Horizontal gene transfer of phytochelatin synthases from bacteria to extremophilic green algae

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ABSTRACT

Transcriptomic sequencing together with bioinformatic analyses and an automated annotation process led us to identify novel phytochelatin synthase (PCS) genes from two extremophilic green algae (*Chlamydomonas acidophila* and *Dunaliella acidophila*). These genes are of intermediate length compared to known PCS genes from eukaryotes and PCS-like genes from prokaryotes. A detailed phylogenetic analysis gives new insight into the complicated evolutionary history of PCS genes and provides evidence for multiple horizontal gene transfer events from bacteria to eukaryotes within the gene family. A separate subgroup containing PCS-like genes within the PCS gene family is not supported since the PCS genes are monophyletic only when the PCS-like genes are included. The presence and functionality of the novel genes in the organisms was verified by genomic sequencing and qRT-PCR. Furthermore, the novel PCS gene in *Chlamydomonas acidophila* showed very strong induction by cadmium. Cloning and expression of the gene in *Escherichia coli* clearly improves its cadmium resistance. The gene in *Dunaliella* was not induced, most likely due to gene duplication.

Keywords: heavy metal tolerance, cadmium, acidophile, *Chlamydomonas acidophila*, *Dunaliella acidophila*

INTRODUCTION

Contamination of soil and water by toxic heavy metals poses a major environmental hazard due to their toxicity and accumulation behavior [1]. Thus, metals are among the most studied polluting agents. Heavy metals are usually toxic because they displace endogenous metal cofactors from their cellular binding sites, undergo aberrant capping reactions with the thiol groups of proteins and thiol containing coenzymes, and promote the formation of active oxygen species [2]. Of the various detoxification pathways activated in plants under heavy metal stress, increased synthesis of sulfur containing defense compounds, namely cysteine, glutathione (GSH) and phytochelatins (PCs), is considered to be of prime importance for their tolerance and survival [3, 4]. In addition to a variety of plants, their synthesis is also increased in algae and fungi [4-5] upon metal exposure.

Phytochelatins (PCs) mediate high-affinity binding and contribute to detoxification of heavy metal ions and metalloids, such as cadmium or arsenic, by promoting the vacuolysosomal sequestration of heavy metals [6]. These compounds are enzymatically synthesized from reduced glutathione (GSH) and related thiols in a γ-glutamyl-cysteinyltranspeptidation reaction catalyzed by phytochelatin synthase (PCS). Genes encoding phytochelatin synthases have been found in all vascular plants as well as some algae, fungi, diatoms and invertebrates [4, 7-9]. However, studies regarding PCS gene expression levels have only been examined in a few vascular plants and, in many cases, the results have been contradictory [10-12]. In addition to the eukaryotic full-length phytochelatin synthase genes, PCS-like or half-size PCS proteins were detected initially in prokaryotes (reviewed by Rea et al. [2]). These proteins were described to have a similar N-terminal but lack the more sequence-variable C-terminal, being therefore shorter (220–237 compared to 421–506 amino acid residues). Since then, PCS-like genes have also been reported in some eukaryotes (e.g. fungi: *Saccharomyces pombe, Phytophtora infestans, Dictyostelium discoideum*; heterokonta: *Tetrahymena thermophila*; metazoan: *Caenorhabditis elegans, Hydra magnipapillata, Strongylocentrotus purpuratus*) with scattered distribution but the functionality of these transcripts has largely remained untested [13-14].

Most studies concerning heavy metals and microorganism physiology are related to environments polluted by industrial and domestic wastes. Only little is known about the toxicity of these substances in environments with a natural, non-anthropogenic source of high levels of

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heavy metals, such as extreme acidic ecosystems. Acidic environments tend to contain unusually high concentrations of heavy metals, because their solubility increases markedly as the pH decreases [15]. Despite these extreme environmental conditions, a large number of prokaryotic and eukaryotic organisms have been identified to live in the presence of high concentrations of heavy metals [16-17]. Acidophiles can survive in ≤1000-fold higher heavy metals amounts than neutrophilic microorganisms [18]. These highly metal resistant organisms seem to have multiple and/or more efficient active resistance systems than neutrophiles [19]. Modern biotechnological methods like high-throughput sequencing of non-model organisms that live in extreme habitats can help to understand the metabolism that allows these organisms to live in their environment and also enable the discovery of novel chemical components.

In a recent study on genes affected by copper excess in a strain of the microalga *Chlamydomonas acidophila* isolated from Río Tinto (one of the largest extreme acidic environments described until now), transcripts with a putative function related to stress and heavy metal detoxification were identified from a *de novo* transcriptomic assembly [20]. Several of these transcripts got a best blast hit in other organisms than green algae. One of these, a transcript annotated as a glutathione gamma-glutamyl-cysteinyl-transferase (synonymous to PCS), was hypothesized to represent a novel gene absent in other green algae. A putative PCS-like transcript was later also found in another microalga, in a *Dunaliella acidophila* strain isolated from the same environment [21]. The present study aims to: 1) Characterize these novel PCS genes by additional sequencing to recover the complete genes. 2) Analyze their protein domains and transmembrane topology. 3) Study their functionality and induction under cadmium exposure. Cadmium was selected because its immense water solubility determines its wide distribution in aquatic systems and, its relatively high mobility in comparison to other metals, makes it a metal of major concern in environmental contamination [22-24]. A phylogenetic analysis was performed in order to uncover relationships between PCS genes in plants, bacteria and green algae and to interpret their evolutionary history.

MATERIAL AND METHODS

Algae cultivation and exposure to Cadmium

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Chlamydomonas acidophila strain RT46 was collected from water samples taken in 2010 at the CEM station of Río Tinto (SW Spain) [17], and isolated to grow in the presence of antibiotics, vancomycin 50 µg/mL, cefotaxime 100 µg/mL and chloramphenicol 15 µg/mL (Sigma Aldrich, MO, US), on agar plates made with 0.22 µm-filtered river water. Clonal cultures were grown in 50-ml flasks in K medium [25] at pH 2 under an illumination of ca. 70 microE s-1 m-2 irradiance provided by day-light fluorescent tubes, 16:8 h LD cycle at 20ºC.

Dunaliella acidophila RT5 was also collected in 2010 at RICHI sampling station, and grown in ARTII medium (developed in our laboratory, see Online Resource 1) at pH 2. When the cultivations reached stationary stage, the cultures were exposed to a Cd solution $CdCl_2$ 2 ½ H_2O (Panreac Química, Barcelona, Spain). Cultures were maintained during 1h, 3h and 24h with a final concentration of 1 µM. Cells were collected in 50 ml Falcon tubes and centrifuged for 5 min at 5000 rpm. Pellets were frozen with liquid nitrogen and stored at -80 °C until RNA extraction.

Nucleic acid extraction

Total RNA was extracted with TRI Reagent® Solution (Thermo Fisher Scientific, MA, US) following manufacturer's protocol. To remove possible DNA contamination the samples were treated with DNase I (RNase free) (Thermo Fisher Scientific). DNA was extracted from cultures not exposed to cadmium using the Wizard® Genomic DNA Purification Kit (Promega Corporation, WI, US) or PurelinkTM Genomic DNA Mini Kit (Thermo Fisher Scientific). DNA and RNA quality were determined spectrometrically ($A_{260}/A_{280} > 1.8$) using a NanoDrop ND-1000 spectrometer (NanoDrop, DE, US).

Primer design

Primers for amplification of the full-length genes (CaPCS1, CaPCS2 and DaPCS1) as well as qRT-PCR, and cloning and expression of CaPCS2 were designed using the program Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) with default settings. Due to high variability in the PCS transcripts, species specific primers were needed. In quantitative reverse transcription PCR (qRT-PCR) 18S rRNA [26] and actin were both used as housekeeping genes to normalize gene expression. All primers used in this study together with optimal annealing temperatures are listed in Table 1.

PCR amplification, cloning and sequencing

PCR amplifications (Bio-Rad Tetrad 2, Bio-Rad, CA, US) of DNA were performed in 25 µl-reactions following the recommendation for GoTaq® DNA Polymerase (Promega Corporation). PCR settings were as follows: 2 min 95°C, 35 cycles (1 min 95°C, 1 min primer specific annealing temperature, 1 min 72°C), 10 min 72°C. The number of cycles to 40 was frequently used for obtaining stronger products. The PCR products were separated on a 1% agarose gel and visualized with ethidium bromide using 50 bp DNA ladder (Thermo Fisher Scientific) for size determination. The amplification specificity and efficiency were tested and one primer pair was discarded due to unspecific amplification. The amplified products for each primer pair were cloned with the TOPO TA Cloning kit (Thermo Fisher Scientific) and sequenced on an Applied Biosystems 3730*xl* DNA Analyzer (Thermo Fisher Scientific) using M13pUC primers and BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific). The sequences were edited manually with PhyDE® v1.0 [27] and primer sequences eliminated before submission to GenBank (accession numbers KT348512 - KT348515).

Occasional non-specific amplification of the target region in *Dunaliella acidophila* was detected. Strong amplification of the non-specific products was detected using a gradient annealing temperature program (1 min 95 °C, 40 cycles (1 min 95 °C, 1 min 53 ºC – 57 ºC, 1 min 72°C, 10 min 72 ºC).

