	-	-
<i>Thalassiosira</i>	<i>pseudonana</i> 35128	HP	62.4	62.4	80%	1.00E-11	10%
<i>Fragilariopsis</i>	<i>cylindrus</i> 186039	N	-	-	-	-	-
<i>Guillardia</i>	<i>theta</i> 905079	HP	125	125	84%	2e-32	13%
<i>Micromonas</i>	<i>pusilla</i> 564608	HP	146	146	81%	4e-41	20%
<i>Phaeodactylum</i>	<i>tricornutum</i> 2850	HP	155	155	91%	2e-44	23%
<i>Pseudo-nitzschia</i>	<i>multiseries</i> 37319	N	-	-	-	-	-
<i>Dictyostelium</i>	<i>discoideum</i> 44689	N	-	-	-	-	-
<i>Volvox</i>	<i>carteri</i> 3067	HP	62	62	64%	2.00E-11	12%
<i>Galdieria</i>	<i>sulphuraria</i> 130081	HP	58.5	58.5	76%	1.00E-10	10%
<i>Homo</i>	<i>sapiens</i> 9606	HP	80.5	80.5	80%	1.00E-16	9%
<i>Mus</i>	<i>musculus</i> 10090	HP	80.5	80.5	80%	7.00E-17	10%
<i>Gallus</i>	<i>gallus</i> 9031	N	-	-	-	-	-

HP – Hypothetical protein

N – No significant hit

Y – Significant hit

Table S5. Taurine dehydrogenase (*Synorhizobium meliloti* SM11 – Accession: AEH83463)

Organisms	Taxid	Result	Max score	Total score	Query cover	E value	Max iden.
<i>Aureococcus</i>	<i>anophagefferens</i> 44056	HP	178	178	82%	2e-48	13%
<i>Bigelowiella</i>	<i>natans</i> 227086	PD	50.8	77.0	22%	2e-08	36%
<i>Coccomyxa</i>	<i>subellipsoidea</i> 574566	HP	157	157	82%	2.00E-42	20%
<i>Chlorella</i>	<i>variabilis</i> 554065	HP	134	134	77%	4.00E-35	17%
<i>Chlamydomonas</i>	<i>reinhardtii</i> 3055	HP	117	117	83%	1.00E-28	15%
<i>Ostreococcus</i>	<i>tauri</i> 70448	HP	89.4	89.4	76%	7.00E-20	12%
<i>Cyanidioschyzon</i>	<i>merolae</i> 45157	HP	144	144	77%	3.00E-37	14%
<i>Thalassiosira</i>	<i>pseudonana</i> 35128	HP	173	173	78%	5.00E-51	16%
<i>Fragilariopsis</i>	<i>cylindrus</i> 186039	N	-	-	-	-	-
<i>Guillardia</i>	<i>theta</i> 905079	HP	172	172	77%	3e-46	14%
<i>Micromonas</i>	<i>pusilla</i> 564608	HP	107	107	80%	2e-25	16%
<i>Phaeodactylum</i>	<i>tricornutum</i> 2850	HP	193	193	83%	1e-53	16%
<i>Pseudo-nitzschia</i>	<i>multiseries</i> 37319	N	-	-	-	-	-
<i>Dictyostelium</i>	<i>discoideum</i> 44689	HP	128	128	80%	1.00E-32	14%
<i>Volvox</i>	<i>carteri</i> 3067	HP	154	154	85%	7.00E-41	19%
<i>Galdieria</i>	<i>sulphuraria</i> 130081	HGD	160	160	79%	3.00E-45	14%
<i>Homo</i>	<i>sapiens</i> 9606	HP	206	206	87%	4.00E-57	16%
<i>Mus</i>	<i>musculus</i> 10090	HP	202	202	80%	6.00E-56	17%
<i>Gallus</i>	<i>gallus</i> 9031	HP	208	208	92%	1.00E-58	16%

HP – Hypothetical protein

HGD – 2-Hydroxylglutarate dehydrogenase isoform 1

PD - Phytoene dehydrogenase

N – No significant hit

Y – Significant hit

Table S6. Sulfoacetaldehyde acetyltransferase (*Desulfarculus baarsii* – Accession: WP_013260015)
AAS – Acetolacetate synthase

