Bioinformatic evaluation of L-arginine catabolic pathways in 24 cyanobacteria and transcriptional analysis of genes encoding enzymes of L-arginine catabolism in the cyanobacterium *Synechocystis* sp. PCC 6803

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Abstract

**Background:** So far very limited knowledge exists on L-arginine catabolism in cyanobacteria, although six major L-arginine-degrading pathways have been described for prokaryotes. Thus, we have performed a bioinformatic analysis of possible L-arginine-degrading pathways in cyanobacteria. Further, we chose *Synechocystis* sp. PCC 6803 for a more detailed bioinformatic analysis and for validation of the bioinformatic predictions on L-arginine catabolism with a transcript analysis.

**Results:** We have evaluated 24 cyanobacterial genomes of freshwater or marine strains for the presence of putative L-arginine-degrading enzymes. We identified an L-arginine decarboxylase pathway in all 24 strains. In addition, cyanobacteria have one or two further pathways representing either an arginase pathway or L-arginine deiminase pathway or an L-arginine oxidase/dehydrogenase pathway. An L-arginine amidinotransferase pathway as a major L-arginine-degrading pathway is not likely but can not be entirely excluded. A rather unusual finding was that the cyanobacterial L-arginine deiminases are substantially larger than the enzymes in non-photosynthetic bacteria and that they are membrane-bound. A more detailed bioinformatic analysis of *Synechocystis* sp. PCC 6803 revealed that three different L-arginine-degrading pathways may in principle be functional in this cyanobacterium. These are (i) an L-arginine decarboxylase pathway, (ii) an L-arginine deiminase pathway, and (iii) an L-arginine oxidase/dehydrogenase pathway. A transcript analysis of cells grown either with nitrate or L-arginine as sole N-source and with an illumination of 50 μmol photons m⁻² s⁻¹ showed that the transcripts for the first enzyme(s) of all three pathways were present, but that the transcript levels for the L-arginine deiminase and the L-arginine oxidase/dehydrogenase were substantially higher than that of the three isoenzymes of L-arginine decarboxylase.

**Conclusion:** The evaluation of 24 cyanobacterial genomes revealed that five different L-arginine-degrading pathways are present in the investigated cyanobacterial species. In *Synechocystis* sp. PCC 6803 an L-arginine deiminase pathway and an L-arginine oxidase/dehydrogenase pathway represent the major pathways, while the L-arginine decarboxylase pathway most likely only functions in polyamine biosynthesis. The transcripts encoding the enzymes of the two major pathways were constitutively expressed with the exception of the transcript for the carbamate kinase, which was substantially up-regulated in cells grown with L-arginine.
Background

L-arginine metabolism is more complex than the majority of other metabolic pathways in living organisms. This is due to (1) the occurrence of a biosynthetic branch point at the level of carbamoylphosphate, a precursor for L-arginine and pyrimidine biosynthesis, (2) the fact that L-arginine is a potential precursor of polyamines, (3) the fact that L-arginine can be a precursor of 4-aminobutyrate, having a role as neurotransmitter in mammals, (4) the function of L-arginine as a precursor for nitric oxide, acting as an abundant signal molecule in bacteria, mammals, and plants, and (5) the existence of an impressive variety of L-arginine-degrading pathways in eubacteria and archaea. Compared to heterotrophically-growing prokaryotes, L-arginine has specific additional roles in cyanobacteria, because some strains have an alternative carbon dioxide fixation pathway with carbamoylphosphate as the first carbon dioxide fixation product. This pathway leads to the formation of L-citrulline and subsequently to L-arginine [1,2]. Moreover, a number of cyanobacteria is able to synthesize the polymer cyanophycin (multi-L-arginyLPoly-L-aspartate), which consists of an aspartic acid backbone with L-arginine residues being attached to the β-carboxyl group of aspartate by isopeptide bonds [3-6]. Cyanophycin has been shown to have a complex dynamic metabolism, which is not yet completely understood [6-12].

L-Arginine serves as a source of nitrogen, carbon, and energy through a variety of catabolic pathways in archaea and eubacteria [13-16]. In eubacteria, six major L-arginine-degrading pathways have been described (Fig. 1). The first enzymes of these six pathways are an arginase, an L-arginine deiminase, an L-arginine decarboxylase, an L-arginine amidino-transferase, an L-arginine succinylltransferase, and an L-arginine oxidase/dehydrogenase, respectively. Heterotrophically growing bacteria contain either only one of these pathways or have multiple catabolic pathways, as e.g. shown for several Pseudomonas species [13,14]. In Pseudomonas putida and Pseudomonas aeruginosa four L-arginine-degrading pathways are functional. The L-arginine succinyltransferase pathway and the L-arginine deiminase pathway serve as major routes of L-arginine catabolism under aerobic and anaerobic conditions, respectively. In addition, an L-arginine oxidase/dehydrogenase pathway also contributes to L-arginine catabolism under aerobic conditions. The role of a fourth pathway, the L-arginine decarboxylase pathway, still remains somewhat unclear. Although it may provide ammonium from L-arginine, it does not seem to play a major role in L-arginine utilization as carbon source. It may have its major function in the biosynthesis of the polyamines agmatine and putrescine [16].

The understanding of cyanobacterial L-arginine catabolism is scarce and only a few studies on L-arginine-degrading enzymes exist. This work includes the detection of arginase and L-arginine deiminase activity in Anabaena cylindrica (being synonymous with Nostoc sp. PCC 7120 and Anabaena sp. PCC 7120) [17]. Anabaena variabilis [18], Aphanocapsa PCC 6308 [19], and Nostoc sp. PCC 73102 [20]. In Synechocystis sp. PCC 6803 two genes encoding ureohydrolase-type enzymes (Sll1077 and Sll0228) have been identified using bioinformatic tools [21]. L-Ornithine was detected as a major initial product of L-arginine degradation. Based on the detected products, a model of L-arginine catabolism with a putative arginase as the first enzyme has been proposed [21]. In this model

Figure 1

Six major L-arginine-degrading pathways have been described in bacteria. The first enzymatic reaction of each pathway is shown. *Transfer of an amidino group to an acceptor such as glycine, L-lysine or inosamine phosphate. **Molecular oxygen or other electron acceptors such as NADP⁺ or quinones.
L-arginine degradation via arginase is suggested to lead to L-ornithine as first product and subsequently to the production of L-glutamate, and also L-proline. Since L-citrulline and a minor amount of argininosuccinate were also detected as products, an urea cycle-type pathway, besides an arginase pathway, was included in the model [21].

In the two closely related strains Synechococcus elongatus PCC 6301 and PCC 7942 an L-amino acid oxidase (AoxA) with a high specificity for basic L-amino acids and with L-arginine as preferred substrate has been partially characterized [22-24]. Recently, such an enzyme has also been identified by enzymatic activity tests in Synechococcus cedrorum PCC 6908 [23]. The aoxA genes in Synechococcus elongatus PCC 6301 and PCC 7942 have also been identified [23].

Since L-arginine catabolism in heterotrophically growing eu-bacteria is very diverse and since the knowledge on L-arginine catabolism in cyanobacteria is rather limited, the genomes of 24 cyanobacterial strains were screened for the presence of genes encoding putative L-arginine-degrading enzymes in order to obtain an overview on L-arginine catabolism in cyanobacteria. We chose Synechocystis sp. PCC 6803 as a model organism and validated the results of our bioinformatic analysis for this strain with a transcript analysis. We chose Synechocystis sp. PCC 6803, because results on the products of L-arginine degradation have been published more recently [21].

Results and Discussion

Evaluation of 24 cyanobacterial genomes for the presence of genes encoding enzymes of L-arginine-degrading pathways

We used a bioinformatic approach to analyze 24 cyanobacterial strains with fully sequenced and annotated genomes for the presence of genes encoding putative enzymes being involved in the degradation of L-arginine. Among the marine cyanobacteria, the genomes of six Prochlorococcus and six Synechococcus species as well as the genomes of two N2-fixing species (Crocosphaera watsonii WH 8501 and Trichodesmium erythraeum IMS 101) were investigated. The investigated freshwater cyanobacteria included three mesophilic strains, Synechococcus elongatus PCC 6301, Synechococcus elongatus PCC 7942, and Synechocystis sp. PCC 6803, and three thermophilic strains, Thermosynechococcus elongatus BP-1, and two Synechococcus Yellowstone species. The latter two thermophilic strains are capable of N2-fixation with a diurnal rhythm. Moreover, three heterocyst-forming N2-fixing species Anaabaena variabilis ATCC 29413, Nostoc sp. PCC 7120, and Nostoc punctiforme PCC 73102 as well as Gloeobacter violaceus PCC 7421, a strain which lacks thylakoid membranes, were investigated. The origins of the evaluated cyanobacterial genome sequences are listed in Table 1. Sequences of genes encoding enzymes involved in L-arginine degradation in various archaea and heterotrophically growing eu-bacteria were used to identify corresponding genes in cyanobacteria (Table 2). The results of the bioinformatic analyses of the 24 cyanobacterial genomes are given in Tables 3 and 4.

In total, we found evidence for the presence of five putative pathways for L-arginine catabolism in the investigated genomes. These are an L-arginine decarboxylase pathway, an arginase pathway, an L-arginine amidinotransferase pathway, an L-arginine deiminase pathway, and an L-arginine oxidase/dehydrogenase pathway. These pathways are outlined (Fig. 2), and the accession numbers of the corresponding genes are given as supplement in Tables 5, 6, 7, 8, 9. No evidence has been found for the presence of an L-arginine succinyl transferase pathway.

L-arginine decarboxylase pathway

One or several genes encoding L-arginine decarboxylase-type enzymes, which catalyze the formation of agmatine from L-arginine, are present in all investigated cyanobacteria (Fig. 2, Tables 3 and 5). A putative agmatinase that converts agmatine to putrescine and urea is present in nineteen cyanobacterial strains. No such gene was identified in Crocosphaera watsonii WH 8501, Synechococcus elongatus PCC 6301, Synechococcus elongatus PCC 7942, Thermosynechococcus elongatus BP-1, and Gloeobacter violaceus PCC 7421. These strains, with the exception of Crocosphaera watsonii WH 8501, convert agmatine to putrescine via an agmatine deiminase and an N-carbamoylputrescine hydrolase. Since in none of the investigated cyanobacteria a putrescine oxidase or a putrescine transaminase encoding gene has been found, we consider the L-arginine decarboxylase pathway to be mainly responsible for the synthesis of the polyamines agmatine and putrescine as well as for production of ammonium from L-arginine. Putrescine can subsequently be converted to spermidine or spermine. Evidence for the utilization of putrescine by γ-glutamylation like in E. coli [25] was not found. However, since transaminases frequently show broad substrate specificity, we can not entirely exclude that a rather unspecific transaminase, which is not annotated as a putrescine transaminase, catalyzes the conversion of putrescine to 4-aminobutyryl aldehyde. The subsequent dehydrogenase that converts the aldehyde to 4-aminobutyrate is present in 23 of the 24 investigated strains. Such an enzyme is absent in Synechococcus sp. WH 7805. The two enzymes, which catalyze the conversion of 4-aminobutyrate to succinate (4-aminobutyrate transaminase and succinate semialdehyde dehydrogenase) are present in all 24 strains. However, since 4-aminobutyrate also is an intermediate of the L-amine oxidase/dehydrogenase pathway and can additionally be formed by decarboxylation of L-glutamate, the presence of genes encoding...
the latter two enzymes not necessarily implies that a complete L-arginine decarboxylase pathway is present. Therefore, the question whether the L-arginine decarboxylase pathway only provides polyamines and ammonium or also allows for utilization of L-arginine as C-source can not be answered on the basis of the bioinformatic considerations.

A phylogenetic tree of the L-arginine decarboxylases, which are present in the investigated cyanobacterial genomes, is given (Fig. 3) and shows that the cyanobacterial L-arginine decarboxylases cluster into four distinct groups. The clusters marked in green and yellow exclusively contain L-arginine decarboxylases of the marine non-N₂-fixing strains, while the red and blue clusters contain L-arginine decarboxylases of freshwater cyanobacteria and of the two marine N₂-fixing species *Crocphaera watsonii* and *Trichodesmium erythraeum* IMS101. It should be pointed out that in species with more than several L-arginine decarboxylase(s) the corresponding enzymes always group into two different clusters. Thus, the marine as well as the fresh water cyanobacteria seem to have two distinct types of L-arginine decarboxylases.