Quantitative reverse transcription PCR (qRT-PCR)

For qRT-PCR, protocols established by Díaz et al. [28] were followed, except that the annealing temperature was 55°C and actin (ACT1) was used in addition to 18S as endogenous control gene. ACT1 primers were modified from the *Tetrahymena* gene TTHERM_00190950 available on the *Tetrahymena* Genome Database (TGD) [29]. The cDNA synthesis was carried out using 3 µg RNA in 20 µl-reaction, according to the protocol supplied by 1st Strand cDNA Synthesis kit (AMV, Roche, Basel, Switzerland). All qRT-PCR reactions were carried out in an iQTM5 multicolor Real-Time PCR detection System (Bio-Rad) apparatus with the following cycling conditions: (i) 5 min at 95°C to denature reverse transcriptase, (ii) 40 cycles of 95°C for 30 s, 55°C for 30 s and 72°C after 20 s. Both NTC (no template control) and RT minus control were negative. The real-time dissociation curve was used to check primer specificity and to confirm the presence of a unique PCR product. Standard curves were obtained using 10-fold serial cDNA dilutions and

determining the Ct (cycle threshold) values. The standard line parameters (amplification efficiency, slope and correlation coefficient) are reported in Online Resource 2. Analysis of relative gene expression was carried out according to the Standard-curve quantification method [30] from at least four independent experiments (each performed in duplicates). Only CaPCS2 was amplified with qRT-PCR, since CaPCS1 was not in the focus of this study and reliable results would not be possible to obtain for DaPCS2 due to assumed gene duplication.

Cloning and expression of the novel PCS gene (CaPCS2) from *C. acidophila*in*E. coli*

The CaPCS2 gene was amplified and cloned in the plasmid pdr111 using the following primers: FQUpHIN and FQLoNHE, which contain the restriction sites HindIII and NheI required for subsequent subcloning. Plasmid pdr111 was a gift from D. Rudner (Harvard Medical School) and contains the hyper-SPANK promoter, upstream of the cloning site, which is inducible by IPTG. Primer FQUpHIN contains a bacterial ribosomal binding sequence to promote the expression the CaPCS2 gene in *E. coli*. PCR reaction was carried out using cDNA as DNA template in 20 μl reaction containing: 1x PCR buffer 1 (Expand Long Template PCR System, Roche) with 17.5 mM MgCl2, 350 μM each dNTP, 300 nM each primer, 3.75 U (0.75μl) Expand Long Template enzyme mix (Roche) and 1 μl of cDNA. Amplification was carried out with the following cycling conditions: 2 min 94°C, 39 cycles (1 min 94°C, 1.5 min 56°C, 1.5 min 72°C), 10 min 72°C.

A unique PCR fragment of approximately 850 bp corresponding to the complete ORF of CaPCS2 gene was gel purified with the QIAquick extraction gel (Qiagen, Hilden, Germany), digested with the restriction endonucleases HindIII (Roche) and NheI (Roche) and ligated into pdr111 plasmid. Chemically competent *Escherichia coli* DH5α were transformed with pdr111 plasmid containing the cloned CaPCS2 gene. Bacterial plasmids were isolated with QIAprep Spin Miniprep kit (Qiagen). The growth medium for transformed bacteria was supplemented with 50 μg ml-1 ampicillin to prevent loss of the plasmid in *E. coli*.

Analysis of Cadmium resistance in *E. coli* clones

The cadmium resistance was analyzed by the minimum inhibitory concentration (MIC) using serial dilutions 0-10of *E. coli* clones in LB solid medium (Luria Bertani medium, Laboratorios Conda, Madrid, Spain) and with growth curves by measured the optical density at 600nm (OD600). Bacteria carrying the plasmid pdr111 without the gene CaPCS2 cloned were used as

negative control. The MIC were determinate pipetting 4 µl of each dilution (0-10^{-4}) onto LB solid medium containing 200-500 µM CdCl$_2$ 2 ½ H$_2$O (Panreac Química). The cultures were maintained during 16-24h at 37ºC. For the growth curves, OD600 was measured every 60 min by using a microplate reader (Tecan Genios, Mannedorf, Switzerland) at 37°C for 50 cycles (49 h) as described in Mirete et al. [31]. At least 3 different clones in LB (non-treated) and LB supplemented with 300 or 500 µM CdCl$_2$ (treated clones) were analyzed. The pdr111 vector contains a hyper-SPANK promoter, which makes it inducible by IPTG. For this reason, MIC determination and growth curves were carried out in the presence or in the absence of 1 mM IPTG.

Characterization of novel genes

To form a complete picture of the genes both genomic and transcriptomic sequences were used. Transcripts were detected from *de novo* assemblies of *C. acidophila* [20] and *D. acidophila* [21] and in addition the corresponding sequences from another assembly for *C. acidophila* (Olsson et al. unpubl.) were retrieved. Sanger sequencing on genomic DNA was used to complement the information. A flowchart (Online Resource 3) outlines the process from culture to extraction to analyses and shows which assemblies are used in the current study.

Protein characterization

The nucleotide sequences were translated to amino acids using ExPASy translation tool [32]. The novel predicted proteins were further characterized using the web-server version of HMMER [33] to identify protein domains and the Phobius web-server [34] to predict transmembrane topology and signal peptides.

Identification of similar PCS coding sequences and phylogenetic analyses

The novel PCS transcripts were used as queries in BLASTx searches [35] against all protein sequences available in GenBank (nr database downloaded in April 2013) and the most similar sequences were downloaded. The number of species and different isoforms in closely related species of well represented groups like land plants were limited. Sequences were aligned with Mafft [36] using alignment option FFT-NS-I. The alignment was manually edited in PhyDE® v1.0 [27] by excluding ends of the alignments which could not be confidently aligned due to length differences and ambiguities in homology assessment, including the variable C-terminal domain.

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Bayesian analyses were performed with MrBayes v3.2.1 [37], applying search strategies suggested by [37-38]. Calculations of the consensus tree and of the posterior probability of clades were performed based upon the trees sampled after the chains converged. Consensus topologies and support values from the different methodological approaches were compiled and drawn using TreeGraph2 [39].

RESULTS AND DISCUSSION

Transcripts coding for novel PCS genes

A full-length PCS from *Chlamydomonas acidophila* was detected and named as CaPCS1. Transcripts annotated as putative phytochelatin synthases or phytochelatin synthase-like genes in *C. acidophila* and *Dunaliella acidophila* were identified as novel genes and named CaPCS2 and DaPCS, respectively. Both of them had best BLAST hits in bacteria, but there are a few eukaryotic sequences among the most similar sequences as well (Blast results are provided in Online Resource 4). The nucleotide sequences have substantial differences between them and only the translated amino acid sequences can be aligned. The genes are illustrated in Figure 1. Additional information on the introns is represented in Online Resource 5.

Characterization of the full-length PCS CaPCS1 from *Chlamydomonas acidophila*

The transcript that was similar to known PCS from plants and green alga from *C. acidophila*, CaPCS1 is 425 amino acids long and contains four introns. Seven different isoforms of the gene were detected. In the predicted 3'UTR (238 bp) two isoforms show a 66 bp long gap, indicating alternative polyadenylation of UTR. Alternative polyadenylation of UTR (APA-UTR) is a widespread phenomenon in eukaryotic cells [40] including *Chlamydomonas reinhardtii* [41] but has not been documented in *C. acidophila*.

There are some differences between the transcripts obtained from the different assemblies used in this study. From the *de novo* assembly by Olsson et al. [20] two different transcripts were obtained of which one (KT348511) is almost identical with the genomic sequence obtained by Sanger sequencing, while the other KT447466 has a unique 58 bp long insertion. Also the sequences from the other transcriptome assembly (Olsson et al. unpubl. corresponding sequences presented in Online resource 6) show several differences, most importantly in the

5'UTR of two of the sequences (comp17619_c0_seq4 and comp17619_c0_seq6 depicted in Fig. 2). Due to these differences it is hypothesized that two copies of the gene exist. The transcript with the longest open reading frame, which was congruent with the genomic sequence (KT348511), was used for the characterization of the gene.

Characterization of CaPCS2 from *Chlamydomonas acidophila*

CaPCS2 consists of four exons and three introns, with a predicted open reading frame of 285 amino acid residues. Alternative polyadenylation of the UTR is present also in this gene; in the eight isoforms covering a 1288 bp long stretch of predicted 5'UTR regions common to all isoforms alter with regions present in only some of the isoforms (Online Resource 7).

A deletion of a nucleotide 235 bp after the beginning of the last exon was detected in the transcript assembled for this study compared to an existing one [20]. Since the deletion occurs in the coding sequence it affects the open reading frame of the gene. The genomic sequence was congruent with the transcript from our previous study (GBAH01012416), which was therefore used for the characterization of the gene for this inconsistent part (depicted in Figure 1).

Characterization of DaPCS from *Dunaliella acidophila*

Two isoforms of DaPCS1 (comp2951_c3_seq1 and comp2951_c3_seq2) were predicted in the *de novo* transcriptome assembly of *Dunaliella acidophila* [21] corresponding accession numbers KT348509 -KT348510). One of them has five and the other eight introns (Figure 1).

The nucleotide sequence of contig comp2951_c3_seq1 is longer than comp2951_c3_seq2 but there is an alternative stop codon TAA (instead of CAA coding alanine), which ends the open reading frame. The open reading frame of contig comp2951_c3_seq2 has 390 amino acid residues including a complete phytochelatin synthase domain (pfam 05023) of 293 amino acids as well as a signal peptide and inwards orientated transmembrane domain. Contig comp2951_c3_seq1 has a shorter open reading frame of only 238 amino acids with a truncated phytochelatin synthase domain and neither signal peptides nor transmembrane domains are detected.

