Bioinformatics International

Short Communication
Published: 08 Feb, 2018

Chitin Synthases in Diatoms

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Abstract

Chitin is the most abundant biopolymer in the oceans and is present in both eukaryotic and prokaryotic organisms. In the ocean diatoms are the most species-rich phytoplankton and some species have proved synthesise chitin. BLAST in chitin synthase genes into the Marine Microbial Eukaryotic Transcriptome Sequencing Project database, which contains 188 diatom transcriptomes with more than 60 different species, we found 46 sequences from 15 different species. This finding reveals that, albeit not all the diatom species identified are proved to synthesise chitin, they do possess the molecular toolkit to synthesise chitin.

Abbreviations

ScCHS: Saccharomyces cerevisiae chitin synthase. Phatri: Phaeodactylum tricornutum CCMP 1055; Chopin: Corethronpennatum strain L29A3; Tri dub: Triceratium dubium strain CCMP147; Minutocellulus polymorphus strain CCMP3303; Stacon: Stauroneis constricta strain1120; Craaus: Craspedostaurosaustralis, strain CCMP3328; Odosin: Odontella sinensis strain Grunow 1884; Thapse: Thalassiosira pseudonana strain CCMP 1335; Cycmen: Cyclotella meneghiniana strain CCMP 338; Skejap: Skeletonema japonicum strain CCMP 2506; Skegre: Skeletonema gretae strain CCMP 1804; Thaoce: Thalassiosirao ceanica strain CCMP1005; Tha ant: Thalassiosira antarctica strain CCMP982; Detcon: Detonula confervacea strain CCMP353; Thawei: Thalassiosira weissflogii strain CCMP1336. On the right-hand side, sequence logo for each clade is reported.

Short Communication

Chitin is the most abundant polymer in the oceans [1] and is spread over numerous taxa in the eukaryotic and prokaryotic kingdoms [2]. It serves mainly a protection function like in fungi, yeasts and arthropods [3,4]. It is composed of N-acetyl glucosamine monomers polymerised by chitin synthase enzymes [5]. Chitin synthase are large enzymes that belong to the family 2 glycosyltransferases (GT2) which include many other enzymes that serve similar functions like cellulose synthase [6].

Diatoms are among the plethora of organisms showing chitin synthase pathways and it has been hypothesised that chitin is involved in cell wall processes [7].

Diatoms are world-wide distributed unicellular protist belonging to Stramenopiles that colonised every humid environment from marine to brackish and fresh waters and also hypogean extreme habitats. Diatoms are the most species-rich phylum in marine phytoplankton with more than 14 thousand species described [8] to date and an extremely wide array of shapes and habits [9]. This group of unicellular protist are characterised by a bipartite external cell wall composed of amorphous orthosilicic acid, called the frustule. The two parts of the frustule (valves) are different in size and fit one onto the other like a lid and a box. Because of this peculiar cellular structure, diatoms have evolved atypical cell and life cycles [10-12]. Besides their proved ecological relevance [13] diatoms are getting economically important because of the interest that biotechnology have demonstrated towards these organisms [14]. Systematically, diatoms belong to the phylum Bacillariophyta [15] that is in turn divided into three classes [8], Bacillariophyceae [16], mainly characterised by bilateral cell ornamentation symmetry) referred to as pennate diatoms; classes Coscinodiscophyceae [9] and Mediophyceae [17], which include cells recognisable by a radial organization of cellular processes and ornamentations. These two classes altogether are referred to as centric diatoms. Among diatoms, the only genera that present clear production of chitin are *Thalassiosira* and *Cyclotella* (Class Mediophyceae). These species produce long chains (colonies) by joining adjacent cells via a chitin thread extruded through cellular processes called the fultoportulae [18,19]. Indeed, chitin was found to be associated to the silica cell wall in *T. pseudonana* [20,21]. It

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Received Date: *18 Jan 2018*
Accepted Date: *31 Jan 2018*
Published Date: *08 Dec 2018*

Citation:
Amato A, Ferrante MI. Chitin Synthases in Diatoms. Bioinform Int. 2018; 1(1): 1001.