It has previously been shown by Sandmeier et al. [26] that amino acid decarboxylases in general can be subdivided into four different groups. These groups seem to be evolutionary unrelated to each other. In these subdivisions, the

Table 1: Origin of the 24 cyanobacterial genome sequences that were used to perform the bioinformatic evaluation of the presence of L-arginine-degrading pathways in cyanobacteria.

| Cyanobacterial strain | Origin of genome sequence* | Reference sequence | GenBank     | Mbps | %GC | Proteins/RNAs |
|-----------------------|-----------------------------|--------------------|-------------|------|-----|---------------|
| *Prochlorococcus marinus* SS 120 | European Union/Genoscope     | NC_005042          | AE017126    | 1.75 | 36.4| 1883/46       |
| *Prochlorococcus marinus* MIT 9211 | Craig Venter Institute      | NZ_AALP00000000   | AALP00000000| 1.84 | 39.7| 2123/45       |
| *Prochlorococcus marinus* MIT 9312 | JGI/MIT/DOE                | NC_007577          | CP000111    | 1.71 | 31.2| 1810/45       |
| *Prochlorococcus marinus* MIT9313 | JGI/DOE                    | NC_005071          | BXS48175    | 2.41 | 50.7| 2269/55       |
| *Prochlorococcus marinus* MED 4 | JGI/DOE                    | NC_005072          | BXS48174    | 1.70 | 30.8| 1717/44       |
| *Prochlorococcus marinus* NATL 2A | JGI/DOE                    | NC_007335          | CP000095    | 1.84 | 35.1| 1892/44       |
| *Synechococcus* sp. WH 8102 | JGI/DOE                    | NC_005070          | BXS48200    | 2.44 | 59.4| 2519/55       |
| *Synechococcus* sp. CC 9902 | JGI/DOE                    | NC_007513          | CP000097    | 2.24 | 54.2| 2307/51       |
| *Synechococcus* sp. RS 9917 | Craig Venter Institute     | NZ_AANP00000000    | AANP00000000| 2.58 | 64.5| 2770/50       |
| *Synechococcus* sp. CC 9605 | JGI/DOE                    | NC_007516          | CP000110    | 2.51 | 59.2| 2645/54       |
| *Synechococcus* sp. WH 5701 | Craig Venter Institute     | NZ_AAN0000000000  | AAN0000000000| 3.04 | 65.4| 3346/55       |
| *Synechococcus* sp. WH 7805 | Craig Venter Institute     | NZ_AAOK0000000000  | AAOK0000000000| 2.62 | 57.6| 2883/51       |
| *Trichodesmium erythraeum* IMS 101 | WHOI/JGI/DOE              | NC_008312          | CP000393    | 7.75 | 34.1| 4451/48       |
| *Crocosphaera watsonii* WH 8501 | WHOI/JGI/DOE              | NC_AADV0000000000  | AADV0000000000| 6.24 | 37.1| 5958/38       |

| Freshwater species |
|-------------------|
| *Synechococcus elongatus* PCC 6301 | Nagoya University | NC_006576 | AP008231 | 2.70 | 55.5| 2527/55       |
| *Synechococcus elongatus* PCC 7942 | JGI/Texas A & M University/DOE | NC_007604 | CP000100 | 2.70 | 55.5| 2612/53       |
| *Synechocystis* sp. PCC 6803 | Kazusa DNA Research Institute | NC_000911 | BA000022 | 3.57 | 47.7| 3172/50       |
| *Gloeobacter violaceus* PCC 7421 | Kazusa DNA Research Institute | NC_005125 | BA000045 | 4.66 | 62.0| 4430/52       |
| *Nostoc* sp. PCC 7120 | Kazusa DNA Research Institute | NC_003272 | BA000019 | 6.41 | 41.3| 5366/64       |
| *Nostoc punctiforme* PCC 73102 | JGI/DOE | NZ_AAY00000000  | AAY0000000000| 9.02 | 41.4| 7672/n.d.     |
| *Anabaena variabilis* ATCC 29413 | Missouri State University/JGI/DOE | NC_007413 | CP000117 | 6.37 | 41.4| 5043/62       |
| *Thermosynechococcus elongatus* BP-1 | Kazusa DNA Research Institute | NC_004113 | BA000039 | 2.59 | 53.9| 2476/49       |
| *Synechococcus Yellowstone A JA-3-3Ab* | TIGR | NC_007775 | CP000239 | 2.93 | 60.2| 2760/55       |
| *Synechococcus Yellowstone B JA-2-3B’a (2–13)* | TIGR | NC_007776 | CP000240 | 3.05 | 58.5| 2862/52       |

*JGI, Joint Genome Research Institute; DOE, Department of Energy USA; WHOI, Woods Hole Oceanographic Institute; MIT, Massachusetts Institute of Technology; TIGR, The Institute for Genomic Research. The strain *Prochlorococcus marinus* SS 120 corresponds to *Prochlorococcus marinus* subsp. *marinus* str. CCMP 1375 and strain *Prochlorococcus marinus* MED 4 corresponds to *Prochlorococcus marinus* subsp. *pastoris* str. CCMP 1986 or CCMP 1378. *Nostoc* sp. PCC 7120 is synonymous to *Anabaena* sp. PCC 7120 as well as *Anabaena cylindrica*. N.d. = not detected.
groups III and IV contain decarboxylases with specificity for basic L-amino acids. In addition, there is evidence that E. coli has two different L-arginine decarboxylases – a biosynthetic and a biodegradable form. The biodegradable L-arginine decarboxylase (P28629 – group III decarboxylase) is only induced in large amounts when cells are grown in rich medium containing L-arginine, while the biosynthetic enzyme (P21170 – group IV decarboxylase) is expressed constitutively [26,27]. On the basis of this classification, the red and green clusters (Fig. 3) contain L-arginine decarboxylases being more similar to group IV L-arginine decarboxylases, while the blue and yellow clusters contain L-arginine decarboxylases with higher similarity to group III L-arginine decarboxylases. The similarity of the biodegradable and the biosynthetic L-arginine decarboxylase of E. coli to selected marine and fresh water cyanobacterial L-arginine decarboxylases is presented in Table 10. E.g. the L-arginine decarboxylases Slr0662 and Slr1312 of Synechocystis sp. PCC 6803 in the red cluster have a higher similarity to the biosynthetic L-arginine decarboxylase (P21170) of group IV than to the biodegradable L-arginine decarboxylase P28629 of group III. In contrast, Slr1683 of Synechocystis sp. PCC 6803 has a higher similarity to P28629 (group III) than to P21170 (group IV) (Table 10). Thus, it is likely that the green and the red cluster (Fig. 3) contain L-arginine decarboxylases of the biosynthetic-type, while the yellow and blue clusters contain L-arginine decarboxylases of the biodegradative type.

Arginase pathway
Urea is released from L-arginine by an arginase in the arginase pathway, and the resulting L-ornithine is further catalyzed to L-glutamate by L-ornithine transaminase and Δ1pyrroline-5-carboxylate dehydrogenase (Fig. 2). In the presence of urease, urea is further degraded to ammonium. The arginase pathway seems to be widely distributed among the investigated cyanobacteria. Genes

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**Table 2: Origin of archaea, eubacterial, and eukaryotic genome sequences used as a reference for the bioinformatic analysis of putative L-arginine-degrading pathways in cyanobacteria.**

| Organism | Origin of genome sequence | Reference sequence | GenBank | Mbps | % GC | Number of Proteins/RNA |
|----------|---------------------------|-------------------|--------|------|------|------------------------|
| *Escherichia coli* K-12 MG1655 | University of Wisconsin-Madison, U.S.A.; *Escherichia coli* Genomic Project NC_000913 | U00096 | 4.64 | 50.8 | 4243/157 |
| *Pseudomonas aeruginosa* PAO1 | PathoGenesis Corporation, Skokie, U.S.A. NC_002516 | AE004091 | 6.30 | 66.6 | 5568/81 |
| *Pseudomonas fluorescens* Pf-5 | DOE Joint Genome Institute, U.S.A. NC_004129 | CP000076 | 7.08 | 63.3 | 6137/87 |
| *Pseudomonas syringae* pv. *syringae* B728a | DOE Joint Genome Institute, U.S.A. NC_007005 | CP000075 | 6.09 | 59.2 | 5089/83 |
| *Bacillus subtilis* subsp. *subtilis* str. 168 | Non-redundant *B. subtilis* database NC_000964 | AL009126 | 4.22 | 43.5 | 4105/119 |
| *Bacillus clausii* KSM-K16 | Kao Corporation, Biological Science Laboratories, Japan NC_006582 | AP006637 | 4.30 | 44.8 | 4096/96 |
| *Bacillus halodurans* C-125 | Extreme Biosphere Research Center MSTC, Japan NC_002570 | BA000004 | 4.20 | 43.7 | 4066/105 |
| *Xanthomonas campestris* pv. *campestris* str. ATCC 33913 | Sao Paulo (State) Consortium NC_003902 | AE008922 | 5.08 | 65.1 | 4181/61 |
| *Corynebacterium glutamicum* ATCC 13032 | Kitazato University, Kitasato, Japan NC_003450 | BA000036 | 3.31 | 53.8 | 2993/81 |
| *Brucella melitensis* 16M | Integrated Genomics Inc., Chicago, U.S.A. NC_003317(chr. I) | AE008917 | 2.12 | 57.2 | 2059/48 |
| *Ralstonia solanacearum* GMI 1000 | Genoscope, Evry cedex, France NC_003318 (chr. II) | AE008918 | 1.18 | 57.3 | 1139/18 |
| *Arabidopsis thaliana* (thale cress) | Arabidopsis Genome Initiative NC_002970 (chr. 1) | AE005172 | 30.43 | 35.7 | 7852/7852 |
| | | AE002093 | 19.71 | 35.9 | 4853/4853 |
| | | BA000014 | 23.47 | 36.3 | 6048/6048 |
| | | AJ270058 | 18.58 | 36.2 | 4655/4655 |
| | | BA000015 | 26.99 | 35.9 | 7072/7072 |

A sequence from *Synechococcus* sp. Yellowstone B JA-2-3B’s 2–13 was used to screen for L-arginine amidinotransferase sequences. The screen for L-arginine oxidase/dehydrogenases was performed with the *aoxA* sequence from *Synechococcus elongatus* PCC 6301/PCC 7942.
encoding the putative second and third enzyme of this pathway, the L-ornithine transaminase and the Δ1pyrroline-5-carboxylate dehydrogenase, are present in all 24 investigated cyanobacteria. A gene encoding a putative arginase is only present in 19 of the investigated genomes (Tables 4 and 6). Such a gene is absent in *Crocosphaera watsonii* WH 8501, *Synechococcus elongatus* PCC 6301, *Synechococcus elongatus* PCC 7942, *Thermosynechococcus elongatus* BP-1, and *Gloeobacter violaceus* PCC 7421.

The likely absence of an arginase-type enzyme in five of the investigated 24 cyanobacterial strains is somewhat surprising, since arginases have been shown to be present in all so far investigated higher plants [28]. However, since plant-type arginases represent a distinct group of ureohydrolases [28] (Fig. 4, ARGAH1 and AtFG08870) and localize in mitochondria [29], they may have originated from the predecessor organism, which gave rise to the evolutionary lineage of mitochondria.

### L-arginine amidinotransferase pathway

In addition to arginases, L-ornithine may also be synthesized by L-arginine amidinotransferases (Fig. 2). A gene for such an enzyme was detected in the N2-fixing species *Nostoc* sp. PCC 7120, *Nostoc punctiforme* PCC 73102, *Anabaena variabilis* ATCC 29413, *Trichodesmium erythraeum* IMS 101, *Crocosphaera watsonii* WH 8501, *Synechococcus* *Yellowstone* sp. JA-2-3Ba’ (2–13), and in the non-N2 fixing cyanobacteria *Synechocystis* sp. PCC 6803, *Gloeobacter violaceus* PCC 7421, and *Synechococcus* sp. PCC 7120 (Table 4 and 7). Three of the five cyanobacteria without an arginase-type enzyme have a putative L-arginine amidinotransferase-type enzyme (*Crocosphaera watsonii* WH 8501, *Synechococcus elongatus* PCC 7421). Thus, *Synechococcus elongatus* PCC 6301 and PCC 7942 are probably the only cyanobacterial strains among the 24 investigated ones, which are unable to form L-ornithine from L-arginine. Interestingly, they

| Pathway | Marine species | L-Arginine decarboxylase |
|----------|---------------|-------------------------|
| Enzymes | A1 | A2.1 | A2.2 | A2.3 | A3 | A4 | A5 | A6 |
| **Marine species** | | | | | | | | |
| *Prochlorococcus marinus* SS 120 | + | + | n.d. | + | n.d. | + | + | + |
| *Prochlorococcus marinus* str. MIT 9211 | + | + | n.d. | + | n.d. | + | + | + |
| *Prochlorococcus marinus* MIT 9312 | + | + | n.d. | + | n.d. | + | + | + |
| *Prochlorococcus marinus* MIT 9313 | + | + | n.d. | + | n.d. | + | + | + |
| *Prochlorococcus marinus* MED 4 | + | + | n.d. | + | n.d. | + | + | + |
| *Prochlorococcus marinus* NATL 2A | + | + | n.d. | + | n.d. | + | + | + |
| *Synechococcus* sp. CC 9605 | + | + | n.d. | + | n.d. | + | + | + |
| *Synechococcus* sp. CC 9902 | + | + | n.d. | + | n.d. | + | + | + |
| *Synechococcus* sp. WH 8102 | + | + | n.d. | + | n.d. | + | + | + |
| *Synechococcus* sp. WH 7805 | + | + | n.d. | + | n.d. | n.d. | + | + |
| *Synechococcus* sp. WH 5701 | + | + | n.d. | + | n.d. | + | + | + |
| *Synechococcus* sp. RS 9917 | + | + | n.d. | + | n.d. | + | + | + |
| *Crocosphaera watsonii* WH 8501 | + | n.d. | n.d. | + | n.d. | + | + | + |
| *Trichodesmium erythraeum* IMS 101 | + | + | n.d. | + | n.d. | + | + | + |

### Table 3: Presence of genes encoding enzymes of the L-arginine-degrading pathways in the genomes of selected marine and freshwater cyanobacteria.