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Amplification of DaPCS yielded in addition to the expected amplification product unspecific amplification products, observed by gel electrophoresis. Two different products differing about 650 bp in size were cloned and sequenced (see material and methods and Online Resource 8). The sequences are otherwise identical for the overlapping parts except for two differing nucleotides, suggesting gene duplication in this haploid organism. The sequence of the larger product is congruent with the other genomic and transcriptomic sequences and thus probably represents the prevalent gene copy.

Gene expression of the novel CaPCS2 gene

Gene expression analysis of the CaPCS2 gene (*Chlamydomonas acidophila*) was carried out under cadmium treatment using qRT-PCR. The relative mRNA expression levels of target gene were normalized against the levels of actin and 18S rRNA. Statistical analysis for qRT-PCR fold induction indicates that there is a significant difference between the cadmium treatment and the control (Pair Wise Fixed Reallocation Randomisation test, $p<0.01$). The gene showed a very strong induction (1275.3 fold induction, ± 218.8 SD) at three hours, which flatten out at 24 hours (47.8 fold change, ± 24.1 SD).

As discussed above, it is likely that DaPCS is duplicated, which explains the difficulties involved in the DNA amplification of this region. For this reasons qRT-PCR amplification was not performed on DaPCS. The focus of our study is on the novel PCS genes, and therefore the function of the full-length CaPCS1 gene was not investigated in detail. Furthermore, different isoforms might be differently expressed thus introducing errors to the measurements. The results by Ramos et al. [10] showed that the regulatory mechanisms of PCS expression in plants is very complex including alternative splicing and gene duplication events.

Expression and cadmium resistance of CaPCS2 in *E. coli* clones

The cadmium resistance analyzed by the minimum inhibitory concentration (MIC) as explained in material and methods clearly shows that the expression of CaPCS2 gene in *E. coli* improves their resistance to Cd (Fig. 3). The effect is observed on LB solid medium with the highest tested concentration (500 µM) of Cd. In addition, the growth curves on liquid LB of the strain expressing CaPCS2 in the presence of Cd confirm this result. The effects of Cd in a liquid medium are different from solid medium, and the improved resistance to cadmium was better observed

in LB broth with 300 µM added Cd (Fig. 4). In spite of the hyper-SPANK promoter in the used pdr111 plasmid, which controls the expression of the inserted gene CaPCS2 and is inducible by IPTG, addition of IPTG has no significant effect on the cell growth. This could be caused by the transcription of the gene from another constitutively promoter in the plasmid, which dims out the effect of IPTG.

Phylogenetic analyses of phytochelatin synthases

The results of the phylogenetic analyses show different topology from the expected one based on known species relationships. The only large clade reflecting phylogenetic relationships of organisms is the clade that contains known PCS genes from land plants and green algae. Even within this group some species, such as lotus and *Volvox carteri*, have different variants or copies of the gene, which were not always monophyletic (Fig. 5). Indeed, many plant species are known to have at least two PCS genes [10, 13]. The PCS genes from other organisms do not form monophyletic groups congruent with species phylogenies and many of the branches remain unresolved or with low support. Some smaller clades containing bacteria, metazoa or fungi get statistical support but none of them includes all the PCS genes of the group of organisms present in the analysis. Genes from cyanobacteria have a basal position in the phylogenetic tree and do not form a clade.

The full-length PCS (CaPCS1) present in *Chlamydomonas acidophila* is located in a clade containing PCS from other green algae. No similar transcripts from *Dunaliella acidophila* or other publicly available *Dunaliella* sequences were found. This could, however, be due to limited data available for the genus *Dunaliella*. The novel PCS transcripts (CaPCS2 from *Chlamydomonas acidophila* and DaPCS1 from *Dunaliella acidophila*) cluster together but are distinguished by long branches due to very different amino acid sequences between them (Fig. 5and Online Resource 9).

The scattered distribution of PCS genes in nature

For a long time, researchers have been puzzled by the high constitutive expression of PCS genes in different plant tissues [2]. This is especially peculiar if we assume the presence of high heavy metal concentrations to be an exclusively recent phenomenon caused by human activity like mining. It might be, however, that high heavy metal concentration has been a much more

severe problem for organisms early in the earth's history and especially arsenic and cadmium comprised a major threat to incipient life forms [42-43]. Indeed, all life forms have evolved strategies to cope with these heavy metals. The best-characterized metalloid resistances are encoded by the *ars* operons of Gram-negative and Gram-positive bacteria, which are non-specialized in the way that they share common organization and genes including repressors of resistance to Zn(II), Cd(II) and Pb(II) (e.g. [44]. If life did emerge in waters rich in ionic metals, resistance to these compounds would probably be a very ancient trait, as suggested by Rosen [45]. Basal plants including mosses and other bryophyte lineages were for a long time supposed not to have PCS genes [13]. Recent results show the opposite, and the presence of constitutively expressed and functional phytochelatin synthases was demonstrated in all the bryophyte lineages by Petraglia et al. [46]. The presence of PCS genes in bryophytes were assayed by western blotting, *in vitro* activity and by measuring glutathione and phytochelatin contents but the genes have not been sequenced and were therefore not included in our phylogenetic analysis. It is thus not clear if PCSs are key innovations to extreme habitats or ancestral traits, and why they haven´t been downregulated or lost through evolution. One plausible explanation would be that PCS serve other physiological functions beyond cadmium or arsenic detoxification, which has been suggested by some authors [9, 14].

According to our results PCS genes probably have a bacterial origin and were subsequently inherited to different groups, in some cases multiple times. Bacterial origin is also supported by the domain structure and size of the PCS genes, the bacterial genes being smaller. It has been noted earlier that PCS genes have a non-monophyletic distribution across kingdoms [14] but a comprehensive phylogenetic analyses including PCS genes from both eukaryotes and prokaryotes has not been made earlier. It has been suggested earlier that there might be horizontal gene transfer events involved in the PCS genes in metazoa and fungi [14] and even among vascular plants [10]. Our results confirm that the evolutionary history of these genes is indeed hard to elucidate and show that prokaryotic genes have to be included to resolve their phylogeny. The complex evolution of the PCS genes involves several gene duplications and losses or independent insertions of the full-length PCS genes in plants and green algae.

The great differences in the amino acid composition and sequence length between the novel PCS genes DaPCS and CaPCS2 point to an ancient horizontal gene transfer event, although two

independent events could also be possible. Horizontal gene transfer (HGT) from bacteria can be beneficial for eukaryotic organisms to survive in adverse conditions [47] and seems to be more common in algae than in vascular plants [48]. Recent studies have emphasized horizontally acquired key genes in adaptation to extreme and rapidly changing environments in other extremophilic algae [49-50]. The novel PCS genes characterized in this study represent a likely example of genes that enable the organism to invade ecological niches otherwise unavailable, in this case an environment toxic for most other organisms.

CONCLUSIONS

Novel PCS genes from two extremophilic green algae are characterized in this study. The results of the phylogenetic analysis oppose the separation of prokaryotic PCS-like genes and suggest multiple horizontal gene transfer events from bacteria to eukaryotes within the PCS gene family. Further functional and structural analyses are needed to understand in detail the role of PCS both in heavy metal detoxification and other possible functions. The gene CaPCS2 increases cadmium tolerance in bacteria but it remains to be seen if the novel genes are transferable to vascular plants since this could be of potential interest for crop science and phytoremediation.

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Table 1. Oligonucleotide primers used in the study. For each region, all forward (F) and reverse (R) primers are indicated as well as optimal annealing temperature, target species and references if not designed for this study. Primers used for quantitative reverse transcription PCR are marked with an asterisk *. Primers used for cloning and expression of the novel PCS gene in *Chlamydomonas acidophila* (CaPCS2) are marked with an exclamation mark !.

FIGURE LEGENDS

Fig. 1 Structures of the predicted genes DaPCS1, CaPCS1 and CaPCS2. Composition of exons (black, numbered E1, E2, etc.) including untranslated regions (gray) and introns (white) of the three genes is given in bp and drawn to scale. An asterisque (*) indicates that only transcriptomic data is available for the UTR

Fig. 2 A partial sequence alignment of the predicted 3'UTR in CaPCS1 from *Chlamydomonas acidophila*. The sequences from different assemblies (2227_isotig04503 from the assembly by Olsson et al. 2015 and comp17619_c0_seq4 and comp17619_c0_seq6 from a Trinity assembly based on merged data from 454- and Illumina sequencing) show differences in both nucleotide composition and length. Clustal format uses special characters to indicate that all residues in the column are identical (*), conserved (:) or semi-conserved substitutions have been observed (.)

Fig. 3 A) Drop assay using serial dilutions 0-10^{-4} of *E. coli* DH5 α negative control and *E. coli* DH5α with CaPCS2 gene cloned into pdr111 vector in LB platessupplemented with 50 µg ml^{-1} ampicillin. B) *E. coli* DH5α negative control in LB with 500 µM Cdand50 µg ml^{-1} ampicillin. C) *E. coli* with CaPCS2 gene cloned into pdr111 vector in LB with 500 µM Cd and50 µg ml^{-1} ampicillin.