Organisms	Taxid	Result	Max score	Total score	Query cover	E value	Max iden.
<i>Aureococcus</i>	<i>anophagefferens</i> 44056	AAS	599	628	97%	0.0	25%
<i>Bigelowiella</i>	<i>natans</i> 227086	N	-	-	-	-	-
<i>Coccomyxa</i>	<i>subellipsoidea</i> 574566	AAS	561	561	95%	0.00E+00	25%
<i>Chlorella</i>	<i>variabilis</i> 554065	HP	565	565	95%	0	23%
<i>Chlamydomonas</i>	<i>reinhardtii</i> 3055	AAS	553	553	95%	0.00E+00	23%
<i>Ostreococcus</i>	<i>tauri</i> 70448	AAS	574	574	95%	0.00E+00	24%
<i>Cyanidioschyzon</i>	<i>merolae</i> 45157	AHS	556	556	95%	0.00E+00	25%
<i>Thalassiosira</i>	<i>pseudonana</i> 35128	HP	280	451	93%	1.00E-83	24%
<i>Fragilariopsis</i>	<i>cylindrus</i> 186039	N	-	-	-	-	-
<i>Guillardia</i>	<i>theta</i> 905079	HP	154	154	89%	3e-40	16%
<i>Micromonas</i>	<i>pusilla</i> 564608	HP	540	540	95%	0.0	22%
<i>Phaeodactylum</i>	<i>tricornutum</i> 2850	HP	54.3	54.3	25%	3e-08	16%
<i>Pseudo-nitzschia</i>	<i>multiseries</i> 37319	N	-	-	-	-	-
<i>Dictyostelium</i>	<i>discoideum</i> 44689	HP	381	381	94%	2.00E-124	21%
<i>Volvox</i>	<i>carteri</i> 3067	HP	557	557	95%	0.00E+00	24%
<i>Galdieria</i>	<i>sulphuraria</i> 130081	AAS	626	626	95%	0.00E+00	25%
<i>Homo</i>	<i>sapiens</i> 9606	HP	454	454	94%	8.00E-152	25%
<i>Mus</i>	<i>musculus</i> 10090	HAL	456	456	95%	3.00E-152	23%
<i>Gallus</i>	<i>gallus</i> 9031	HAL	466	466	95%	5.00E-157	22%

AHS – Acetohydroxyacid synthase

HAL – 2- Hydroxyacyl – CoA lyase 1

HP – Hypothetical Protein

N – No significant hit

Table S7. Taurine-pyruvate aminotransferase (*Marinomonas posidonica* – Accession: WP_013796546)

Organisms	Taxid	Result	Max score	Total score	Query cover	E value	Max iden.
<i>Aureococcus</i>	<i>anophagefferens</i> 44056	HP	522	522	96%	0.0	36%
<i>Bigelowiella</i>	<i>natans</i> 227086	GSA	313	313	91%	4e-102	20%
<i>Coccomyxa</i>	<i>subellipsoidea</i> 574566	AOAT	451	451	96%	2.00E-155	26%
<i>Chlorella</i>	<i>variabilis</i> 554065	HP	444	444	97%	2.00E-152	25%
<i>Chlamydomonas</i>	<i>reinhardtii</i> 3055	AOAT	426	426	97%	3.00E-145	23%
<i>Ostreococcus</i>	<i>tauri</i> 70448	AOAT	372	372	89%	2.00E-125	27%
<i>Cyanidioschyzon</i>	<i>merolae</i> 45157	AOAT	444	444	92%	2.00E-152	27%
<i>Thalassiosira</i>	<i>pseudonana</i> 35128	AOAT	427	427	96%	6.00E-146	25%
<i>Fragilariopsis</i>	<i>cylindrus</i> 186039	N	-	-	-	-	-
<i>Guillardia</i>	<i>theta</i> 905079	AOAT	409	409	96%	3e-138	26%
<i>Micromonas</i>	<i>pusilla</i> 564608	GSA	300	300	91%	3e-96	19%
<i>Phaeodactylum</i>	<i>tricornutum</i> 2850	AOAT	412	412	90%	2e-139	25%
<i>Pseudo-nitzschia</i>	<i>multiseries</i> 37319	N	-	-	-	-	-
<i>Dictyostelium</i>	<i>discoideum</i> 44689	AOAT	446	446	98%	2.00E-153	27%
<i>Volvox</i>	<i>carteri</i> 3067	AOAT	429	429	96%	3.00E-146	24%
<i>Galdieria</i>	<i>sulphuraria</i> 130081	AOAT	426	426	90%	4.00E-145	28%
<i>Homo</i>	<i>sapiens</i> 9606	HP	441	441	96%	9.00E-151	26%
<i>Mus</i>	<i>musculus</i> 10090	HP	441	441	95%	2.00E-150	27%
<i>Gallus</i>	<i>gallus</i> 9031	OAT	451	451	95%	7.00E-155	27%