**Pathway** | **Enzymes**
--- | ---
**L-Arginine decarboxylase** | **A1** | **A2.1** | **A2.2** | **A2.3** | **A3** | **A4** | **A5** | **A6**
**Marine species** | | | | | | | | | |
| *Prochlorococcus marinus* SS 120 | + | + | n.d. | + | n.d. | + | + | + |
| *Prochlorococcus marinus* str. MIT 9211 | + | + | n.d. | + | n.d. | + | + | + |
| *Prochlorococcus marinus* MIT 9312 | + | + | n.d. | + | n.d. | + | + | + |
| *Prochlorococcus marinus* MIT 9313 | + | + | n.d. | + | n.d. | + | + | + |
| *Prochlorococcus marinus* MED 4 | + | + | n.d. | + | n.d. | + | + | + |
| *Prochlorococcus marinus* NATL 2A | + | + | n.d. | + | n.d. | + | + | + |
| *Synechococcus* sp. CC 9605 | + | + | n.d. | + | n.d. | + | + | + |
| *Synechococcus* sp. CC 9902 | + | + | n.d. | + | n.d. | + | + | + |
| *Synechococcus* sp. WH 8102 | + | + | n.d. | + | n.d. | + | + | + |
| *Synechococcus* sp. WH 7805 | + | + | n.d. | + | n.d. | n.d. | + | + |
| *Synechococcus* sp. WH 5701 | + | + | n.d. | + | n.d. | + | + | + |
| *Synechococcus* sp. RS 9917 | + | + | n.d. | + | n.d. | + | + | + |
| *Crocosphaera watsonii* WH 8501 | + | n.d. | n.d. | + | n.d. | + | + | + |
| *Trichodesmium erythraeum* IMS 101 | + | + | n.d. | + | n.d. | + | + | + |

**Freshwater species**

| Marine species | | | | | | | | |
| **Synechococcus elongatus** sp. PCC 6301 | + | n.d. | + | + | n.d. | + | + | + |
| *Synechococcus* elongatus sp. PCC 7942 | + | n.d. | + | + | n.d. | + | + | + |
| *Synechococcus* Yellowstone sp. JA-3-3-AB | + | + | n.d. | + | n.d. | + | + | + |
| *Synechococcus* Yellowstone sp. BJ-A-2-3B’a (2–13) | + | + | n.d. | + | n.d. | + | + | + |
| *Thermosynechococcus elongatus* BP-1 | + | n.d. | + | + | n.d. | + | + | + |
| *Synechocystis* sp. PCC 6803 | + | + | n.d. | + | n.d. | + | + | + |
| *Gloeobacter violaceus* PCC 7421 | + | n.d. | + | n.d. | + | + | + | + |
| *Nostoc* sp. PCC 7120 | + | + | n.d. | + | n.d. | + | + | + |
| *Nostoc* punctiforme PCC 73102 | + | + | n.d. | + | n.d. | + | + | + |
| *Anabaena variabilis* ATCC 29413 | + | + | n.d. | + | n.d. | + | + | + |

L-ornithine is formed from L-arginine by the enzymes arginase or L-arginine amidinotransferase. It is also formed in the 2nd reaction of the L-arginine deiminase pathway. Enzymes A5 and E3 are identical enzymes and both represent a 4-aminobutyrate transaminase. Enzymes A6 and E4 are identical and both represent a succinate semialdehyde dehydrogenase (Fig. 2). Enzymes A2.1, B1, and C1 represent ureohydrolases, and the same gene(s) is (are) annotated as an agmatinase (A2.1), an arginase (B1) or a 4-guanidinobutyrase (E2). The genes encoding the enzymes C1 and D1 are annotated as L-arginine amidinotransferase as well as L-arginine deiminase (see text for further details). N.d. = not detected.
have a very active L-amino acid oxidase (AoxA) with high specificity for basic amino acids and a preference for L-arginine, utilizing molecular oxygen as an electron acceptor [22-24].

**L-arginine deiminase pathway**

The L-arginine deiminase pathway is widely distributed among eubacteria and archaea [13,14,16] and has also been discovered in a few primitive eukaryotes, e.g. in *Giardia intestinalis* [30], *Trichomonas vaginalis* [31], and *Tritrichomonas foetus* [32]. However, it has so far not been detected in multi-cellular organisms. The L-arginine deiminase pathway consists of three enzymes and catalyzes the production of ATP in its final enzymatic step. The first enzyme of this pathway is an L-arginine deiminase, which irreversibly converts L-arginine to L-citrulline and ammonium. The second and third enzymes are an L-ornithine transcarbamoylase and a carbamate kinase, respectively (Fig. 2). A gene encoding a putative L-arginine deiminase was detected in the N2-fixing species *Nostoc* sp. PCC 7120, *Nostoc punctiforme* PCC 73102, *Anabaena variabilis* ATCC 29413, *Trichodesmium erythraeum* IMS 101, *Crocosphaera watsonii* WH 8501, and *Synechococcus* sp. CC 9902 (Tables 4 and 8).

### Table 4: Presence of genes encoding enzymes of the L-arginine-degrading pathways in the genomes of selected marine and freshwater cyanobacteria.

| Pathway | Arginase | L-Arginine amidinotransferase | L-Arginine deiminase | L-Arginine oxidase/dehydrogenase |
|---------|----------|------------------------------|----------------------|---------------------------------|
| Enzymes | B1 | B2 | B3 | C1 | C2 | C3 | D1 | D2 | D3 | D4 | D5 | E1 | E2 | E3 | E4 |
| Marine species | | | | | | | | | | | | | | | |
| Prochlorococcus marinus SS 120 | + | + | n.d. | + | + | n.d. | + | n.d. | + | n.d. | + | + |
| Prochlorococcus marinus str. MIT 9211 | + | + | n.d. | + | + | n.d. | + | n.d. | + | n.d. | + | + |
| Prochlorococcus marinus MIT 9312 | + | + | n.d. | + | + | n.d. | + | n.d. | + | n.d. | + | + |
| Prochlorococcus marinus MIT 9313 | + | + | n.d. | + | + | n.d. | + | n.d. | + | n.d. | + | + |
| Prochlorococcus marinus MED 4 | + | + | n.d. | + | + | n.d. | + | n.d. | + | n.d. | + | + |
| Prochlorococcus marinus NATL 2A | + | + | n.d. | + | + | n.d. | + | n.d. | + | n.d. | + | + |
| Synechococcus sp. CC 9605 | + | + | n.d. | + | + | n.d. | + | n.d. | + | n.d. | + | + |
| Synechococcus sp. CC 9902 | + | + | n.d. | + | + | n.d. | + | n.d. | + | n.d. | + | + |
| Synechococcus sp. WH 8102 | + | + | n.d. | + | + | n.d. | + | n.d. | + | n.d. | + | + |
| Synechococcus sp. WH 7805 | + | + | n.d. | + | + | n.d. | + | n.d. | + | n.d. | + | + |
| Synechococcus sp. WH 5701 | + | + | n.d. | + | + | n.d. | + | n.d. | + | n.d. | + | + |
| Synechococcus sp. RS 9917 | + | + | n.d. | + | + | n.d. | + | n.d. | + | n.d. | + | + |
| Crocosphaera watsonii WH 8501 | n.d. | + | + | + | + | + | n.d. | n.d. | + | n.d. | n.d. | + |
| Trichodesmium erythraeum IMS 101 | + | + | + | + | + | + | n.d. | n.d. | + | n.d. | n.d. | + |

| Freshwater species | | | | | | | | | | | | | | | |
| Synechococcus elongatus sp. PCC 6301 | n.d. | + | + | n.d. | + | + | n.d. | + | n.d. | + | + | n.d. | + |
| Synechococcus elongatus sp. PCC 7942 | n.d. | + | + | n.d. | + | + | n.d. | + | n.d. | + | + | n.d. | + |
| Synechococcus Yellowstone sp. A JA-3-3-AB | + | + | n.d. | + | + | n.d. | n.d. | n.d. | + | n.d. | + | + |
| Synechococcus Yellowstone sp. B JA-2-3B'a (2–13) | + | + | + | + | + | n.d. | + | n.d. | + | n.d. | + | + |
| Thermosynechococcus elongatus BP-1 | n.d. | + | + | + | + | + | n.d. | + | n.d. | n.d. | + |
| Synechocystis sp. PCC 6803 | + | + | + | + | + | + | n.d. | + | + | n.d. | + |
| Gloeobacter violaceus PCC 7421 | n.d. | + | + | + | + | + | n.d. | + | + | n.d. | + |
| Nostoc sp. PCC 7120 | + | + | + | + | + | + | n.d. | + | + | + | + |
| Nostoc punctiforme PCC 73102 | + | + | + | + | + | + | n.d. | + | + | + | + |
| Anabaena variabilis ATCC 29413 | + | + | + | + | + | + | n.d. | n.d. | + | n.d. | + | + |

These species have a very active L-amino acid oxidase (AoxA) with high specificity for basic amino acids and a preference for L-arginine, utilizing molecular oxygen as an electron acceptor [22-24].
L-arginine oxidase/dehydrogenase pathway

The fifth putative L-arginine catabolic pathway starts with an L-arginine oxidase/dehydrogenase-type enzyme. In this pathway L-arginine is converted to succinate via 2-ketoarginine, 4-guanidinobutyrate, and 4-aminobutyrate with a concomitant production of ammonium, carbon dioxide, and urea (Fig. 2). Ten out of 24 cyanobacterial species have one or two gene(s) encoding an L-arginine oxidase/dehydrogenase (Tables 4 and 9), which is similar to an L-amino acid oxidase that is present in the two closely related strains *Synechococcus elongatus* PCC 6301 and PCC 7942 [22-24]. The corresponding L-amino acid oxidase of these two cyanobacteria is encoded by the *aoxA* genes YP_171306 and ZP_00164087 for *Synechococcus elongatus* PCC 6301 and PCC 7942, respectively, and has been purified and partially characterized. This AoxA has a high specificity for basic L-amino acids as substrate with a preference for L-arginine. AoxA converts L-arginine to 2-

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**Figure 2**
Schematic presentation of putative L-arginine-degrading pathways in cyanobacteria with the corresponding enzymes, intermediate metabolites, and final products. Numbering of enzymes refers to the one used in Table 3, 4, and 5–9.
ketoarginine and ammonium and utilizes oxygen as electron acceptor. When hydrogen peroxide is not removed by hydrogen peroxide decomposing enzymes, 2-ketoarginine is converted to 4-guanidinobutyrate in a non-enzymatic reaction. Seven of the 10 cyanobacteria, which have a putative L-arginine oxidase/dehydrogenase, Table 5: Database entries of genes from 24 cyanobacterial genomes encoding putative L-arginine decarboxylases (A1), agmatinases (A2.1), agmatine deiminases (A2.2), N-carbamoylpentrescine hydrolases (A2.3), putrescine oxidases or putrescine transaminases (A3), and 4-aminobutyraldehyde dehydrogenases (A4) of the L-arginine decarboxylase pathway.