Fig. 4 Growth curves of *Escherichia coli* in LB broth supplemented with 300 µM of Cd in presence (+IPTG) or absence (-IPTG) of 1mM IPTG. NC= *Escherichia coli* negative control. CaPCS2= *Escherichia coli* cells carrying plasmids with CaPCS2 gene.

Fig.5 Phylogenetic relationships based on the novel PCS-like genes and the most similar genes obtained with a BLASTX search against NCBI'sdatabases. The tree represents the majority consensus of trees sampled after stationarity in the Bayesian analysis. Posterior probability values from the Bayesian inference equal or greater than 0.50 are shown above each branch. The tree is unrooted. The amino acid alignment was 383 aa long. The kingdoms of each clade are indicated. A miniature phylogram of the consensus tree is depicted to show the branch lengths

ONLINE RESOURCE CAPTIONS

Online Resource 1 ART II medium used for the cultivation of *Dunaliella acidophila* strain RT5. The volume was brought to 1l with distilled water and the pH adjusted with 0.1N H_2SO_4.

Online Resource 2 Quantitative reverse transcription PCR standard-curve parameters for target gene (novel phytochelatin synthase in *Chlamydomonas acidophila* CaPCS2) and the expression control (housekeeping) genes 18S rRNA and actin. E= amplification efficiency, S= slope, R^2= correlation coefficient.

Online Resource 3 Flow chart on the process from culture to extraction to analyses for the sequences used in the current study.

Online Resource 4 Results for BLASTx search using the open reading frame of transcripts coding for CaPCS2 (isotig12422) and DaPCS (comp2951_c3_seq1 and comp2951_c3_seq2) as query.

Online Resource 5 Introns in the predicted PCS genes from *C. acidophila* and *D. acidophila*. There are three spliceosomal introns in CaPCS2, four in CaPCS1 and three in DaPCS1. The introns are either the most common GT-AG splice site or variations of it.

Online Resource 6 Different contigs of component comp17619_c0 coding for the CaPCS1 gene in *Chlamydomonas acidophila*.

22

Online Resource 7 Different contigs of component comp11852_c0 coding for the CaPCS2 gene in *Chlamydomonas acidophila*.

Online Resource 8 PCR products amplified in *Dunaliellaacidophila* strain RT5 using gradient annealing run on 1% agarose gel (1X TBE). The gel electrophoresis shows amplification of products sized 1198 bp and 542 bp and several non-specific amplification products. The intensity of the non-specific bands depends on the used annealing temperature and PCR program. Wells 1: Ladder 1kb (Promega Corporation), marker sizes from 250 kb to 10000 kb, well2: annealing 52-55°C. Well 3: annealing 55-60°C.

Online Resource 9 Phylogram based on the novel phytochelatin synthase genes and the most similar genes obtained with a BLASTx search against NCBI's databases. The tree represents the majority consensus of trees sampled after stationarity in the Bayesian analysis. PP values from the Bayesian inference > 0.50 are indicated above each branch. The amino acid alignment was 383 aa long.

Primer name	5' Sequence 3'	F/R	Ann. °C	Target species	Author
CaPCH2_1F	TCACACGAAGGACAAAAGGC	F	57	*C. acidophila*	This study
CaPCH2_2F	CCAGGGTCATCACTCTCCAA	F	53	*C. acidophila*	This study
CaPCH2_1R	TGCAATTGGAGAGTGATGACC	R	57	*C. acidophila*	This study
CaPCH2_3F*	TGGGATTGGGATATTGTGCT	F	55	*C. acidophila*	This study
CaPCH2_2R*	ATCTGTTTATGCCCCTGCAC	R	55	*C. acidophila*	This study
CaPCH2_3R	AGTGTCATCAAATGATGTTGCAG	R	57	*C. acidophila*	This study
CaPCH1_1F	TGAATACAACAGCGCCACAT	F	60	*C. acidophila*	This study
CaPCH1_1R	GCTCTCCACAAACATGCTCA	R	60	*C. acidophila*	This study
CaPCH1_2R	TCCTCCCCACTCCTACACAC	R	60	*C. acidophila*	This study
DaPCH2_1F	TTGTGAAGATGAGGGCATGT	R	57	*D. acidophila*	This study
DaPCH2_2F	ACATCAGGCCCAACATAACG	F	57	*D. acidophila*	This study
DaPCH2_1R	GCTGCGTCTGAACATCTCAA	R	57	*D. acidophila*	This study
DaPCH2_3F	GCTTGTCCCTTGAAGACCTG	F	57	*D. acidophila*	This study
DaPCH2_2R	TGCACGTTACATTCCTGCTC	R	57	*D. acidophila*	This study
18SrRNAF*	TCAACTTTCGATGGTAGGATAGTG	F	55	*D. acidophila, C. acidophila, C. reinhardtii*	Fei et al. 2010
18SrRNAR*	CCGTGTCAGGATTGGGTAATTT	R	55	*D. acidophila, C. acidophila, C. reinhardtii*	Fei et al. 2010
RT46_ACT1*	CTCACTCTCAACATTCCAGCAA	F	55	*C. acidophila,*	Modified from TTHERM_00190950
RT46_ACT2*	GGGCCCGCTCTCATCATACTC	R	55	*C. acidophila*	Modified from TTHERM_00190950
FQUpHIN[!]	TCAGTAAAGCTTAAAGGTGGTGAACTACTATGC AGGACCCTGCTTCA	Left	58	*C. acidophila*	This study
FQ_Lo_NHE[!]	ATCGTAGCTAGCAACAGCGCACAGTGGTGATA	Right	58	*C. acidophila*	This study

a)

DaPCS1

5'-UTR — 917*
E1 — 108
E2 — 84
E3 — 123
E4 — 60
E5 — 174
E6 — 145
E7 — 168
E8 — 86
E9 — 421
3'-UTR — 3154*

92, 282, 238, 444, 109, 130

- Isoform 1, mRNA

5'-UTR E1 E2 E3 E4 E5 E6 E7 E8 E9 3'-UTR AAAA

1 917 1517 5440

- Isoform 2, mRNA

5'-UTR E1 E2 E3 E4 E5 E7 E9 3'-UTR AAAA

1 917 1973 5127

b)

CaPCS1

5'-UTR — 238
E1 — 274
E2 — 73
E3 — 132
E4 — 43
E5* — 753*
3'-UTR — 2572*

66, 169, 121, 150, 269

- Isoform 1, mRNA

5'-UTR E1 E2 E3 E4 E5* 3'-UTR AAAA

1 239 1514 4086

c)

CaPCS2

5'-UTR — 274
E1 — 66 114
E2 — 174
E3 — 86
E4 — 481
3'-UTR — 5*

214, 189, 251, 273

- Isoform 1, mRNA

5'-UTR E1 E2 E3 E4 3'-UTR AAAA

1 340 1194 1119
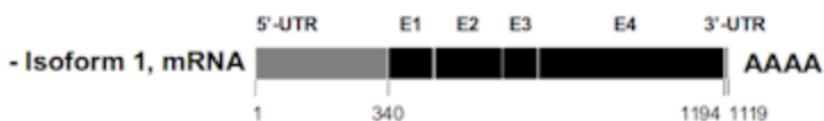
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RT46_2227_Ctg_i  CATAAACATGGCCTTCAAGAAACTTGCCAGACCAAGGATGACAATGACAATCATTTCACA
comp17619_c0_se  CATAAACATGGCCTTCAAGAAACTTGCCAGACCAAGGATGACAATGACAATCATTTCACA
comp17619_c0_se  CTTAAACATGGCCTTCAAGAACCTTGCCAGACCTAG---GACAATGACACACATTTCACA
                 * ****************** ********** **    *********  *********

RT46_2227_Ctg_i  ACGCCCTAAGCAGTCATTTCACGACGCTCTAAGCAGTCGTTGTAAACTTGTAAACAATAC
comp17619_c0_se  ACGCCCTAAGCAGTCATTTCACGACGCTCTAAGCAGTCGTTGTAAACTTGTAAACAATAC
comp17619_c0_se  ACGCCCTAAGCAGTCGTTG----------------------------TAAACAATA-
                 ***************.**  .   **.*..**** * .* **