AOAT – Acetyloronithine aminotransferase

GSA - Glutamate 1-semialdehyde 2,1-aminomutase

HP – Hypothetical protein

OAT – Ornithine aminotransferase

N – No significant hit

Table S8. 3'-phosphoadenylyl sulfate: 2-aminoacrylate C-sulfotransferase (*Arabidopsis thaliana* – NP_178471.1)

Organisms	Taxid	Result	Max score	Total score	Query cover	E value	Max iden.
<i>Aureococcus anophagefferens</i>	44056	HP	193	193	81%	4e-59	26%
<i>Bigelowiella natans</i>	227086	N	-	-	-	-	-
<i>Coccomyxa subellipsoidea</i>	574566	HP	32.2	32.2	47%	0.095	8%
<i>Chlorella variabilis</i>	554065	HP	112	112	77%	1.00E-28	19%
<i>Chlamydomonas reinhardtii</i>	3055	Y	77.4	77.4	77%	2.00E-16	16%
<i>Ostreococcus tauri</i>	70448	Y	84.7	84.7	79%	3.00E-19	17%
<i>Cyanidioschyzon merolae</i>	45157	HP	34.3	34.3	54%	0.021	9%
<i>Thalassiosira pseudonana</i>	35128	Y	90.9	90.9	81%	3.00E-21	25%
<i>Dictyostelium discoideum</i>	44689	HP	58.2	58.2	79%	2.00E-9	16%
<i>Fragilariopsis cylindrus</i>	186039	N	-	-	-	-	-
<i>Guillardia theta</i>	905079	HP	155	155	80%	7e-44	24%
<i>Micromonas pusilla</i>	564608	HP	154	154	76%	5e-44	21%
<i>Phaeodactylum tricornutum</i>	2850	HP	124	124	79%	1e-32	22%
<i>Pseudo-nitzschia multiseriis</i>	37319	N	-	-	-	-	-
<i>Volvox carteri</i>	3067	HP	46.6	46.6	40%	9.00E-6	12%
<i>Galdieria sulphuraria</i>	130081	HP	30.8	30.8	64%	0.29	10%
<i>Homo sapiens</i>	9606	Y	305	305	81%	4.00E-101	27%
<i>Mus musculus</i>	10090	Y	305	305	81%	3.00E-104	28%
<i>Gallus gallus</i>	9031	Y	293	293	81%	5.00E-97	28%

HP – Hypothetical protein

N – No significant hit

Y – Significant hit

Table S9. Sulfate adenylyltransferase (*Porphyra purpurea* – Accession: AAP97122)

Organisms	Taxid	Result	Max score	Total score	Query cover	E value	Max iden.
<i>Aureococcus</i>	<i>anophagefferens</i> 44056	HP	402	402	94%	3e-132	25%
<i>Bigelowiella</i>	<i>natans</i> 227086	N	-	-	-	-	-
<i>Coccomyxa</i>	<i>subellipsoidea</i> 574566	Y	441	441	96%	5.00E-153	25%
<i>Chlorella</i>	<i>variabilis</i> 554065	HP	410	410	97%	5.00E-140	25%
<i>Chlamydomonas</i>	<i>reinhardtii</i> 3055	Y	451	451	96%	1.00E-155	25%
<i>Ostreococcus</i>	<i>tauri</i> 70448	Y	409	409	96%	2.00E-140	26%
<i>Cyanidioschyzon</i>	<i>merolae</i> 45157	Y	399	399	97%	6.00E-136	57%
<i>Thalassiosira</i>	<i>pseudonana</i> 35128	Y	310	310	93%	6.00E-96	22%
<i>Fragilariopsis</i>	<i>cylindrus</i> 186039	N	-	-	-	-	-
<i>Guillardia</i>	<i>theta</i> 905079	HP	404	404	97%	3e-138	25%
<i>Micromonas</i>	<i>pusilla</i> 564608	HP	422	422	96%	3e-145	27%
<i>Phaeodactylum</i>	<i>tricornutum</i> 2850	HP	427	427	96%	3e-147	26%
<i>Pseudo-nitzschia</i>	<i>multiseries</i> 37319	N	-	-	-	-	-
<i>Dictyostelium</i>	<i>discoideum</i> 44689	Y	477	477	98%	5.00E-164	26%
<i>Volvox</i>	<i>carteri</i> 3067	HP	451	451	97%	2.00E-156	25%
<i>Galdieria</i>	<i>sulphuraria</i> 130081	Y	389	389	97%	5.00E-131	21%
<i>Homo</i>	<i>sapiens</i> 9606	Y	432	432	96%	5.00E-145	23%
<i>Mus</i>	<i>musculus</i> 10090	Y	432	432	96%	2.00E-148	22%
<i>Gallus</i>	<i>gallus</i> 9031	P	442	442	95%	2.00E-148	22%