| Enzyme | A1 | A2.1 | A2.2 | A2.3 | A3 | A4 |
|--------|----|------|------|------|----|----|
| Marine species | | | | | | |
| Prochlorococcus marinus SS 120 | Pro1112, Pro0049 | Pro1849 | n.d | Pro1045 | n.d | Pro1319 |
| Prochlorococcus marinus str. MIT 9211 | P211_03242, P211_08607 | P9211_09067 | n.d | P9211_03592 | n.d | P9211_07012 |
| Prochlorococcus marinus MIT 9312 | PML312_1095, PML312_0646 | PML312_1779 | n.d | PML312_0615 | n.d | PML312_0337 |
| Prochlorococcus marinus MIT 9313 | PML1066, PML2150 | PML2214 | n.d | PML0395 | n.d | PML0191 |
| Prochlorococcus marinus MED 4 | PMM1084, PMM0045 | PMM1686 | n.d | PMM0615 | n.d | PMM1215, PMM0331 |
| Prochlorococcus marinus NATL 2A | PMNA2A_0665, PMNA2A_1378 | PMNA2A_1287 | n.d | PMNA2A_0502 | n.d | PMNA2A_1709 |
| Synechococcus sp. CC 9605 | Sync9605_1621, Sync9605_2513 | Sync9605_1082 | Sync9605_2591 | Sync9605_0497 |
| Synechococcus sp. CC 9902 | Sync9902_1380, Sync9902_2172 | Sync9902_2230 | n.d | Sync9902_1838 |
| Synechococcus sp. WH 8102 | SYN90949, SYN92359 | SYN91412, SYN92242 | n.d | SYW1008 | n.d | SYNW_1956 |
| Synechococcus sp. WH 7085 | WH7805_04481, WH7805_10353 | WH7805_09974 | n.d | WH7805_01902 | n.d | n.d |
| Synechococcus sp. WH 5701 | WH7501_04905, WH7501_0310 | WH7501_03684, WH7501_03860 | n.d | WH7501_06196 |
| Synechococcus sp. RS 9917 | R590917_01007, R590917_06495 | R590917_06190 | n.d | R590917_11395 | n.d | R590917_02641 |
| Crocosphaera watsonii WH 8501 | CwatDRAFT_1880 | n.d | n.d | CwatDRAFT_4111 | n.d | CwatDRAFT_1681, CwatDRAFT_9242 |
| Trichodesmium erythraeum IMS 101 | TeryDRAFT_0894, TeryDRAFT_0959, TeryDRAFT_0311 | TeryDRAFT4567 | n.d | TeryDRAFT_0835 | n.d | TeryDRAFT_3296, TeryDRAFT_3923 |

| Freshwater species | | | | | | |
|-------------------|----|------|------|------|----|----|
| Synechococcus elongatus sp. PCC 6301 | Syc0823_d, Syc0510_d | n.d | Syc1703_c, Syc1643_d | Syc1946_d, Syc1745_c | n.d | Syc1030_d |
| Synechococcus elongatus sp. PCC 7942 | Sync7942_0707, Sync7942_1037 | n.d | Sync794212420, Sync79422461 | Sync79422145, Sync79422358 | n.d | Sync7942_0489 |
| Synechococcus Yellowstone sp. JA-3-3-AB | CYA_1002, CYA_0128 | CYA_0859 | n.d | CYA_0364 |
| Synechococcus Yellowstone sp. JA-2-3Ba (2-13) | CYB_2779, CYB_0482 | CYB_1744 | n.d | CYB_1181 | n.d | CYB_0715, CYB_1893 |
| Thermosynechococcus elongatus BP-1 | Tlr1866, Tlr1807 | n.d | Tlr1811, Tlr0920 | n.d | Tlr0221 |
| Synechocystis sp. PCC 6803 | SiI1173, SiI1460 | SiI1495, SiI0370 |
| Gloeobacter violaceus PCC 7421 | Gil4070, Gil3478 | n.d | Gil1681, Gil1682, Gil2043 | Gil2207, Gil1504, Gil3848, Gil3380 | n.d | Gil2207, Gil1504, Gil3848, Gil3380 |
| Nostoc sp. PCC 7120 | All401, All4887 | All2310 | n.d | All2826, All3771, All3556, All5022 |
| Nostoc punctiforme PCC 73102 | Npun02000556, Npun02000612 | Npun02002114 | n.d | Npun02002053 | n.d | Npun02003427, Npun02002895, Npun02002692, Npun02003702 |
| Anabaena variabilis ATCC 29413 | Ava_2157, Ava_3423 | Ava_0127 | n.d | Ava_0561 | n.d | Ava_1107, Ava_1554, Ava_3334, Ava_2258 |

N.d. = not detected.
also have a gene encoding a putative 4-guanidino butyrase ([**Synechococcus** sp. CC 9605, **Synechococcus** sp. WH 7805, **Synechococcus** sp. WH 5701, **Trichodesmium erythraeum** IMS 101, **Synechocystis** sp. PCC 6803, **Nostoc** sp. PCC 7120, and **Nostoc punctiforme** PCC 73102), while the enzyme is absent in **Synechococcus elongatus** PCC 6301, **Synechococcus elongatus** PCC 7942, and **Gloeobacter violaceus** PCC 7421.

The genes encoding the two enzymes which convert 4-aminobutyrate to succinate (4-aminobutyrate transaminase and succinate semialdehyde dehydrogenase) are present in all investigated cyanobacteria. The fact that 4-aminobutyrate is also an intermediate in the L-arginine decarboxylase pathway and can additionally be formed by decarboxylation of L-glutamate might explain the presence of these two enzymes even in those cyanobacteria that do not have an L-arginine oxidase/dehydrogenase. An L-arginine oxidase/dehydrogenase pathway, converting L-arginine to 4-aminobutyrate, was first described on the basis of detected products for **Streptomyces griseus** [34] and is also present in **Pseudomonas putida** (Trevisan) Migula P2 ATCC 25571. However, the first enzyme has not yet been characterized biochemically [16,35,36].

**L-arginine succinyl transferase pathway**

We did not find evidence for the presence of an L-arginine succinyl transferase pathway in the genome sequences of the investigated 24 cyanobacterial strains. This pathway is suggested to be mainly limited to those heterotrophically
growing eubacteria that have the ability to use L-arginine as both, a nitrogen and a carbon source [13,14,16].

Problems related to the bioinformatic analysis

All 24 investigated cyanobacterial genomes have a putative L-arginine decarboxylase pathway and one or several additional L-arginine-degrading pathways. These can either be an arginase pathway, an L-arginine amidinotransferase pathway, an L-arginine deiminase or an L-arginine oxidase/dehydrogenase pathway. Thus, all investigated cyanobacteria have at least two putative L-arginine-degrading pathways. However, the performed similarity searches do not always allow a statement whether all enzymes of the corresponding pathways are present and whether the gene products have indeed the enzymatic activity that has been assigned to them on the basis of the corresponding similarity searches and domain predictions. No matter what similarity search results suggest, a proof is only provided by activity measurements with purified enzymes. Therefore, uncertainties related to this aspect will be briefly discussed with respect to the enzymes being annotated as ureohydrolases [37] and enzymes being annotated as L-arginine amidinotransferases or L-arginine deiminases. The latter two types of enzymes belong to the family of guanidino group modifiers [38].

Ureohydrolases

The bioinformatic evaluation of the 24 cyanobacterial genome sequences suggests the presence of (a) gene(s) encoding an arginase, an agmatinase, or a 4-guanidino butyrase in 19 cyanobacterial genomes. Five cyanobacterial species have neither an arginase- nor an agmatinase- nor a 4-guanidino butyrase-encoding gene (Tables 4 and 11). Arginases, agmatinases, and 4-guanidino butyrases release urea from L-arginine (guanidino amino acid),

Table 7: Database entries of genes from 24 cyanobacterial genomes encoding putative L-arginine amidinotransferases (C1), L-ornithine transaminases (C2), and Δ1 pyrroline-5-carboxylate dehydrogenases (C3) of the L-arginine amidinotransferase pathway.

| Enzyme | C1 | C2 | C3 |
|--------|----|----|----|
| **Marine species** | | | |
| Prochlorococcus marinus SS 120 | n.d. | Pro1375, Pro1626 | Pro0374 |
| Prochlorococcus marinus str. MIT 9211 | n.d. | P9211_02002, P9211_0217 | P9211_07012 |
| Prochlorococcus marinus MIT 9312 | n.d. | PMT9312_1397, PMT9312_1565 | PMT9312_0337 |
| Prochlorococcus marinus MIT 9313 | n.d. | PMT0331, PMT1493 | PMT0191 |
| Prochlorococcus marinus MED 4 | n.d. | PMM1301, PMM1472 | PMM0331 |
| Prochlorococcus marinus NATL 2A | n.d. | PMN2A_0867, PMN2A_1003 | PMN2A_1709 |
| Synechococcus sp. CC 9605 | n.d. | Syncc9605_0858, Syncc9605_2052, Syncc9605_0569 | Syncc9605_0497 |
| Synechococcus sp. CC 9902 | n.d. | Syncc9902_1534, Syncc9902_0620 | Syncc9902_1838 |
| Synechococcus sp. WH 8102 | n.d. | SYNW1634, SYNW0629 | SYNW1956 |
| Synechococcus sp. WH 7805 | n.d. | WH7805_0565, WH7805_12388, WH7805_13803 | WH7805_06416 |
| Synechococcus sp. WH 5701 | n.d. | WH5701_07406, WH5701_15376 | WH5701_0696 |
| Synechococcus sp. RS 9917 | n.d. | R59917_02041, R59917_05240 | R59917_02641 |
| Crocosphaera watsonii WH 8501 | n.d. | CwatDRAFT_0830 | CwatDRAFT_5161 |
| Trichodesmium erythraeum IMS 101 | n.d. | TeryDRAFT_2282 | TeryDRAFT_3251 |

| **Freshwater species** | | | |
| Synechococcus elongatus sp. PCC 6301 | n.d. | Syc0599_c, Syc1466_c | Syc1030_d |
| Synechococcus elongatus sp. PCC 7942 | n.d. | Synpcc7942_0943, Synpcc7942_0031 | Synpcc7942_0489 |
| Synechococcus Yellowstone sp. JA-3-3-AB (2-13) | n.d. | CYA_1537, CYA_0889 | CYA_0364 |
| Synechococcus Yellowstone sp. JA-2-38a | n.d. | CYB_0250 | CYB_0516, CYB_0715, CYB_1893 |
| Thermosynechococcus elongatus BP-1 | n.d. | TII0507 | TII1328, TII0408, TII1935 |
| Synechocystis sp. PCC 6803 | n.d. | SII1336 | SII0416, SII0221 |
| Gloeobacter violaceus PCC 7421 | n.d. | Girs1758 | Girs1561, Girs0370, Girs0091 |
| Nostoc sp. PCC 7120 | n.d. | Air2398, Air1080, Air0396 | Air0540, Air3771, Air1556, Air10022 |
| Nostoc punctiforme PCC 73102 | n.d. | Npun02001803 | Npun02003702, Npun02006572, Npun02002795, Npun02002692 |
| Anabaena variabilis ATCC 29413 | n.d. | Ava_2273 | Ava_2942, Ava_1354, Ava_3534, Ava_2238 |

N.d. = not detected.
agmatine (guanidino amine) or 4-guanidino butyrate (guanidino acid), respectively. All three types of enzymes belong to the group of ureohydrolases (C-N hydrolases), require the cofactor manganese, and might have an identical evolutionary origin. This implies that an ancient enzyme with broad substrate specificity has progressively been evolved to gain narrower substrate specificity during evolution. Therefore, it is extremely difficult to annotate

Table 8: Database entries of genes from 24 cyanobacterial genomes encoding putative L-arginine deiminases (D1), L-ornithine transcarbamoylases (D2), carbamate kinases (D3), L-ornithine transaminases (D4), and Δ¹-pyrroline-5-carboxylate dehydrogenases (D5) of the L-arginine deiminase pathway.