RT46_2227_Ctg_i  GGCTCTTATTAGTC
comp17619_c0_se  GGCTCTTATTAGTC
comp17619_c0_se  --CTCTTATTACTC
```

10^0 10^{-1} 10^{-2} 10^{-3} 10^{-4}

E. coli negative control
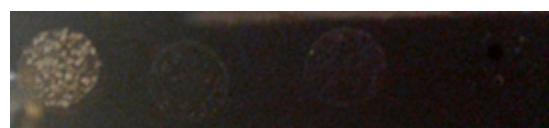
E. coli CaPCS2

E. coli negative control (500 μM Cd)

E. coli CaPCS2 (500 μM Cd)
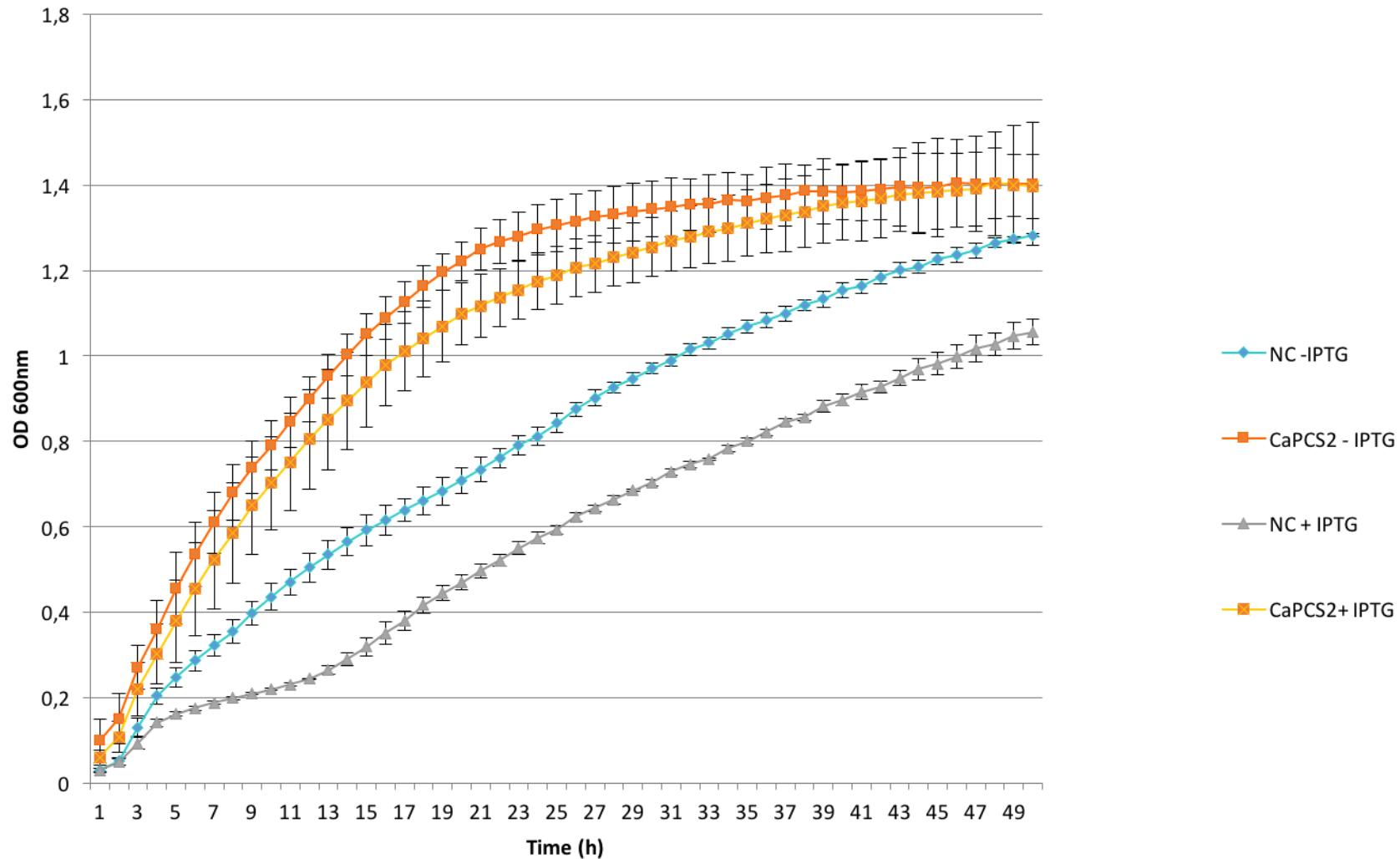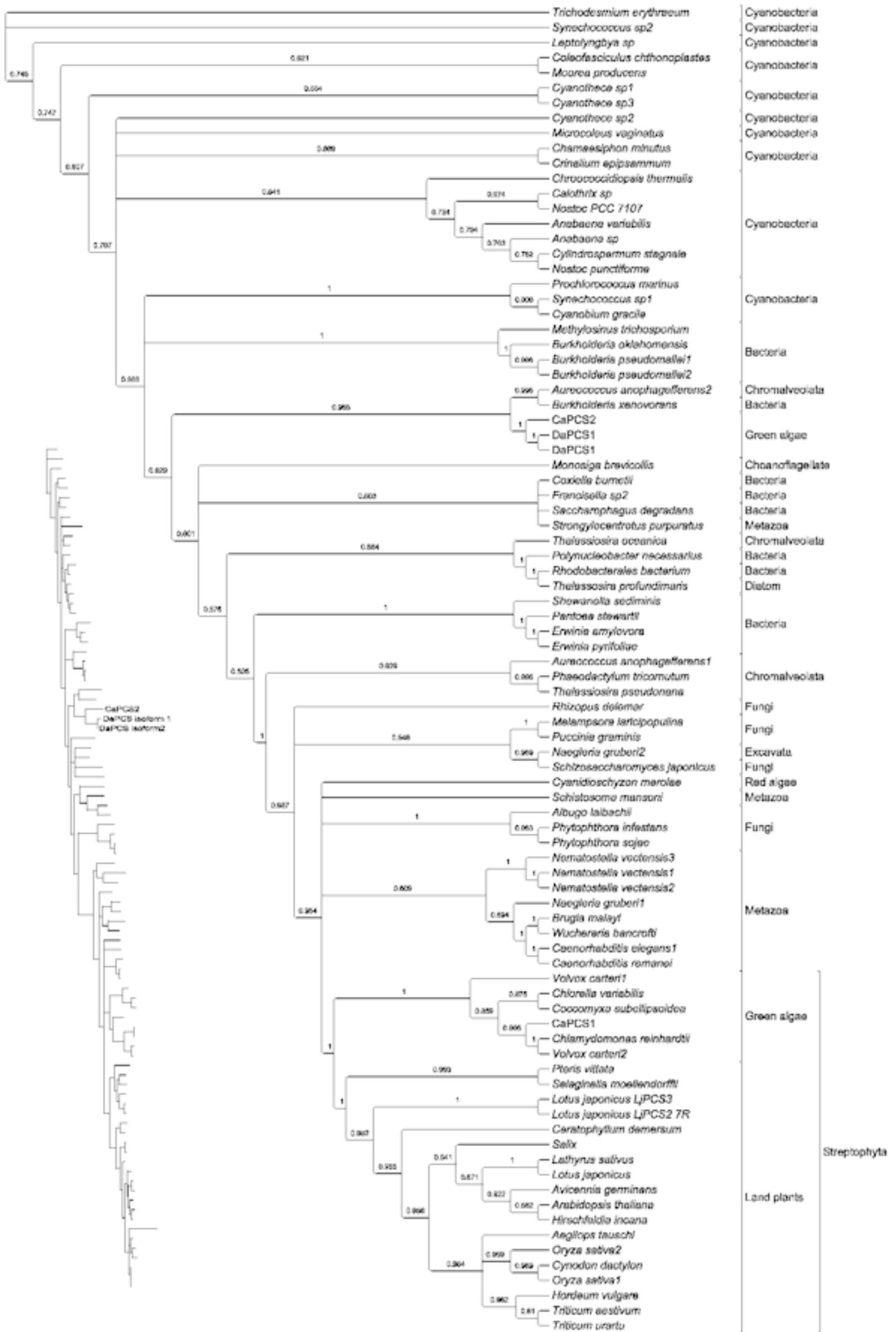
Horizontal gene transfer of phytochelatin synthases from bacteria to extremophilic green algae; Microbial Ecology; Olsson S*, Penacho V, Puente-Sánchez F, Díaz S, Aguilera A; * corresponding author, Department of Agricultural Sciences, P.O. Box 27, 00014 University of Helsinki, Finland, sanna.olsson@helsinki.fi

Online Resource 1 ART II medium used for the cultivation of *Dunaliella acidophila* strain RT5. The volume was brought to 1l with distilled water and the pH adjusted with 0.1N H_2SO_4.

Stock Solution	ml/l
0.5M $ZnSO_4 \cdot 7H_2O$	3.5 ml
0.5M $CuSO_4 \cdot 5H_2O$	8.1 ml
0.5M $Mn(NO_3)_2 \cdot 6H_2O$	2.0 ml
0.5M $Mg\ SO_4 \cdot 7H_2O$	27 ml
0.5M $FeSO_4 \cdot 7H_2O$	30 ml
0.5M $NiSO_4 \cdot 6H_2O$	80 μl
0.5M $KCl \cdot 7H_2O$	380 μl
0.5M $CoCl_2 \cdot 6H_2O$	190 μl
0.5M $KBr \cdot 5H_2O$	30 μl

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Online Resource 2 Quantitative reverse transcription PCR standard-curve parameters for target gene (novel phytochelatin synthase in *Chlamydomonas acidophila* CaPCS2) and the expression control (housekeeping) genes 18S rNA and actin. E= amplification efficiency, S= slope, R2= correlation coefficient.

Gene	E	S	R^2
PCS	1,95	-3,43	0,99
18S rRNA	2,09	-3,12	0,98
Actin	2,10	-3,2	0,98

Horizontal gene transfer of phytochelatin synthases from bacteria to extremophilic green algae; Microbial Ecology; Olsson S*, Penacho V, Puente-Sánchez F, Díaz S, Aguilera A; * corresponding author, Department of Agricultural Sciences, P.O. Box 27, 00014 University of Helsinki, Finland, sanna.olsson@helsinki.fi

Online Resource 3 Flow chart on the process from culture to extraction to analyses for the sequences used in the current study.
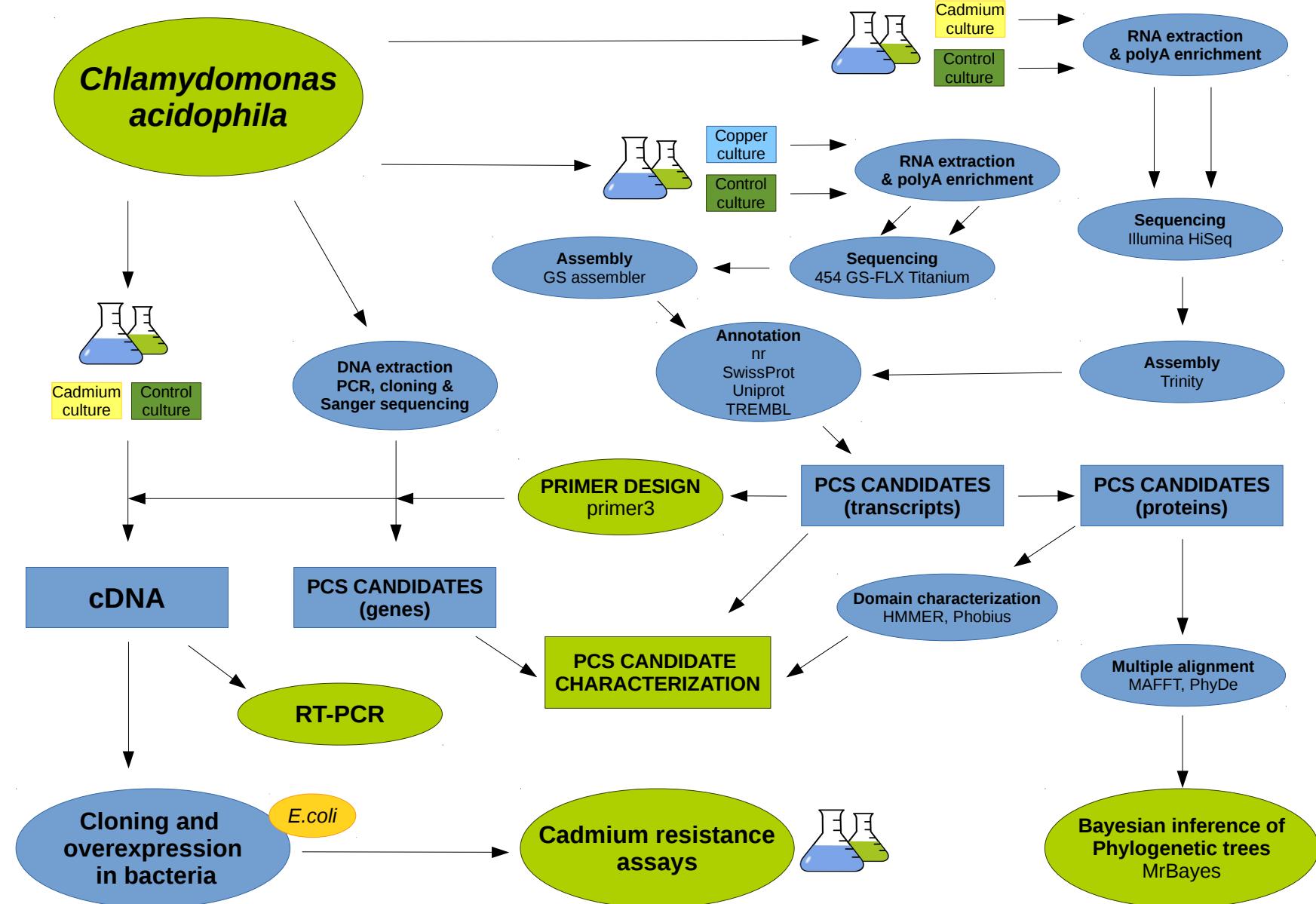
Electronic Supplementary Material 4. Results for BLASTx search using the open reading frame of transcripts coding for CaPCS2 (isotig12422) and DaPCS (comp2951_c3_seq1 and comp2951_c3_seq2) as query.

ORF_isotig12422   gi|821561551|ref|WP_046868556.1| 48.98   147 70 5   37   474 95   237 3e-27   117 ORF_isotig12422 gi|504945810|ref|WP_015132912.1| 42.67   150 80   4   31   474 96 241 2e-25   112 ORF_isotig12422 gi|981461217|ref|WP_059669889.1| 40.65   155 85   4   31   489 92   241 2e-24   109 ORF_isotig12422 gi|938273386|gb|KPQ08481.1| 44.76   143 75   3   46   474 89   227 3e-24   108 ORF_isotig12422 gi|981383968|ref|WP_059597148.1| 41.10 146 81   3   37   474 84   224 3e-24   108 ORF_isotig12422 gi|981251097|ref|WP_059470554.1| 41.10   146 81   3   37   474 93 233 4e-24   108 ORF_isotig12422 gi|981645748|ref|WP_059844321.1| 40.91   154 86   4   37   495 94   243 9e-24   107 ORF_isotig12422 gi|705475049|gb|KGS04910.1| 40.52   153 84   4   37   489 83   230 1e-23   107 ORF_isotig12422 gi|740960548|ref|WP_038745121.1| 40.52 153 84   4   37   489 94   241 1e-23   107 ORF_isotig12422 gi|782673738|ref|WP_045600604.1| 40.52   153 88   3   37   495 94 243 1e-23   107 ORF_isotig12422 gi|497590677|ref|WP_009904861.1| 40.52   153 88   3   37   495 94   243 1e-23   107 ORF_isotig12422 gi|584107904|gb|AHI79522.1| 40.91   154 86   4   37   495 92   241 1e-23   107 ORF_isotig12422 gi|497576287|ref|WP_009890471.1| 40.91 154 86   4   37   495 94   243 1e-23   107 ORF_isotig12422 gi|981433289|ref|WP_059643234.1| 40.52   153 84   4   37   489 94 241 1e-23   107 ORF_isotig12422 gi|773064690|gb|AJY42591.1| 41.22 148 84   3   31   474 82   226 2e-23   106 ORF_isotig12422 gi|505013627|ref|WP_015200729.1| 38.22   157 88   3   19   471 88 241 2e-23   106 ORF_isotig12422 gi|976471317|gb|KVD74754.1| 39.73 146 83   3   37   474 84   224 2e-23   106 ORF_isotig12422 gi|492895672|ref|WP_006026078.1| 39.35   155 89   3   31   495 92 241 2e-23   106 ORF_isotig12422 gi|705585042|gb|KGS47698.1| 41.10 146 83   3   37   474 84   226 3e-23   105 ORF_isotig12422 gi|814343774|emb|CPH68122.1| 41.29   155 87   4   10   474 84   234 3e-23   105 ORF_isotig12422 gi|981296166|ref|WP_059513522.1| 42.00 150 84   3   25   474 89   235 4e-23   105 ORF_isotig12422 gi|925230922|gb|KOS75550.1| 41.10   146 83   3   37   474 19   161 4e-23   103 ORF_isotig12422 gi|686848709|gb|KGC28119.1| 41.22   148 84   3   37   480 84   228 4e-23   105 ORF_isotig12422 gi|686840559|gb|KGC20015.1| 41.10   146 83   3   37   474 84   226 4e-23   105 ORF_isotig12422 gi|976553572|gb|KVE52227.1| 39.73   146 83   3   37   474 84   224 4e-23   105 ORF_isotig12422 gi|714646342|gb|KGW52040.1| 41.10   146 83   3   37   474 84   226 4e-23   105 ORF_isotig12422 gi|741015566|ref|WP_038797782.1| 41.10 146 83   3   37   474 94   236 4e-23   105 ORF_isotig12422 gi|714526348|gb|KGV56958.1| 41.10   146 83   3   37   474 92   234 4e-23   105 ORF_isotig12422 gi|714575209|gb|KGW05595.1| 41.10   146 83   3   37   474 95   237 5e-23   105 ORF_isotig12422 gi|648412694|ref|WP_026104445.1| 43.62   149 78   5   34   474 99 243 5e-23   105 ORF_isotig12422 gi|497606907|ref|WP_009921091.1| 41.10   146 83   3   37   474 94   236 5e-23   105 ORF_isotig12422 gi|740942487|ref|WP_038727502.1| 41.10   146 83   3   37   474 97 239 5e-23   105 ORF_isotig12422 gi|740984489|ref|WP_038768164.1| 41.78   146 82   3   37   474 94   236 5e-23   105 ORF_isotig12422 gi|740980945|ref|WP_038764816.1| 41.22   148 84   3   37   480 97 241 6e-23   105 ORF_isotig12422 gi|752526330|ref|WP_041197894.1| 41.10   146 83   3   37   474 94   236 6e-23   105 ORF_isotig12422