HP – Hypothetical protein

P - Predicted

Y – Significant hit

Table S10. Adenylylsulfate kinase (*Chlamydomonas reinhardtii* – Accession: BAF46286)

Organisms	Taxid	Result	Max score	Total score	Query cover	E value	Max iden.
<i>Aureococcus</i>	<i>anophagefferens</i> 44056	HP	216	216	77%	4e-70	44%
<i>Bigelowiella</i>	<i>natans</i> 227086	N	-	-	-	-	-
<i>Coccomyxa</i>	<i>subellipsoidea</i> 574566	Y	244	244	88%	9.00E-81	62%
<i>Chlorella</i>	<i>variabilis</i> 554065	HP	325	325	89%	5.00E-112	62%
<i>Chlamydomonas</i>	<i>reinhardtii</i> 3055	Y	360	360	100%	6.00E-126	100%
<i>Ostreococcus</i>	<i>tauri</i> 70448	Y	244	244	91%	8.00E-81	65%
<i>Cyanidioschyzon</i>	<i>merolae</i> 45157	Y	234	234	82%	6.00E-78	50%
<i>Thalassiosira</i>	<i>pseudonana</i> 35128	Y	237	237	81%	4.00E-78	49%
<i>Fragilariopsis</i>	<i>cylindrus</i> 186039	N	-	-	-	-	-
<i>Guillardia</i>	<i>theta</i> 905079	HP	254	254	70%	4e-84	56%
<i>Micromonas</i>	<i>pusilla</i> 564608	HP	297	297	91%	5e-101	65%
<i>Phaeodactylum</i>	<i>tricornutum</i> 2850	HP	210	210	72%	2e-62	47%
<i>Pseudo-nitzschia</i>	<i>multiseries</i> 37319	N	-	-	-	-	-
<i>Dictyostelium</i>	<i>discoideum</i> 44689	Y	186	186	73%	2.00E-54	44%
<i>Volvox</i>	<i>carteri</i> 3067	Y	57.8	57.8	44%	7.00E-10	22%
<i>Galdieria</i>	<i>sulphuraria</i> 130081	Y	93.6	93.6	46%	4.00E-24	41%
<i>Homo</i>	<i>sapiens</i> 9606	Y	222	222	82%	8.00E-67	44%
<i>Mus</i>	<i>musculus</i> 10090	Y	227	227	87%	1.00E-68	44%
<i>Gallus</i>	<i>gallus</i> 9031	P	239	239	82%	9.00E-74	45%

HP – Hypothetical protein

P – Predicted protein

Y – Significant hit

Table S11. Serine dehydratase (*Mus musculus* – Accession: EDL19770)