| Enzyme                          | D1       | D2       | D3       | D4       | D5       |
|--------------------------------|----------|----------|----------|----------|----------|
| Marine species                 |          |          |          |          |          |
| Prochlorococcus marinus SS 120 | n.d.     | Pro1337, | Pro0262  | n.d.     | Pro1375, |
|                                |          | Pro1626  |          |          | Pro1626  |
| Prochlorococcus marinus str. MIT 9211 | n.d. | P9211_0227, P9211_07567 | n.d. | P9211_02002, P9211_0127 | P9211_07012 |
| Prochlorococcus marinus MIT 9312 | n.d.     | P9213_1357 | n.d. | P9213_1397, P9213_1565 | P9213_0337 |
| Prochlorococcus marinus MIT 9313 | n.d.     | PMT0379, PMT1807 | n.d. | PMT0331, PMT1493 | PMT0191 |
| Prochlorococcus marinus MED 4   | n.d.     | PPM1263, PPM0233 | n.d. | PPM1301, PPM1472 | PPM0331 |
| Prochlorococcus marinus NATL 2A | n.d.     | PMN2S_0829 | n.d. | PMN2A_0867, PMN2A_1003 | PMN2A_1709 |
| Synechococcus sp. CC 9605       | n.d.     | Syncc9605_0926, Syncc9605_0292, Syncc9605_2634 | n.d. | Syncc9605_0858, Syncc9605_2032, Syncc9605_0659 | Syncc9605_0497 |
| Synechococcus sp. CC 9902       | n.d.     | Syncc9902_1482, Syncc9902_2261, Syncc9902_2051 | n.d. | Syncc9902_1534, Syncc9902_0620 | Syncc9902_1838 |
| Synechococcus sp. WH 8102       | n.d.     | SYNW1586, SYNW2454, SYNW0296 | n.d. | SYNW1634, SYNW0629 | SYNW1956 |
| Synechococcus sp. WH 7805       | n.d.     | WH7805_05251, WH7805_09779, WH7805_07451 | n.d. | WH7805_05656, WH7805_12388, WH7805_13803 | WH7805_06416 |
| Synechococcus sp. WH 5701       | n.d.     | WH5701_15491, WH5701_01185 | n.d. | WH5701_07406, WH5701_15376 | WH5701_06196 |
| Synechococcus sp. RS 9917       | n.d.     | RS_01761, RS_10896, RS_03633 | n.d. | RS9917_02041, RS9917_05240 | RS9917_02641 |
| Crocosphaera watsonii WH 8501   | CwatDRAFT_0830 | CwatDRAFT_4406, CwatDRAFT_6596 | n.d. | CwatDRAFT_5161 | CwatDRAFT_0865, CwatDRAFT_0842, CwatDRAFT_0969 |
| Trichodesmium erythraeum IMS 101 | TeryDRAFT_2282 | TeryDRAFT_0921, TeryDRAFT_1912 | n.d. | TeryDRAFT_3251 | TeryDRAFT_2672, TeryDRAFT_3296, TeryDRAFT_3923 |
| Freshwater species              |          |          |          |          |          |
| Synechococcus elongatus sp. PCC 6301 | n.d. | Syc1592_c, Syc0859_c | n.d. | Syc0599_c, Syc1466_c | Syc1030_d |
| Synechococcus elongatus sp. PCC 7942 | n.d. | Syncc7942_2514, Syncc7942_0670 | n.d. | Syncc7942_0943, Syncc7942_0031 | Syncc7942_0489 |
| Synechococcus Yellowstone sp. JA-3-3-AB | n.d. | CYA_2817, CYA_1730 | n.d. | CYA_1537, CYA_0689 | CYA_0364 |
| Synechococcus Yellowstone sp. JA-2-3Ba (2-13) | CYB_0250 | CYB_0821, CYB_1917 | n.d. | CYB_1419, CYB_2128 | CYB_0516, CYB_0715, CYB_1899 |
| Thermosynechococcus elongatus BP-1 | TII0507 | TII106, TII158 | n.d. | TII1328, TII0408, TII1935 | TII0416, TII0221 |
| Synechocystis sp. PCC 6803      | SII1336  | SII0902, SII1476 | SII0573  | SII1022  | SII1561, SII0370, SII0991 |
| Gloeobacter violaceus PCC 7421  | GII1758  | GII3101, GII2875 | n.d. | GII0547, GII3849, GII2223 | GII2755, GII3848, GII504, GII2805 |
| Nostoc sp. PCC 7120             | Air4495  | Air4907, Air1681 | n.d. | Air2398, Air1080, Air0396 | Air0540, Air3771, Air3556, Air0522 |
| Nostoc punctiforme PCC 73102    | Npun02001803 | Npun02004258, Npun02007755 | n.d. | Npun02005728, Npun02001164, Npun02001509 | Npun02003702, Npun02006572, Npun02002895, Npun02002692 |
| Anabaena variabilis ATCC 29413  | Ava_2273 | Ava_2197, Ava_1174 | n.d. | Ava_0214, Ava_3730, Ava_2839 | Ava_2942, Ava_1554, Ava_3534, Ava_2258 |

N.d. = not detected.
these genes correctly with respect to the nature of their true substrate [37,39]. According to Sekowska et al. [37], we constructed a phylogenetic distance tree (Fig. 4) with 20 sequences of arginases or agmatinases (given in that paper) as well as the sequences of two arginases from *Arabidopsis thaliana* and the sequences of cyanobacterial ureo-

| Enzymes | E1   | E2              | E3          | E4        |
|---------|------|----------------|-------------|----------|
| Prochlorococcus marinus SS 120 | n.d. | Pro1849, P9211_09067 | Pro1375, Pro0482, Pro1626 | Pro0374 |
| Prochlorococcus marinus str. MIT 9211 | n.d. | P9211_02002, P9211_06427, P9211_10217 | P9211_00350, P9211_07012 | P9211_00350, P9211_07012 |
| Prochlorococcus marinus MIT 9312 | n.d. | P9211_02002, P9211_06427, P9211_10217 | P9211_00350, P9211_07012 | P9211_00350, P9211_07012 |
| Prochlorococcus marinus MIT 9313 | n.d. | P9211_02002, P9211_06427, P9211_10217 | P9211_00350, P9211_07012 | P9211_00350, P9211_07012 |
| Prochlorococcus marinus MED 4 | n.d. | P9211_02002, P9211_06427, P9211_10217 | P9211_00350, P9211_07012 | P9211_00350, P9211_07012 |
| Prochlorococcus marinus NATL 2A | n.d. | P9211_02002, P9211_06427, P9211_10217 | P9211_00350, P9211_07012 | P9211_00350, P9211_07012 |
| Synechococcus sp. CC 9605 | n.d. | Sync9605_0521, Sync9605_0745 | Sync9605_0521, Sync9605_0745 | Sync9605_0521, Sync9605_0745 |
| Synechococcus sp. CC 9902 | n.d. | Sync9605_0521, Sync9605_0745 | Sync9605_0521, Sync9605_0745 | Sync9605_0521, Sync9605_0745 |
| Synechococcus sp. WH 8102 | n.d. | Sync9605_0521, Sync9605_0745 | Sync9605_0521, Sync9605_0745 | Sync9605_0521, Sync9605_0745 |
| Synechococcus sp. WH 7805 | n.d. | Sync9605_0521, Sync9605_0745 | Sync9605_0521, Sync9605_0745 | Sync9605_0521, Sync9605_0745 |
| Synechococcus sp. WH 5701 | n.d. | Sync9605_0521, Sync9605_0745 | Sync9605_0521, Sync9605_0745 | Sync9605_0521, Sync9605_0745 |
| Synechococcus sp. RS 9917 | n.d. | Sync9605_0521, Sync9605_0745 | Sync9605_0521, Sync9605_0745 | Sync9605_0521, Sync9605_0745 |
| Crocosphaera watsonii WH 8501 | n.d. | n.d. | n.d. | n.d. |
| Trichodesmium erythraeum IMS 101 | n.d. | TrichDRAFT0956, TrichDRAFT0956 | TrichDRAFT3251, TrichDRAFT3251 | TrichDRAFT3296, TrichDRAFT3296 |

| Enzymes | C1     | C2     | C3*    | C4**   |
|---------|--------|--------|--------|--------|
| Synechococcus elongatus sp. PCC 6301 | n.d. | Syc0596_c, Syc1144_c | Syc1666_c, Syc0881_c | Syc1030_c |
| Synechococcus elongatus sp. PCC 7942 | n.d. | Sync7942_0943, Sync7942_0369 | Sync7942_0943, Sync7942_0369 | Sync7942_0943, Sync7942_0369 |
| Synechococcus Yellowstone sp. JA-3-AB | n.d. | CYB_1744 | CYB_1419, CYB_2128, CYB_1012 | CYB_1893, CYB_1419, CYB_0715 |
| Synechococcus Yellowstone sp. JA-2-3A (2-13) | n.d. | CYB_1744 | CYB_1419, CYB_2128, CYB_1012 | CYB_1893, CYB_1419, CYB_0715 |
| Thermosynechococcus elongatus BP-1 | n.d. | n.d. | n.d. | n.d. |
| Synechocystis sp. PCC 6803 | n.d. | n.d. | n.d. | n.d. |
| Gloeobacter violaceus PCC 7421 | n.d. | n.d. | n.d. | n.d. |
| Nostoc sp. PCC 7120 | n.d. | n.d. | n.d. | n.d. |
| Nostoc punctiforme PCC 73102 | n.d. | n.d. | n.d. | n.d. |
| Anabaena variabilis ATCC 29413 | n.d. | n.d. | n.d. | n.d. |

N.d. = not detected.
hydrolases (Table 11). The eukaryotic non-plant arginases cluster in one group (marked in red), while the majority of the cyanobacterial enzymes form two clusters containing either the enzymes from marine cyanobacteria (marked in yellow) or from freshwater cyanobacteria (marked in blue). The two plant arginases form a separate group [28] and are more closely related to agmatinas (encoded by \textit{speB}) than to the arginas from non-photosynthetic organisms of the red cluster. The green cluster contains 4-guanidino butyrases from \textit{Pseudomonas aeruginosa} and \textit{Pseudomonas putida} (GbuA\textsubscript{Paeru} and GbuA\textsubscript{Pputi}) and the cyanobacterial enzyme Sll1077 of \textit{Synechocystis} sp. PCC 6803 (for relevance of this finding see below) as well as the enzymes of \textit{Synechococcus} sp. CC 9605, \textit{Synechococcus} sp. WH 8102, and \textit{Synechococcus} sp. WH 5701. The similarity of these cyanobacterial enzymes to known 4-guanidino butyrases [40] suggests that these enzymes also have a 4-guanidino butyrase activity (Fig. 4). Since all other cyanobacterial ureohydrolases group into two separate clusters (blue and yellow cluster), it is likely that they do not represent 4-guanidino butyrases, but represent either an arginase or an agmatinase or an enzyme with both activities – albeit with different substrate affinities. It has been shown that the two arginas of \textit{Lycopersicon esculentum} (tomato), which have an arginase activity, also have a very low agmatinase activity (0.2–0.5% of the arginase activity) [28]. Since the blue cluster contains sll0228 of \textit{Synechocystis} sp. PCC 6803, which has been shown to encode an agmatinase [21,37], it is likely that at least some of the enzymes in the blue cluster are true agmatinas. To further investigate the real activity of the putative cyanobacterial ureohydrolases, the expression of the corresponding proteins in \textit{E. coli} is required to allow activity measurements as was done for Sll0228 and Sll1077 of \textit{Synechocystis} sp. PCC 6803. Although originally being annotated as arginas, neither Sll0228 nor Sll1077 have arginase activity [21,37]. Sll0228 has been shown to have agmatinase activity, while Sll1077 has neither arginase nor an agmatinase activity [37] and thus, most likely is a 4-guanidino butyrase (alignment of Sll1077 and GbuA from \textit{Pseudomonas putida} F1, ZP_00902038 is given in Fig. 5).
Enzymes modifying the guanidino group

This family of enzymes comprises L-arginine deiminases and L-arginine amidinotransferases [38,41], which share common structural features [41]. L-arginine deiminases participate in L-arginine catabolism and are found in prokaryotes [13,16,42] and primitive eukaryotes [30]. L-arginine amidinotransferases have been shown to have a function as L-arginine:glycine amidinotransferase in creatine biosynthesis in vertebrates [43,44], as L-arginine:glycine amidinotransferase in the biosynthesis of the toxin cylindrospermopsin in various cyanobacteria [45], as L-arginine:inosamine phosphate amidinotransferase in streptomycin biosynthesis in Streptomyces spp. [45], and as L-arginine:L-lysine amidinotransferase in the phaseolotoxin biosynthesis in Pseudomonas syringae pv. phaseolicola [46]. In nine cyanobacteria an identical gene was annotated as L-arginine amidinotransferase as well as L-arginine deiminase (Table 4). Thus, a decision, which of the two putative pathways is present, can not be made with certainty. The similarity of the cyanobacterial enzymes to characterized L-arginine deiminases is rather low and is even lower to L-arginine amidinotransferases (Table 12). However, since L-arginine amidinotransferases have so far only been shown to function in antibiotic or toxin biosynthesis in prokaryotes and since an L-arginine deiminase activity has been detected in several fresh water cyanobacteria [17-20], we think that it is more likely that the corresponding gene in the nine cyanobacteria (Tables 4, 7, and 8) encodes an L-arginine deiminase and not an L-arginine amidinotransferase. One reason,
why these genes have not yet been annotated as L-arginine deiminases in the databases, may be related to the fact that so far well characterized prokaryotic L-arginine deiminases consist of about 400 amino acid residues (Table 12) [47-49] and that the L-arginine deiminase of the primitive eukaryote Giardia intestinalis consists of 580 amino acid residues [30]. In contrast, the corresponding nine cyanobacterial genes encode proteins of 699 to 710 amino acid residues length with a molecular mass of 77.5 to 78.3 kDa. Among the cyanobacterial proteins a high similarity of about 80% exists (Table 12). Another unique property of cyanobacterial L-arginine deiminases is that they contain two transmembrane helixes in their C-terminal region. This implies that the cyanobacterial enzymes are membrane-bound or at least membrane-associated. Whether the enzymes are bound to the cytoplasmic or the thylakoid membrane is not yet known.