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gi|686936961|gb|KGD15055.1|    41.10    146  83   3    37   474  52   194
6e-23    104 ORF_isotig12422 gi|740959588|ref|WP_038744210.1|    41.10
146  83   3    37   474  94   236  6e-23    105 ORF_isotig12422
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6e-23    105 ORF_isotig12422 gi|490304232|ref|WP_004199532.1|    41.10
146  83   3    37   474  84   226  7e-23    104 ORF_isotig12422
gi|484585464|gb|AGK47089.1|    40.54    148  85   3    31   474  82   226
7e-23    104 ORF_isotig12422 gi|814476642|emb|CPI32267.1|    41.10    146
83   3    37   474  92   234  8e-23    104 ORF_isotig12422
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236  9e-23    104 ORF_isotig12422 gi|714591842|gb|KGW22095.1|    41.10
146  83   3    37   474  84   226  9e-23    104 ORF_isotig12422
gi|754360430|ref|WP_041861630.1|    40.54    148  85   3    31   474  92
236  9e-23    104 ORF_isotig12422 gi|490685647|ref|WP_004550267.1|
41.10    146  83   3    37   474  92   234  1e-22    104 ORF_isotig12422
gi|913356948|ref|WP_050376444.1|    41.10    146  83   3    37   474  94
236  1e-22    104 ORF_isotig12422 gi|800785064|emb|CFL25926.1|    41.10
146  83   3    37   474  92   234  1e-22    104 ORF_isotig12422
gi|953804284|ref|WP_058039816.1|    41.10    146  83   3    37   474  94
236  1e-22    104 ORF_isotig12422 gi|490301683|ref|WP_004197081.1|
41.10    146  83   3    37   474  94   236  1e-22    104 ORF_isotig12422
gi|953803876|ref|WP_058039691.1|    41.10    146  83   3    37   474  94
236  1e-22    104 ORF_isotig12422 gi|981741072|ref|WP_059934588.1|
38.71    155  90   3    31   495  92   241  1e-22    104 ORF_isotig12422
gi|981354997|ref|WP_059570263.1|    39.61    154  88   3    37   498  94
242  1e-22    104 ORF_isotig12422 gi|751301901|ref|WP_041010414.1|
38.61    158  91   4    28   492  86   240  1e-22    104 ORF_isotig12422
gi|740967447|ref|WP_038751917.1|    41.10    146  83   3    37   474  94
236  1e-22    104 ORF_isotig12422 gi|751277775|ref|WP_040986624.1|
39.47    152  86   4    28   474  86   234  2e-22    103 ORF_isotig12422
gi|751301199|ref|WP_041009719.1|    38.61    158  91   4    28   492  86
240  2e-22    103 ORF_isotig12422 gi|913438795|ref|WP_050418395.1|
40.26    154  87   4    13   474  88   236  2e-22    103 ORF_isotig12422
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2e-22    103 ORF_isotig12422 gi|806494169|gb|KKC16091.1|    40.41    146
84   3    37   474  84   226  2e-22    103 ORF_isotig12422
gi|568795567|gb|AHE26011.1|    41.10    146  83   3    37   474  84   226
3e-22    103 ORF_isotig12422 gi|772972989|gb|AJX33047.1|    39.73    146
83   3    37   474  92   232  3e-22    103 ORF_isotig12422
gi|756829253|ref|WP_042646352.1|    39.47    152  86   4    28   474  86
234  3e-22    103 ORF_isotig12422 gi|741019975|ref|WP_038801977.1|
39.73    146  83   3    37   474  93   233  3e-22    103 ORF_isotig12422
gi|740943421|ref|WP_038728427.1|    40.41    146  84   3    37   474  94
236  4e-22    103 ORF_isotig12422 gi|740986897|ref|WP_038770440.1|
41.10    146  83   3    37   474  94   236  4e-22    103 ORF_isotig12422
gi|785594494|ref|WP_045719475.1|    40.41    146  84   3    37   474  94
236  4e-22    102 ORF_isotig12422 gi|741007236|ref|WP_038789710.1|
40.41    146  84   3    37   474  94   236  4e-22    102 ORF_isotig12422
gi|493029431|ref|WP_006099075.1|    42.36    144  77   5    46   474  104
242  4e-22    102 ORF_isotig12422 gi|237502694|gb|ACQ95012.1|    41.10
146  83   3    37   474  198  340  5e-22    103 ORF_isotig12422
gi|714902087|gb|KGX30928.1|    41.10    146  83   3    37   474  206  348
5e-22    103 ORF_isotig12422 gi|157806771|gb|EDO83941.1|    41.10    146
83   3    37   474  212  354  6e-22    103 ORF_isotig12422
gi|160698859|gb|EDP88829.1|    41.10    146  83   3    37   474  190  332
7e-22    103 ORF_isotig12422 gi|689678301|emb|CEF48871.1|    38.36    146
87   3    37   474  94   236  7e-22    102 ORF_isotig12422
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7e-22     103 ORF_isotig12422 gi|126229014|gb|ABN92554.1|    41.10    146
83   3    37   474  199  341  7e-22     103 ORF_isotig12422
gi|685679385|gb|AIO66505.1|    39.73    146  83   3    37   474  92   232
8e-22     102 ORF_isotig12422 gi|126219717|gb|ABN83223.1|    41.10    146
83   3    37   474  203  345  8e-22     103 ORF_isotig12422
gi|169654349|gb|EDS87042.1|    41.10    146  83   3    37   474  209  351
8e-22     103 ORF_isotig12422 gi|184209881|gb|EDU06924.1|    41.10    146
83   3    37   474  205  347  8e-22     103 ORF_isotig12422
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233  8e-22     102 ORF_isotig12422 gi|157936951|gb|EDO92621.1|    41.10
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8e-22     103 ORF_isotig12422 gi|217395731|gb|EEC35749.1|    41.10    146
83   3    37   474  222  364  8e-22     103 ORF_isotig12422
gi|225927831|gb|EEH23872.1|    41.10    146  83   3    37   474  225  367
8e-22     103 ORF_isotig12422 gi|134251017|gb|EBA51096.1|    41.10    146
83   3    37   474  220  362  8e-22     103 ORF_isotig12422
gi|501733845|ref|WP_012629148.1|    41.18    153  79   5    31   474  95
241  8e-22     102 ORF_isotig12422 gi|927917924|emb|CDX53272.1|    39.47