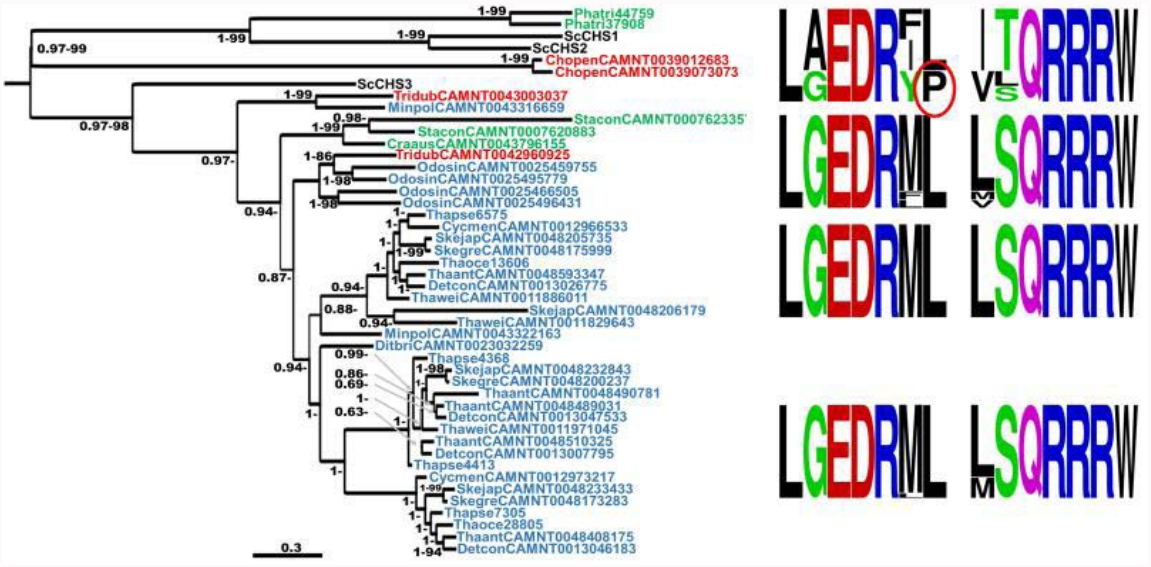

Figure 1: Bayesian phylogenetic tree of the CHS protein sequences (split frequency σ: 0.001).

was also demonstrated that in silica starvation *Skeletonema costatum* (Class Mediophyceae) synthesise 6s chitin [21]. *In vivo* and *in vitro* chitin interacts with silica [22,23] and possibly it is involved in frustule formation. Recently it has been shown that some diatoms, other than *Thalassiosira* and *Cyclotella* species, have the molecular tool kit to synthesise chitin [2,21] but the number of species tested is only a small representation of those inhabiting our planet. For this reason in the present work we searched for chitin synthase genes in the Marine Microbial Eukaryote Transcriptome Sequencing Project [24,25] database. The MMETSP contains a total of 678 transcriptomes from marine organisms, of which 188 are diatoms with more than 60 different species.

The amino acid sequence of the Saccharomyces cerevisiae chitin synthase proteins, ScCHS1 ScCHS2 and ScCHS3, were used as query for a tblastn search in MMETSP database. For one or two representatives of each diatom genus with a good hit (e-value cut-off 10-10), all the transcripts were downloaded, translated and blasted in Pfam, UniProt and HMMER in order to verify the presence of chitin synthase domains [21]. Sequences(Supplementary file 1) were visualized in the BioEdit Sequence Alignment Editor 7.0.9.0 [26] software and aligned by Clustal W. The alignment was manually curated. Maximum Likelihood (ML) and Bayesian phylogenetic analyses were performed using MEGA7 [27,28] software respectively. The LG (Le and Gascuel, 2008) +G+I evolution model was chosen for ML by running modeltest [29] in MEGA7. The analysis was supported by 10,000 bootstrap replicates. For Bayesian inference, four parallel and totally independent Markov Chain Monte Carlo (MCMC) runs were carried out on data matrices. Eight chains (seven hot and one cold) drove each analysis. Ten million generations (sampling frequency every 100) were set. The analyses were forced to jump among the evolutionary models for protein sequence alignments implemented in the software. The first 25% of the samples from the cold chain were discarded in order to stabilise the algorithm, reduce the variability among results and have a more robust analysis. Consensus tree with posterior probability (PP) of each node and branch length are reported after a 50% majority-rule consensus. Phylogenetic trees were visualised and edited in the FigTree (Tree Figure Drawing Tool Version 1.4.2) software (http://tree.bio.ed.ac.uk/).