Identification of genes encoding enzymes of L-arginine catabolizing pathways in Synechocystis sp. PCC 6803
We chose Synechocystis sp. PCC 6803 as a model organism to present more details on the enzymes of the L-arginine-degrading pathways and to validate the bioinformatic results by a transcript analysis. The reason for choosing this cyanobacterium is based on previously published results, showing that Synechocystis sp. PCC 6803 possesses a very effective uptake system for L-arginine [50]. Moreover, several products of L-arginine degradation have already been identified [51]. In addition, substantial differences in the utilization of L-arginine as sole N-source in the growth medium have been observed between Synechocystis sp. PCC 6803 WT and a PsbO-free Synechocystis mutant [10].

Synechocystis sp. PCC 6803 contains genes encoding enzymes of a putative L-arginine decarboxylase pathway, an L-arginine deiminase pathway, and an L-arginine oxidase/dehydrogenase pathway (Tables 3, 4, 13, and Fig. 6).
Three genes, slr1683, slr0662, and slr1312, encoding enzymes with similarity to L-arginine decarboxylases, are present. As shown in Table 10, Sll1683 has a higher similarity to the biodegradable than to the biosynthetic L-arginine decarboxylase of E. coli. In contrast, Slr0662 and Slr1312 have higher similarity to the biosynthetic than to the biodegradable enzyme. Moreover, two genes, sll1077 and sll0228, encoding proteins with similarity to ureohydrolases, were detected. Sll0228, but not Sll1077, has been shown to have agmatinase activity, catalyzing the synthesis of putrescine [21,37]. However, no true putrescine oxidase or putrescine transaminase encoding genes were found in the genome of Synechocystis sp. PCC 6803. Therefore, the L-arginine decarboxylase pathway may mainly serve as a route for polyamine biosynthesis and for the production of ammonium from L-arginine. This assumption is in agreement with results obtained for pseudomonads, which were shown to an L-arginine decarboxylase pathway [13,14,16].

Sll1336 has the common features of an L-arginine amidinotransferase as well as of an L-arginine deiminase. However, since L-arginine amidinotransferases are predominantly involved in antibiotic or toxin synthesis in prokaryotes, it is more likely that Sll1336 is an L-arginine deiminase. This is supported by the fact that Sll1336 has a slightly higher similarity to sequenced L-arginine deiminases than to L-arginine amidinotransferases (Table 12). The highest similarity of Sll1336 (705 aa) exists to the L-arginine deiminase ArcA from Giardia intestinalis (580 aa, 43% overall similar amino acid residues: 10% identical, 19% strongly similar, and 14% weakly similar amino acid residues). Thus, Sll1336 (705 aa) is substantially larger than the average L-arginine deiminases of primitive eukaryotes (~580 aa) or of heterotrophically growing

| Strain | Database entry* | AA | MM (kDa) | pI |
|--------|-----------------|----|----------|----|
| Marine species | | | | |
| Prochlorococcus marinus SS 120 | Pro1849 | 303 | 33.6 | 6.32 |
| Prochlorococcus marinus str. MIT 9211 | P9211_09067 | 296 | 32.7 | 6.45 |
| Prochlorococcus marinus MIT 9312 | PMT9312_1779 | 293 | 32.6 | 5.38 |
| Prochlorococcus marinus MIT 9313 | PMT2214 | 304 | 32.8 | 5.55 |
| Prochlorococcus marinus MED 4 | PMM1686 | 294 | 32.6 | 5.13 |
| Prochlorococcus marinus NATL 2A | PMN2A_1287 | 299 | 32.9 | 5.01 |
| Synechococcus sp. CC 9605 | Syncc9605_1082 | 396 | 43.8 | 5.03 |
| Synechococcus sp. CC 9605 | Syncc9605_2591 | 291 | 31.3 | 4.91 |
| Synechococcus sp. CC 9902 | Syncc9902_2230 | 287 | 30.8 | 5.10 |
| Synechococcus sp. WH 8102 | SYN_W1412 | 426 | 46.8 | 5.48 |
| Synechococcus sp. WH 7805 | STNW2422 | 286 | 30.4 | 4.68 |
| Synechococcus sp. WH 7805 | WH7805_06086 | 492 | 53.8 | 4.48 |
| Synechococcus sp. WH 5701 | WH5701_03860 | 401 | 44.1 | 5.35 |
| Synechococcus sp. WH 5701 | WH5701_03684 | 308 | 32.6 | 4.96 |
| Synechococcus sp. RS 9917 | RS9917_06190 | 286 | 30.9 | 5.06 |
| Crocosphaera watsonii WH 8501 | n.d. | n.d. | n.d. | n.d. |
| Trichodesmium erythraeum IMS 101 | Tery_3780 | 303 | 34.0 | 4.80 |
| Freshwater species | | | | |
| Synechococcus elongatus sp. PCC 6301 | n.d. | n.d. | n.d. | n.d. |
| Synechococcus elongatus sp. PCC 7942 | n.d. | n.d. | n.d. | n.d. |
| Synechococcus Yellowstone sp. JA-3-3-AB | CYA_0859 | 301 | 33.1 | 5.51 |
| Synechococcus Yellowstone sp. JA-2-3Bl (2–13) | CYB_1744 | 307 | 33.7 | 5.23 |
| Thermosynechococcus elongatus BP-1 | n.d. | n.d. | n.d. | n.d. |
| Synechocystis sp. PCC 6803 | Sll1077 | 390 | 42.9 | 5.06 |
| Synechocystis sp. PCC 6803 | Sll0228 | 306 | 33.5 | 4.90 |
| Gloeobacter violaceus PCC 7421 | n.d. | n.d. | n.d. | n.d. |
| Nostoc sp. PCC 7120 | Air2310 | 346 | 38.6 | 4.69 |
| Nostoc punctiforme PCC 73102 | Npun02002114 | 347 | 38.5 | 4.53 |
| Anabaena variabilis ATCC 29413 | Ava_0127 | 346 | 38.5 | 4.66 |

N.d. = not detected. *These ureohydrolases are annotated as arginases, as agmatinases as well as 4-guanidino butyrases. The (+) in Table 3 for A2.1, B1, and E2 refers to an identical gene, because the gene annotation does not distinguish between arginases, agmatinases, and 4-guanidino butyrases. A classification is only possible in a few cases, in which enzymatic activity has been measured or the similarity values are very high to already biochemically well-characterized enzymes (see text for details).
**Figure 5**
ClustalW alignment of the putative 4-guanidino butyrase Sll1077 of *Synechocystis* sp. PCC 6803 and the 4-guanidino butyrase GbuA from *Pseudomonas putida* F1 (GbuA_Pputi, ZP_00902038; 25% identical, 20% similar, and 15% weakly similar amino acid residues). * Identical amino acid residues, : similar amino acid residues (A/V/F/P/M/I/L/W, D/E, R/H/K, S/T/Y/H/C/N/G/Q, and : weakly similar amino acid residues). Gaps were introduced into the sequences to maintain an optimal alignment.

| Sll1077 | GbuA |
|---------|------|
| GbuA    | GbuA |
| Sll1077 | Sll1077 |
| GbuA    | GbuA |
| Sll1077 | Sll1077 |
| GbuA    | GbuA |
| Sll1077 | Sll1077 |
| GbuA    | GbuA |
| Sll1077 | Sll1077 |
| GbuA    | GbuA |
| Sll1077 | Sll1077 |
| GbuA    | GbuA |
| Sll1077 | Sll1077 |
| GbuA    | GbuA |
| Sll1077 | Sll1077 |
| GbuA    | GbuA |
| Sll1077 | Sll1077 |
| GbuA    | GbuA |
| Sll1077 | Sll1077 |
| GbuA    | GbuA |
| Sll1077 | Sll1077 |
| GbuA    | GbuA |
| Sll1077 | Sll1077 |
| GbuA    | GbuA |
| Sll1077 | Sll1077 |
| GbuA    | GbuA |
| Sll1077 | Sll1077 |
| GbuA    | GbuA |
| Sll1077 | Sll1077 |
| GbuA    | GbuA |
| Sll1077 | Sll1077 |
| GbuA    | GbuA |
| Sll1077 | Sll1077 |
| GbuA    | GbuA |
| Sll1077 | Sll1077 |
| GbuA    | GbuA |

Like all other investigated cyanobacteria, *Synechocystis* sp. PCC 6803 has an L-ornithine transcarbamoylase (Slr1022), but it is the only species among the investigated strains, which has a gene encoding a carbamate kinase (*sll0573*). This enzyme shows an intriguingly high degree of similarity to carbamate kinases from other eubacteria. *Sll0573* (32 kDa and calculated pl 5.66) has an overall similarity of 71% (41% identical, 19% strongly similar, and 11% weakly similar amino acid residues) to the carbamate kinase ArcC from *Enterococcus faecalis* (32.9 kDa and calculated pl 5.13) and an overall similarity of 82% (55% identical, 18% strongly similar, 9% weakly similar amino acid residues) to ArcC from *Pseudomonas aeruginosa* (33 kDa and calculated pl 5.25) (Fig. 8). Thus, it is likely that the second possible route for L-arginine degradation in *Synechocystis* sp. PCC 6803 is an L-arginine deiminase pathway leading to synthesis of L-citrulline and subsequently to L-ornithine, carbon dioxide, ammonium, and ATP (Fig. 6). L-ornithine becomes further metabolized to L-glutamate by an L-ornithine transaminase (Slr1022) and a Δ^1^pyrroline-5-carboxylate dehydrogenase (Slr0370) (Table 11). This pathway also leads to the synthesis of L-proline via a Δ^1^pyrroline-5-carboxylate reductase (ProC, Slr0661), and L-proline can be converted back to this intermediate by a proline oxidase (PutA, Slr1561) [21].

The third possible route of L-arginine catabolism in *Synechocystis* sp. PCC 6803 may be an L-arginine oxidase/dehydrogenase pathway. The gene *slr0782* encodes a putative L-arginine oxidase/dehydrogenase, *sll0177* and *sll0228* encode putative ureohydrolases, *slr1022* and *sll0017* encode putative 4-aminobutyrate transaminases, and *slr0370, slr1561,* and *slr0091* encode putative succinate semialdehyde dehydrogenases. Thus, L-arginine becomes degraded to succinate, carbon dioxide, and ammonium, via 2-ketoarginine, 4-guanidinobutyrate, and 4-amino- butyrate. Since the ureohydrolase Sll1077 groups with known 4-guanidino butyrases (Fig. 4), and the heterologously expressed enzyme has neither an arginase nor an
agmatinase activity [37], this enzyme may indeed be a 4-guanidino butyrase. An alignment of the enzyme with the biochemically identified 4-guanidino butyrase of *Pseudomonas putida* strain F1 (ZP_00902038) is given (Fig. 5).

The first enzyme of the L-arginine oxidase/dehydrogenase pathway (Slr0782) in *Synechocystis* sp. PCC 6803 has 58% similarity (20% identical, 24% similar, and 14% weakly similar amino acid residues) to an L-amino acid oxidase (AoxA) from *Synechococcus elongatus* PCC 6301, encoded by the *aoxA* gene (YP_171306) [22-24]. This enzyme catalyzes the oxidative deamination of basic L-amino acids with a preference for L-arginine. An alignment of Slr0782 with AoxA of *Synechococcus elongatus* PCC 6301 is given and shows that Slr0782 has a dinucleotide-binding site (GxGxxG) [55] like the AoxA enzyme (Fig. 9). Thus, Slr0782 may also be a FAD-containing enzyme. Since we were never able to detect an L-arginine oxidizing activity with utilization of molecular oxygen in intact cells or cell extracts of *Synechocystis* sp. PCC 6803 so far (unpublished results), it is more likely that Slr0782 interacts in a complex not yet understood way with the electron transport chain. This is in agreement with the fact that the enzyme has two hydrophobic regions possibly being transmembrane helices. We would like to also point out that *Synechococcus elongatus* PCC 6301 has an additional gene encoding a protein called AoxB (YP_171854), which has 59% similarity (25% identical, 21% similar, and 13% weakly similar amino acid residues) to AoxA [24]. AoxB has not yet been characterized biochemically. Slr0782 of *Synechocystis* sp. PCC 6803 has a higher similarity to AoxB (in total 66% similarity: 31% identical, 22% similar, and 13% weakly similar amino acid residues) than to AoxA (in total 58% similarity). It should also be mentioned that the genomes of different *Pseudomonas* species contain a gene encoding an enzyme, which has similarity to Slr0782 (*P. putida* KT2440, NP_747085; *P. putida* F1, ZP_00902633; *P. aeruginosa* PAO-1, NP_249112; *P. fluorescens* Pf0-1, YP_348469). The similarity of Slr0782 to the enzyme of *P. fluorescens* corresponds to 47% (27% identical, 17% similar, and 13% weakly similar amino acid residues). All these enzymes contain a dinucleotide-binding GxGxxG