152  86   4    28   474  86   234  1e-21     101 ORF_isotig12422
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241  2e-21     100 ORF_isotig12422 gi|503088795|ref|WP_013323642.1|
41.98    162  81   6    13   474  87   243  3e-21     100 ORF_isotig12422
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229  4e-21     100 ORF_isotig12422 gi|504949901|ref|WP_015137003.1|
44.52    155  76   7    25   474  93   242  5e-21     100 ORF_isotig12422
gi|685089209|emb|CDX24205.1|    36.71    158  94   4    28   492  86   240
7e-21     99.4 ORF_isotig12422    gi|751264288|ref|WP_040974001.1|
38.16    152  88   4    28   474  88   236  8e-21     99.4
ORF_isotig12422    gi|966518160|ref|WP_058534654.1|    39.51    162  85
5    13   480  75   229  9e-21     99.0 ORF_isotig12422
gi|974600092|ref|WP_059190108.1|    36.71    158  94   4    28   492  86
240  1e-20     98.6 ORF_isotig12422    gi|764950513|ref|WP_044520790.1|
38.18    165  90   6    19   492  80   239  2e-20     98.2
ORF_isotig12422    gi|17130321|dbj|BAB72932.1|    38.18    165  90   6
19   492  83   242  2e-20     98.2 ORF_isotig12422
gi|652914138|ref|WP_027168079.1|    38.82    152  87   4    28   474  86
234  2e-20     97.8 ORF_isotig12422    gi|701274860|gb|AIV79086.1|
39.61    154  82   5    37   474  84   234  3e-20     97.8
ORF_isotig12422    gi|75702893|gb|ABA22569.1|    38.36    159  87   5
19   474  80   234  3e-20     97.4 ORF_isotig12422
gi|759580794|ref|WP_043299684.1|    39.61    154  82   5    37   474  94
244  4e-20     97.4 ORF_isotig12422    gi|657935844|ref|WP_029636980.1|
34.81    158  94   3    19   474  88   242  4e-20     97.4
ORF_isotig12422    gi|496440563|ref|WP_009149408.1|    39.33    150  85
4    31   474  109  254  5e-20     97.4 ORF_isotig12422
gi|504970487|ref|WP_015157589.1|    40.49    163  82   6    13   474  80
236  6e-20     96.7 ORF_isotig12422    gi|505018197|ref|WP_015205299.1|
37.04    162  89   4    13   474  86   242  1e-19     95.5
ORF_isotig12422    gi|748165414|ref|WP_039738990.1|    37.11    159  89
5    19   474  88   242  2e-19     95.5 ORF_isotig12422
gi|953510847|dbj|BAT53535.1|    35.44    158  93   3    19   474  80   234
2e-19     95.5 ORF_isotig12422    gi|501377640|ref|WP_012409206.1|
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32.30      161  98   3    16    474  82    239   2e-19         95.5
ORF_isotig12422      gi|85544315|pdb|2BTW|B    38.18      165  90   6    19
492  94    253  2e-19        95.5 ORF_isotig12422      gi|85544314|pdb|2BTW|A
38.18      165  90   6    19    492  94    253  2e-19         95.1
ORF_isotig12422      gi|737172391|ref|WP_035158595.1|    41.26      143  79
4    49    474  101   239  2e-19        95.1 ORF_isotig12422
gi|515888082|ref|WP_017318665.1|    35.19      162  92   5    16    474  85
242  3e-19        94.7 ORF_isotig12422      gi|505020123|ref|WP_015207225.1|
35.86      145  90   2    37    471  100   241  3e-19        94.7
ORF_isotig12422      gi|489705594|ref|WP_003609731.1|    39.24      158  90
4    1     474  86    237  3e-19        94.7 ORF_isotig12422
gi|504891436|ref|WP_015078538.1|    38.10      147  86   4    37    474  100
242  4e-19        94.4 ORF_isotig12422      gi|763312982|ref|WP_044171432.1|
38.41      151  86   4    31    474  87    233  9e-19        93.2
ORF_isotig12422      gi|658419581|ref|WP_029646255.1|    37.24      145  87
3    34    468  100   240  1e-18        93.2 ORF_isotig12422
gi|516956783|ref|WP_018183684.1|    38.82      152  87   4    28    474  87
235  1e-18        93.2 ORF_isotig12422      gi|497231444|ref|WP_009545706.1|
42.76      145  76   6    46    474  99    238  2e-18        92.8
ORF_isotig12422      gi|746961585|gb|KIF19114.1|    42.21      154  77   8
31    474  92    239  2e-18        92.4 ORF_isotig12422
gi|917776332|ref|WP_052290273.1|    42.21      154  77   8    31    474  83
230  2e-18        92.4 ORF_isotig12422      gi|500232799|ref|WP_011902392.1|
37.41      147  83   4    31    462  87    227  3e-18        91.7
ORF_isotig12422      gi|515356232|ref|WP_016865554.1|    35.29      153  93
3    25    474  92    241  3e-18        91.7 ORF_isotig12422
gi|751564302|ref|WP_041033204.1|    36.42      162  93   6    1     474  87
242  4e-18        91.7 ORF_isotig12422      gi|656037395|ref|WP_029076335.1|
35.26      156  96   3    25    492  89    239  5e-18        90.9
ORF_isotig12422      gi|522080484|ref|WP_020591693.1|    38.46      143  84
3    46    474  95    233  5e-18        90.9 ORF_isotig12422
gi|495550750|ref|WP_008275329.1|    42.57      148  78   6    46    483  99
241  6e-18        90.9 ORF_isotig12422      gi|496167609|ref|WP_008892116.1|
39.16      143  83   3    46    474  91    229  6e-18        90.9
ORF_isotig12422      gi|427346779|gb|AFY29492.1|    37.84      148  87   4
34    474  81    224  6e-18        90.9 ORF_isotig12422
gi|504925739|ref|WP_015112841.1|    37.66      154  88   5    25    474  93
242  7e-18        90.9 ORF_isotig12422      gi|817130062|ref|WP_046502581.1|
37.76      143  85   3    46    474  99    237  7e-18        90.9
ORF_isotig12422      gi|1000089089|gb|KXJ56520.1|    39.16      143  83   3
46    474  91    229  7e-18        90.5 ORF_isotig12422
gi|522054471|ref|WP_020565680.1|    39.04      146  85   3    37    474  108
249  7e-18        90.9 ORF_isotig12422      gi|504967338|ref|WP_015154440.1|
38.16      152  86   5    31    474  94    241  1e-17        90.5
ORF_isotig12422      gi|917319449|ref|WP_051926161.1|    38.19      144  84