Organisms	Taxid	Result	Max score	Total score	Query cover	E value	Max iden.
<i>Aureococcus</i>	<i>anophagefferens</i> 44056	HP	298	298	95%	3e-96	27%
<i>Bigelowiella</i>	<i>natans</i> 227086	N	-	-	-	-	-
<i>Coccomyxa</i>	<i>subellipsoidea</i> 574566	Y	232	232	95%	3.00E-73	26%
<i>Chlorella</i>	<i>variabilis</i> 554065	HP	280	280	96%	7.00E-91	24%
<i>Chlamydomonas</i>	<i>reinhardtii</i> 3055	Y	229	229	94%	6.00E-72	27%
<i>Ostreococcus</i>	<i>tauri</i> 70448	Y	221	221	94%	3.00E-67	20%
<i>Cyanidioschyzon</i>	<i>merolae</i> 45157	Y	188	188	97%	5.00E-57	35%
<i>Thalassiosira</i>	<i>pseudonana</i> 35128	Y	246	246	94%	8.00E-79	22%
<i>Fragilariopsis</i>	<i>cylindrus</i> 186039	N	-	-	-	-	-
<i>Guillardia</i>	<i>theta</i> 905079	HP	354	354	96%	8e-118	30%
<i>Micromonas</i>	<i>pusilla</i> 564608	HP	285	285	98%	2e-91	28%
<i>Phaeodactylum</i>	<i>tricornutum</i> 2850	HP	253	253	96%	7e-80	28%
<i>Pseudo-nitzschia</i>	<i>multiseries</i> 37319	N	-	-	-	-	-
<i>Dictyostelium</i>	<i>discoideum</i> 44689	Y	249	249	96%	9.00E-80	40%
<i>Volvox</i>	<i>carteri</i> 3067	Y	231	231	93%	7.00E-73	25%
<i>Galdieria</i>	<i>sulphuraria</i> 130081	Y	333	333	94%	2.00E-109	29%
<i>Homo</i>	<i>sapiens</i> 9606	Y	293	293	98%	1.00E-94	71%
<i>Mus</i>	<i>musculus</i> 10090	Y	392	392	99%	4.00E-135	100%
<i>Gallus</i>	<i>gallus</i> 9031	Y	231	231	96%	2.00E-72	51%

Y – Significant hit

Table S12. Glutamate decarboxylase (*Ostreococcus tauri* – Accession: XP_003075194)

Organisms	Taxid	Result	Max score	Total score	Query cover	E value	Max iden.
<i>Aureococcus</i>	<i>anophagefferens</i> 44056	HP	174	174	84%	1e-49	18%
<i>Bigelowiella</i>	<i>natans</i> 227086	GSA	44.7	44.7	56%	9e-07	14%
<i>Coccomyxa</i>	<i>subellipsoidea</i> 574566	Y	254	254	98%	3.00E-79	16%
<i>Chlorella</i>	<i>variabilis</i> 554065	HP	235	235	94%	2.00E-71	14%
<i>Chlamydomonas</i>	<i>reinhardtii</i> 3055	AAD	244	244	93%	2.00E-75	25%
<i>Ostreococcus</i>	<i>tauri</i> 70448	Y	450	450	100%	8.00E-157	100%
<i>Cyanidioschyzon</i>	<i>merolae</i> 45157	Y	204	204	99%	2.00E-59	29%
<i>Thalassiosira</i>	<i>pseudonana</i> 35128	HP	247	247	80%	6.00E-78	36%
<i>Fragilariopsis</i>	<i>cylindrus</i> 186039	N	-	-	-	-	-
<i>Guillardia</i>	<i>theta</i> 905079	HP	295	295	99%	8e-94	35%
<i>Micromonas</i>	<i>pusilla</i> 564608	PDD	358	358	100%	3e-114	64%
<i>Phaeodactylum</i>	<i>tricornutum</i> 2850	HP	204	204	78%	2e-61	22%
<i>Pseudo-nitzschia</i>	<i>multiseries</i> 37319	N	-	-	-	-	-
<i>Dictyostelium</i>	<i>discoideum</i> 44689	Y	248	248	96%	6.00E-77	16%
<i>Volvox</i>	<i>carteri</i> 3067	HP	184	184	77%	1.00E-52	23%
<i>Galdieria</i>	<i>sulphuraria</i> 130081	Y	240	240	98%	4.00E-74	16%
<i>Homo</i>	<i>sapiens</i> 9606	Y	383	383	100%	1.00E-126	42%
<i>Mus</i>	<i>musculus</i> 10090	Y	380	380	100%	1.00E-128	42%
<i>Gallus</i>	<i>gallus</i> 9031	Y	377	377	100%	1.00E-124	41%

AAD – Aromatic-aminoacid decarboxylase

GSA - Glutamate 1-semialdehyde 2,1-aminomutase

HP – Hypothetical protein

PDD – Pyridoxal dependent decarboxylase

Y – Significant hit