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**Table 12: Comparison of cyanobacterial putative L-arginine deiminases or L-arginine amidinotransferases to selected prokaryotic sequences and a sequence of a primitive eukaryote**

| Strain                                      | Database entry | AA   | MM (kDa) | pl     | Identity/similarity/gaps vs. Sll1336 (%) |
|---------------------------------------------|----------------|------|----------|--------|----------------------------------------|
| *Cyanobacterial L-arginine deiminases or L-arginine amidinotransferases* |                |      |          |        |                                        |
| *Synechocystis* sp. PCC 6803               | Sll1336        | 705  | 78.3     | 5.40   | 100.0/100.0/0.0                         |
| *Crocospaera watsonii* VWH 8501            | CwatDRAFT_0830 | 703  | 78.0     | 5.15   | 78.0/88.8/0.3                          |
| *Trichodesmium erythraeum* IMS 101         | Tery_4659      | 703  | 77.8     | 5.43   | 74.3/85.7/1.1                          |
| *Synechococcus* Yellowswater sp. JA-2-3Bx (2–13) | YP_476511 | 710  | 78.2     | 5.75   | 64.1/79.0/2.1                          |
| *Thermosynechococcus elongatus* BP-1       | Tll0507        | 699  | 77.5     | 5.53   | 71.3/84.9/1.4                          |
| *Gloeobacter violaceus* PCC 742I           | Glr1758        | 699  | 77.5     | 5.53   | 63.7/78.6/2.1                          |
| *Nostoc* sp. PCC 7120                      | Atr4995        | 703  | 77.9     | 5.41   | 73.4/85.7/0.8                          |
| *Nostoc punctiforme* PCC 73102             | Npun0201803    | 703  | 77.9     | 5.48   | 74.6/86.6/1.4                          |
| *Anabaena variabilis* ATCC 29413           | Aya_2273       | 703  | 78.2     | 5.38   | 73.7/86.6/0.8                          |

**L-arginine deiminases of prokaryotes and a primitive eukaryote**

| Strain       | Database entry | AA   | MM (kDa) | pl     | Identity/similarity/gaps vs. Sll1336 (%) |
|--------------|----------------|------|----------|--------|----------------------------------------|
| *Giardia intestinales* | AAC06116 | 580  | 64.1     | 6.11   | 13.9/22.3/53.1                         |
| *Thermoplasma volcanium* GSS1 | NP_110996 | 418  | 48.1     | 5.32   | 10.2/18.1/65.7                         |
| *Thermoplasma acidophilum* DSM 1728 | NP_394444 | 418  | 47.7     | 5.20   | 8.7/17.5/65.5                         |
| *Pseudomonas aeruginosa* | P13981 | 418  | 45.4     | 5.52   | 7.3/12.0/74.9                         |
| *Enterococcus faecalis*        | CAC41341     | 408  | 46.7     | 4.87   | 7.4/14.8/71.6                         |
| *Bacillus licheniformis*       | AUA25597     | 411  | 47.2     | 5.28   | 7.8/13.2/73.3                         |

**Characterized L-arginine amidinotransferases**

| Strain                  | Database entry | AA   | MM (kDa) | pl     | Identity/similarity/gaps vs. Sll1336 (%) |
|-------------------------|----------------|------|----------|--------|----------------------------------------|
| *Rattus norvegicus*     | AAX21250       | 423  | 48.2     | 7.17   | 6.3/9.5/82.1                          |
| *Streptomyces griseus*  | CAA68517       | 347  | 38.7     | 5.12   | 9.0/12.7/72.5                         |
| *Aphanizomenon ovalisporum* | AAM3469 | 392  | 44.8     | 5.40   | 8.0/13.2/74.3                         |

L-arginine deiminases and L-arginine amidinotransferases belong to a superfamily of enzymes that catalyze the modification of guanidino groups. The number of amino acid residues, the molecular mass, and the calculated isoelectric point is given. Moreover, the similarity of the selected reference enzymes to Sll1336 from *Synechocystis* sp. PCC 6803 is given. Values for % identity and similarity to Sll1336 were determined with the EMBOSS Pairwise alignment algorithm [65]. The percentage identity and similarity does not include weakly similar amino acid residues.
motif and thus, are likely FAD-containing dehydrogenases and not aminotransferases [35,36]. For *Pseudomonas putida* (Trevisan) Migula P2 ATCC 2557 the Rodwell group has indeed suggested that an L-amino acid oxidase is the first enzyme degrading L-arginine via 2-ketoarginine, 4-

guanidinobutyrate, and 4-aminobutyrate to succinate in Synechocystis sp. PCC 6803 genome encoding putative enzymes of an L-arginine decarboxylase-, an L-arginine deiminase-, and an L-arginine oxidase/dehydrogenase pathway.

### Table 13: Presence of genes in the Synechocystis sp. PCC 6803 genome encoding putative enzymes of an L-arginine decarboxylase-, an L-arginine deiminase-, and an L-arginine oxidase/dehydrogenase pathway.

| L-Arginine-degrading pathways in Synechocystis sp. PCC 6803 | ORF | Database # | Length (aa) | pI | MW (kDa) | Best hit vs. gene | Organism | E-value (ident./pos. aa) | Similarity |
|----------------------------------------------------------|-----|------------|-------------|----|----------|------------------|----------|----------------------------|------------|
| L-Arginine decarboxylase (A1)                            | sll1683 | NP_440109 | 483         | 5.44 | 51.84 | speA | B. subtilis | 5.0e-103 | 40/61 |
| Agmatinase (A2.1)                                        | slr0662 | NP_442871 | 695         | 5.08 | 78.24 | speA | X. campestris | 2.0e-134 | 41/56 |
| Agmatinase (A2.1)                                        | slr1712 | NP_439907 | 659         | 5.30 | 74.48 | speA | X. campestris | 5.0e-121 | 38/56 |
| Putrescine oxidase or transaminase (A3)                  | sll1077 | NP_440618 | 390         | 5.06 | 42.96 | speB2 | P. aeruginosa | 1.1e-40  | 33/41 |
| Putrescine oxidase or transaminase (A3)                  | slr0228 | NP_440030 | 306         | 4.90 | 33.46 | speB | B. subtilis | 1.6e-22  | 30/45 |
| 4-Aminobutyraldehyde dehydrogenase (A4)                  | sll1495 | NP_442886 | 397         | 8.43 | 43.54 | BMEII0291 | B. melitensis | 1.2e-93  | 42/61 |
| 4-Aminobutyrate transaminase (A5)                        | slr1022 | NP_440479 | 429         | 5.11 | 46.54 | gabT | P. aeruginosa | 6.7e-58  | 33/50 |
| Succinate semialdehyde dehydrogenase (A6)                | sll0017 | NP_442115 | 433         | 5.13 | 45.87 | gabT | E. coli | 5.7e-41  | 30/44 |
| Succinate semialdehyde dehydrogenase (A6)                | slr0370 | NP_442020 | 454         | 5.02 | 48.75 | gabD | X. campestris | 5.0e-121 | 47/65 |
| L-Arginine deiminase (D1)                                | sll1336 | NP_442829 | 705         | 5.40 | 78.33 | cyb_250 | S. yellowstone | 0.0       | 61/79 |
| L-Ornithine transcarbamoylase (D2)                       | slr0902 | NP_442776 | 308         | 5.38 | 33.62 | argF | P. aeruginosa | 1.1e-77  | 47/66 |
| L-Ornithine transcarbamoylase (D2)                       | slr1476 | NP_441572 | 331         | 6.53 | 33.39 | argF | P. aeruginosa | 7.2e-13  | 26/42 |
| Carbamate kinase (D3)                                    | sll0573 | NP_443041 | 308         | 5.66 | 32.93 | ygcA | E. coli | 8.1e-52  | 41/58 |
| L-Ornithine transaminase (D4)                            | slr1022 | NP_440479 | 429         | 5.11 | 46.54 | rocD | B. subtilis | 2.1e-61  | 32/52 |
| L-Ornithine transaminase (D4)                            | sll0017 | NP_442115 | 433         | 5.13 | 45.87 | gabT | E. coli | 5.7e-41  | 30/44 |
| L-Ornithine transaminase (D4)                            | slr0370 | NP_442020 | 454         | 5.02 | 48.75 | gabD | X. campestris | 5.0e-121 | 47/65 |
| L-Ornithine transaminase (D4)                            | sll1561 | NP_441689 | 990         | 5.46 | 110.03 | gabD | P. aeruginosa | 2.7e-66  | 17/25 |

The letters with numbers in parenthesis behind the enzyme names correspond to those given in Tables 3 and 4, and Fig. 2. In Synechocystis sp. PCC 6803 the gene slr1022 has similarity to L-ornithine transaminases and to 4-aminobutyrate transaminases. The L-ornithine transferase (D2) and the 4-aminobutyrate transferase (E3) both belong to the group of class III aminotransferases (InterProScan), which explains why the same gene slr1022 is annotated either as L-ornithine transaminase or as 4-aminobutyrate transaminase. The gene slr0370 has similarity to the Δ1pyrroline-5-carboxylate dehydrogenase (D5) and to succinate semialdehyde dehydrogenase (E4). Both enzymes belong to the NAD-dependent aldehyde dehydrogenases (InterProScan), which explains why the same gene slr0370 is either annotated as Δ1pyrroline-5-carboxylate dehydrogenase or succinate semialdehyde dehydrogenase. Thus, it can not be decided in a bioinformatic approach whether the gene products Slr1022 and Slr0370 are components of the L-arginine deiminase pathway or the L-arginine oxidase/dehydrogenase pathway or of both pathways. N.d. = not detected.
Detection of transcripts for L-arginine-degrading enzymes in Synechocystis sp. PCC 6803

The bioinformatic evaluation suggests the presence of three putative L-arginine-degrading pathways in Synechocystis sp. PCC 6803. These putative pathways are an L-arginine decarboxylase pathway (three isoenzymes as first enzyme: Sll1683, Slr0662, and Slr1312), an L-arginine deiminase pathway (first enzyme Sll1336), and an L-arginine oxidase/dehydrogenase pathway (first enzyme Slr0782) (Fig. 6).

For detection of the corresponding transcripts, Synechocystis sp. PCC 6803 was cultivated with nitrate or with L-arginine as sole N-source and with an illumination of 50 μmol photons m⁻² s⁻¹ for three days. These growth conditions were similar to those published previously [51] for experiments to determine products of L-arginine degradation. The growth curves and the chlorophyll content are given in Fig. 10. Synechocystis sp. PCC 6803 grew about equally well with nitrate as with L-arginine. Total RNA was isolated from the corresponding cultures and was applied to RNA slot-blot hybridization with selected Dig-DUTP-labeled gene-specific DNA probes (Fig. 11). Equal length, concentration, almost equal GC-content of the probes, and equal exposure time allowed for semi-quantitative comparison of mRNA levels of all five investigated transcripts: sl11683, sl1336, and slr1312 encoding isoenzymes of L-arginine decarboxylases, sl1336 encoding an L-arginine deiminase, and slr0782 encoding an L-arginine oxidase/dehydrogenase. The transcript level for the three L-arginine decarboxylase-encoding genes was low when the cells grew with nitrate and did not or only slightly...
increase when the cells grew with L-arginine as sole N-source. A low steady-state mRNA level was also observed for **sll0228** transcript (not shown), which encodes an agmatinase-type enzyme [37,51] – the second enzyme in the L-arginine decarboxylase pathway. This implies that the L-arginine decarboxylase pathway probably has its only function in polyamine biosynthesis and does not represent a major pathway for L-arginine degradation in *Synechocystis* sp. PCC 6803 when cells grew with L-arginine as sole N-source.

As shown in Fig. 11, the transcript levels for the L-arginine deiminase (Sll1336) as well as for the L-arginine oxidase/dehydrogenase (Slr0782) were substantially higher than for the three L-arginine decarboxylase isoenzymes. The steady-state transcript levels for these two enzymes were as high in nitrate-grown cells as in L-arginine-grown cells. This suggests that these two genes are transcribed constitutively. The same is true for the transcripts of the subsequent enzymes of the two pathways with the exception of the carbamate kinase transcript (Fig. 12 and 13). The mRNA for the carbamate kinase was lower than for the other enzymes and the steady-state transcript level was found to be highly increased in L-arginine-grown cells.