All the putative diatom chitin synthase proteins investigated here contained the two main functional sites identified by [30], i.e. the acceptor-deprotonation (A/D) and the catalytic sites, corroborating the identification as putative chitin synthases. Interestingly, among MMESTP BLAST results most of the diatom species that produced a good hit belonged to the Mediophyceae class. Only two Coscinodiscophyceae (*Choretronpennatum* and *Triceratium dubium*) and two Bacillariophyceae (*Craspedostaurosaustralis* and *Stauroneis constricta*) representatives were present (Figure 1). In the genome of the pennate diatom *Phaeodactylum tricornutum* [31] two genes are annotated as GT2 (protein ID: 44759, chromosome 5:155409-158214; 37908, chromosome 14:373305-376179) and in previous analyses [21] both clustered in a basal position to all the other diatom chitin synthase *P. tricornutum* protein sequences are quite divergent from the other diatoms (with an average sequence identity of around 9%). Moreover, these two sequences show the highest identity with ScCHS1 and ScCHS2 and the lowest with ScCHS3. Consistently, blasting. cerevisiae chitin synthases in the *P. tricornutum* genome, only ScCHS1 and ScCHS2 produced sound hits. Interestingly, *P. tricornutum* sequences present the A/D and the catalytic sites but are the only sequences showing a Proline instead of a Leucine in position +2 respect to the A/D site.

Noteworthy, apart from the two members of the class Bacillariophyceae, no other pennate diatoms produced any sound hits, although numerous transcriptomes from different species are present in the MMESTP database. In order to further verify this result, a BLAST search was performed in the genome and in the transcriptome of the pennate diatom *Pseudo-nitzschia multistriata* [32] and in the genomes of the congeneric P. multi-series and the very closely related Fragilariopsis cylindrus; no hits were found in any of the datasets. This finding corroborates the BLAST search in MMESTP where 14 transcriptomes for six different Pseudo-nitzschia and eight from *Fragilariopsis kerguelensis* are present.

Our phylogenetic analyses showed that most of the diatom chitin synthases robustly (PP 0.97) cluster together in a separate

clade with ScCHS3 while *P. tricornutum* and *Corethronpennatum* (Calss Coscinodiscophyceae) cluster with ScCHS1 and ScCHS2. This finding, corroborated by sequence inspection, suggests that this centric species in fact shares the same genes with *P. tricornutum*. Surprisingly, the pennate species *Craspedostaurosaustralis* and *Stauroneis constricta* cluster in the ScCHS3 clade. This clade clustered, in a series of basal bifurcations, Bacillariophyceae, Mediophyceae and Coscinodiscophyceae. All the other Mediophyceae sequences grouped in two separate well supported clades. These proteins can be the result of an ancient gene duplication that in this class promoted the use of chitin in the cell wall. The distribution of orthologous or paralogous CHS3 genes over the species is not constant. In fact, *Triceratium dubium* presented two *CHS3* while *Odontella sinensis* had four. All the other species (apart the pennate) have at least two sequences clustering in one clade and at least one in the other.

The role of chitin synthases in diatoms is not clear, especially in those species that do not synthesise chitin. Functional studies would be needed to unravel this issue also benefitting of the progresses in molecular techniques available for diatoms [33-38]. The analyses involved amino acid 46 sequences and 1034 positions with gaps. Line color indicates Bayesian posterior probability (color bar range 0.6 – 1.0). Close to each node the posterior probability and the bootstrap value (where applicable) are reported. Sequence logos for the A/D and the catalytic sites are reported for each clade. Sequence ID color code: green: class Bacillariophyceae; red: class Coscinodiscophyceae; blue: class Mediophyceae. Protein ID (for *P. tricornutum*, *T. pseudonana* and *T. oceanica*) and transcript ID are reported after the species.

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