### ClustalW alignment of the putative L-arginine deiminase Sll1336 of *Synechocystis* sp. PCC 6803 and the L-arginine deiminase ArcA from the primitive eukaryote *Giardia intestinalis*

Both proteins share 43% overall similarity (10% identical, 19% strongly similar, 14% weakly similar amino acid residues). * Identical amino acid residues, : similar amino acid residues (A/V/F/P/M/I/L/W, D/E, R/H/K, S/T/Y/H/C/N/G/Q, and • weakly similar amino acid residues. Gaps were introduced into the sequences to maintain an optimal alignment. Two putative transmembrane helices of Sll0573 are boxed (see text for details).

| Sll1336 | MADDIRILMCPPDHYDVDYVINPWMEGNIHKSSQERAVEQWKKLHQTIKECAIVDLVKPA 60 |
|---------|---------------------------------------------------------------|
| ArcA    | ------------------------------------------------------------- |
|         | EFEIYIWGDPKDHGSTLEGVDVMIGN---GVLIOMGERSRQ 247               |
| Sll1336 | KGWPDMVFTANAGLVLGENVVLSRFYHKERQGEEFYKAFWFEENQTYLFLPDLPFGA 120 |
| ArcA    | LAHQR1TPNCDELFDOVYWYV---QPKHRDHFVYTKMRGIDVLWHELHNLTTT- 79 |
| Sll1336 | GDALFDEMWGILWRSLVWGLFHYLHDLFDDLCPFLPSGGY 180                |
| ArcA    | IQQKELVWLDRTAIDSVGLSRLSELESFPRKLA 122                        |
| Sll1336 | LLYPFADAFAYNVRIMYFIPPEKRIVEELDAVNFACNAVNVDDYIMLVSRTLEKKL 240 |
| ArcA    | LIYYG-----------------------------------VAADDLPAEGAN--------ILKMYREY 148 |
| Sll1336 | ELCDINETVGGGVAFDFFYSTIYPTEVKNCWME VQTVQRMDAAIVTVSNPPARC 420 |
| ArcA    | A1GQVAQSLFARG----AEERVIVAGFLKSSRAHMHTFVSCDRO-----LTVTFEVEKRE 301 |
| Sll1336 | LLRLQGVIRGVVQEGRTKKVESHEGTRKKNKPEAFMAAEGSVCEERVVLEIQAW 480 |
| ArcA    | VPFSFLPSSESYGMN-----IRREE-----KTFLEVASELGLKLKLVETGNSFAA 350   |
| Sll1336 | EMQGKEQCGGIKVYAGKPVPHVHTQGGLVMHLCVYRHRRGAVAGIAVHEDQATMG 540 |
| ArcA    | VREPKEQVGNVLCFLPQVYGVD-----KRTYNTLLRLKEGVITVESLGERGRO 400 |
| Sll1336 | LGVDMQRGPhVRGHRHHLKVINSRYGGQRQAVEAGFKGSVMYEVCVNKNFPCAGLS 600 |
| ArcA    | R---------------GGHCMTCPVRDIDY--------------------------------- 418 |

Figure 7

ClustalW alignment of the putative L-arginine deiminase Sll1336 of *Synechocystis* sp. PCC 6803 and the L-arginine deiminase ArcA from the primitive eukaryote *Giardia intestinalis*. Both proteins share 43% overall similarity (10% identical, 19% strongly similar, 14% weakly similar amino acid residues). * Identical amino acid residues, : similar amino acid residues (A/V/F/P/M/I/L/W, D/E, R/H/K, S/T/Y/H/C/N/G/Q, and • weakly similar amino acid residues. Gaps were introduced into the sequences to maintain an optimal alignment. Two putative transmembrane helices of Sll0573 are boxed (see text for details).
ylase-, an arginase-, an L-arginine amidinotransferase-, an L-arginine deiminase-, and an L-arginine oxidase/dehydrogenase pathway in the investigated cyanobacteria (Tables 3 and 4, and Fig. 2). All investigated strains contain an L-arginine decarboxylase pathway, which most likely mainly facilitates polyamine biosynthesis. Since extracellularly added putrescine has been shown to be toxic, at least for some cyanobacteria [56], it is unlikely that this pathway is a major pathway for L-arginine degradation. In addition to the L-arginine decarboxylase pathway, one or two further L-arginine-degrading pathway(s) is (are) present, which is either an arginase pathway, an L-arginine deiminase pathway or an L-arginine oxidase/dehydrogenase pathway. Although an L-arginine amidinotransferase pathway can not be excluded entirely, this pathway is rather unlikely to have a major function in L-arginine degradation, since L-arginine amidinotransferases seem to mainly function in antibiotic and toxin production in prokaryotes [44-46].

An interesting result of the bioinformatic analysis is the observation that the cyanobacterial L-arginine deiminases, being present in nine cyanobacterial strains (Table 4), are substantially larger than the corresponding enzymes from non-photosynthetic eubacteria (Table 12). Further, they seem to be bound either to the cytoplasmic or the thylakoid membrane. In bacteria it has been shown that the L-arginine deiminase pathway is regulated in a rather complex way in dependence of the L-arginine and oxygen concentration, the redox poise, and/or energy status of the cell [13,14,48,49]. On the basis of the larger size and the predicted membrane association of the cyanobacterial L-arginine deiminases, the regulation of the L-arginine deiminase pathway in cyanobacteria maybe even more complex than in bacteria. This has also to be seen under the aspect that this pathway leads to ATP synthesis in the last enzymatic step providing an additional substrate-level phosphorylation site.

The second rather unexpected observation is the presence of a putative L-arginine oxidase/dehydrogenase pathway in ten cyanobacteria (Table 4). The first enzyme of this pathway has similarity to an L-amino acid oxidase, catalyzing the oxidative deamination of basic L-amino acids with a preference for L-arginine and with oxygen as electron acceptor in *Synechococcus elongatus* PCC 6301 and PCC 7942. This pathway has not yet been investigated in detail. However, preliminary results, which had been obtained with *Synechocystis* sp. PCC 6803, suggest that the first enzyme of this pathway does not represent an L-arginine oxidase with oxygen as electron acceptor, but rather represents an L-arginine dehydrogenase, which interacts in a complex not yet understood with the electron transport chain. An interaction of amino acid dehydrogenases with the respiratory electron transport chain has previously been shown for *E. coli* [57].

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**Figure 8**

ClustalW alignment of the putative carbamate kinase Sll0573 of *Synechocystis* sp. PCC 6803 and the carbamate kinase ArcC from *Pseudomonas aeruginosa*. Both proteins share 82% overall similarity (55% identical, 18% strongly similar, 9% weakly similar amino acid residues. * Identical amino acid residues, : similar amino acid residues (A/V/F/P/M/I/L/W, D/E, R/H/K, S/T/Y/H/C/N/G/Q, and • weakly similar amino acid residues. Gaps were introduced into the sequences to maintain an optimal alignment. Two putative transmembrane helices of Sll0573 are boxed (see text for details).
ClustalW alignment of the putative L-arginine oxidase/dehydrogenase Slr0782 from Synechocystis sp. PCC 6803 with the characterized L-amino acid oxidase AoxA from Synechococcus elongatus PCC 6301 (P72346) [23]. Both proteins share an overall similarity of 57% (21% identical, 23% similar, and 13% weakly amino acid residues). The dinucleotide binding motif GxGxxG is boxed. * Identical amino acid residues, : similar amino acid residues (A/V/F/P/M/I/L/W, D/E, R/H/K, S/T/Y/H/C/N/G/Q, and • weakly similar amino acid residues. Gaps were introduced into the sequences to maintain an optimal alignment. Two putative transmembrane helices (aa 628–648; aa 670–690) were detected for Slr0782 using the DAS TM prediction algorithm [52]. Slr0782 also has 66% similarity (31% identical; 22% strongly similar, and 13% weakly similar amino acid residues) to AoxB of Synechococcus elongatus PCC 6301, an enzyme not yet characterized.

Figure 10
Growth and phenotypical appearance of Synechocystis sp. PCC 6803 cells grown in the presence of nitrate or L-arginine as sole N-source and with a light intensity of 50 μmol photons m⁻² s⁻¹ for 24, 48 or 72 hours.
In addition to the overview on L-arginine-degrading pathways in 24 cyanobacteria, we have performed a more detailed evaluation of the pathways in Synechocystis sp. PCC 6803. This investigation provided evidence that Synechocystis sp. PCC 6803 has three putative L-arginine-degrading pathways, being an L-arginine decarboxylase pathway, an L-arginine deiminase pathway, and an L-arginine oxidase/dehydrogenase pathway. An arginase pathway does not seem to exist, since the two proteins, originally annotated as arginases, do not possess an arginase activity [37,51]. Transcript analyses revealed that the mRNA levels for the three isoenzymes of L-arginine decarboxylase (Slr1312, Slr0662, and Sll1683) and also for the agmatinase Sll0228 were rather low in Synechocystis sp. PCC 6803 in nitrate- or L-arginine-grown cells. Thus, this pathway probably has its major function in polyamine biosynthesis. In contrast, the transcript levels for a putative L-arginine deiminase pathway (first enzyme: Sll1336) and an L-arginine oxidase/dehydrogenase pathway (first enzyme: Slr0782) were high whether L-arginine or nitrate was the N-source, suggesting that these two pathways are the major L-arginine-degrading pathways and that they are expressed constitutively. The only exception is the carbamoyl kinase, whose transcript was found at elevated levels in L-arginine-grown cells. The lack of a substantial up-regulation of these transcripts, when cells were transferred from a nitrate-containing medium to an L-arginine-containing medium and an illumination of 50 μmol photons m⁻² s⁻¹ light, suggests that these pathways, besides having

![Slot-blot transcript analysis of the genes encoding the first putative enzymes of the L-arginine deiminase pathway (sll1336), the L-arginine oxidase/dehydrogenase pathway (slr0782), and the L-arginine decarboxylase pathway in Synechocystis sp. PCC 6803.](image1)

![Slot-blot transcript analysis of the genes encoding the putative enzymes of the L-arginine deiminase pathway in Synechocystis sp. PCC 6803.](image2)
a function in the utilization of extracellular L-arginine, have a role in the complex dynamic metabolism of cyanophycin, which is not yet fully understood [8]. Such a functional L-arginine deiminase pathway would account for the products of L-arginine degradation identified in Synechocystis sp. PCC 6803 [51]. The bioinformatic evaluation in combination with the transcript analysis suggests that Synechocystis sp. PCC 6803 has an unusual L-arginine deiminase and an unusual L-arginine oxidase/dehydrogenase as the major L-arginine-degrading enzymes. An extended biochemical investigation of these two enzymes and the corresponding pathways is required before a statement can be made on how these two pathways are integrated in the overall C- and N-metabolism in Synechocystis sp. PCC 6803.

**Methods**

**Bioinformatic analyses and tools for the interpretation of genomic DNA sequences**

Bacterial genome sequences were obtained from the Kyoto Encyclopedia of Genes and Genomes database (KEGG). Database searches and similarity searches were done as described in Rueckert et al. [58] with nucleotide and amino acid sequences using the BlastN- and BlastP-algorithms [59]. Multiple sequence alignments were performed using the DIALIGN2 software [60]. The phylogenetic trees were calculated using the neighbor-joining method [61], which is integrated in the ClustalX software package [62]. The results were visualized as a radial tree with the interactive phylogenetic tree plotting program TreeTool [63].

**Cyanobacterial strains, growth conditions, and cell harvest**

Synechocystis sp. strain PCC 6803 was obtained from the Pasteur Culture Collection of Cyanobacterial Strains, Paris, France. Cells were grown in gas wash bottles with a capacity of 250 ml in a stream of 2% carbon dioxide in air at 30°C. Growth either with nitrate or L-arginine as sole nitrogen source was performed basically according to Stephan et al. [10] except that the light intensity has been reduced from 200 to 50 μmol photons m⁻² s⁻¹. Under these conditions the Synechocystis sp. PCC 6803 can grow with L-arginine without a stress phenotype. The standard inoculation corresponded to an absorbance of 0.3 at 750 nm (OD₇₅₀ nm). Growth was determined as OD 750 nm of Synechocystis sp. PCC 6803 cultures. After 24, 48, and 72 h cells were mixed 1:1 with crushed ice and harvested by centrifugation for 5 min at 4.000 × g in a table top centrifuge. Isolation of total RNA was performed as described previously [64] combined with an on-column DNase digestion step with the RNase-free DNase set from Qiagen (Qiagen, Hilden, Germany).

**Quantification of steady-state mRNA pools of selected transcripts with slot-blot RNA hybridization analysis**

For slot-blot RNA hybridization experiments, 5 μg RNA were denatured for 10 min at 68°C in a formaldehyde/formamide-containing buffer and transferred to HybondN⁺ membranes (Amersham Pharmacia Biotech, Freiburg, Germany) using the BioRad-Dot-blot SF Microfiltration Apparatus (BioRad) as described in the corresponding manual. RNA was UV cross-linked to the membrane and samples were probed with different PCR-derived digoxigenin-dUTP (Dig-dUTP) labeled gene-specific DNA probes (Table 14). Slot-blot RNA detection were performed using the CDP-Star ready-to-use system (Roche, Mannheim, Germany) according to the manufacturer’s recommendation. The rnpB probe was used in all experiments to ensure equal loading.

**Authors’ contributions**

SS performed the bioinformatic and the transcript analyses. CR aided the bioinformatic analyses and performed the phylogenetic analyses. EKP provided the knowledge and expertise on L-arginine catabolism and in part wrote the paper. KPM supervised the research and provided...
tables and figures. DS and all other authors have read and approved the final manuscript.

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