4    46    474  103   242  1e-17        90.5 ORF_isotig12422
gi|746710169|ref|WP_039669460.1|    34.23      149  97   1    37    483  19
166  1e-17        88.6 ORF_isotig12422      gi|759607566|ref|WP_043325766.1|
37.84      148  87   4    34    474  92    235  1e-17        90.1
ORF_isotig12422      gi|501565810|ref|WP_012570258.1|    34.23      149  97
1    37    483  19    166  1e-17        88.2 ORF_isotig12422
gi|648291774|ref|WP_026072923.1|    38.41      151  87   5    34    483  98
243  2e-17        89.7 ORF_isotig12422      gi|827519218|gb|KLN61905.1|
37.06      143  86   3    46    474  83    221  2e-17        89.4
ORF_isotig12422      gi|920578875|ref|WP_053005650.1|    37.06      143  86
3    46    474  99    237  2e-17        89.4 ORF_isotig12422
gi|740234212|ref|WP_038075400.1|    37.16      148  88   4    37    474  92
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236  2e-17      89.4 ORF_isotig12422      gi|652338179|ref|WP_026735392.1|
34.64      153 94   3    25   474 93   242 2e-17      89.4
ORF_isotig12422      gi|515385832|ref|WP_016877723.1|    35.95      153 92
3    25   474 93   242 3e-17      89.0 ORF_isotig12422
gi|915474789|ref|WP_050814322.1|    36.62      142 87   3    10   432 9
148 3e-17      87.4 ORF_isotig12422      gi|916294818|ref|WP_051029864.1|
34.38      160 96   3    13   474 84   240 3e-17      89.0
ORF_isotig12422      gi|91691483|gb|ABE34681.1|    37.84      148 88   3
31   474 99   242 3e-17      89.0 ORF_isotig12422
gi|156355949|ref|XP_001623696.1|    50.00      82  38   1    256  501 155
233 3e-17      89.0 ORF_isotig12422      gi|872579356|ref|WP_048533013.1|
37.67      146 87   3    37   474 188  329 4e-17      89.7
ORF_isotig12422      gi|648499655|ref|WP_026191406.1|    36.14      166 97
4    16   495 82   244 4e-17      88.6 ORF_isotig12422
gi|697780908|gb|AIT63532.1|    34.23      149 97   1    37   483 78   225
4e-17      88.2 ORF_isotig12422      gi|207082025|gb|ABS78257.2|    34.23
149 97   1    37   483 99   246 4e-17      88.6 ORF_isotig12422
gi|165917203|gb|EDR35807.1|    34.23      149 97   1    37   483 137  284
4e-17      89.0 ORF_isotig12422      gi|737933697|ref|WP_035898371.1|
38.22      157 91   5    10   474 84   236 5e-17      88.2
ORF_isotig12422      gi|918692141|ref|WP_052561880.1|    35.46      141 87
3    52   474 88   224 6e-17      87.8 ORF_isotig12422
gi|738342341|ref|WP_036294947.1|    36.14      166 97   4    16   495 87
249 6e-17      88.2 ORF_isotig12422      gi|941312503|ref|WP_055116231.1|
38.85      157 90   5    10   474 84   236 6e-17      87.8
ORF_isotig12422      gi|749301086|ref|WP_040125311.1|    37.84      148 88
3    31   474 149  292 8e-17      88.2 ORF_isotig12422
gi|916990632|ref|WP_051597344.1|    37.21      129 79   2    34   420 99
225 1e-16      87.4 ORF_isotig12422      gi|692118754|ref|WP_032075052.1|
33.56      149 98   1    37   483 19   166 1e-16      85.9
ORF_isotig12422      gi|797211671|ref|WP_045872614.1|    35.00      160 91
5    19   474 88   242 1e-16      87.4 ORF_isotig12422
gi|494317175|ref|WP_007177410.1|    37.25      153 92   3    31   489 145
293 1e-16      87.8 ORF_isotig12422      gi|772943476|gb|AJX80078.1|
34.93      146 69   3    37   474 84   203 1e-16      86.7
ORF_isotig12422      gi|497652379|ref|WP_009966563.1|    34.93      146 69
3    37   474 94   213 2e-16      86.3 ORF_isotig12422
gi|493165631|ref|WP_006170876.1|    35.34      133 85   1    34   432 125
256 2e-16      87.0 ORF_isotig12422      gi|656719681|gb|KEF42411.1|
37.41      147 87   4    37   474 79   221 2e-16      86.7
ORF_isotig12422      gi|504939174|ref|WP_015126276.1|    36.55      145 88
3    40   474 108  248 4e-16      85.9 ORF_isotig12422
gi|501395618|ref|WP_012427184.1|    37.84      148 88   3    31   474 136
279 6e-16      85.5 ORF_isotig12422      gi|651604058|ref|WP_026598946.1|
36.08      158 92   4    16   471 77   231 7e-16      84.7
ORF_isotig12422      gi|755598209|ref|WP_042525907.1|    32.89      149 99
1    37   483 19   166 7e-16      83.6 ORF_isotig12422
gi|640368848|ref|WP_024880427.1|    36.08      158 92   4    16   471 82
236 1e-15      84.3 ORF_isotig12422      gi|1001837663|gb|KXS32259.1|
36.99      146 88   3    37   474 99   240 1e-15      84.0
ORF_isotig12422      gi|931478713|gb|KPK59845.1|    37.80      127 77   2
52   432 73   197 2e-15      82.8 ORF_isotig12422
gi|926791675|ref|XP_013904311.1|    35.83      120 74   2    142  501 19
135 2e-15      81.6 ORF_isotig12422      gi|156394135|ref|XP_001636682.1|
48.28      87  42   2    241  501 157  240 3e-15      83.2
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3    46   483 90   231 4e-15      82.4 ORF_isotig12422
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gi|754969497|ref|WP_042325399.1|    36.71       158  95   4    1     474  119
271  7e-15      82.4 ORF_isotig12422    gi|156379434|ref|XP_001631462.1|
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ORF_isotig12422    gi|752594371|ref|WP_041263758.1|    34.69       98   61
2    181  474  6    100  9e-15       78.6 ORF_isotig12422
gi|738534441|ref|WP_036482850.1|    31.79       151  96   4    28    474  103
248  1e-14      81.3 ORF_isotig12422    gi|499442921|ref|WP_011130385.1|
36.57      134  82   3    34   432  105  236  2e-14       80.5
ORF_isotig12422    gi|739030777|ref|WP_036911908.1|    34.81       135  83
4    34   432  105  236  4e-14       79.7 ORF_isotig12422
gi|499786267|ref|WP_011467001.1|    42.57       101  53   2    196  498  222
317  5e-14      80.5 ORF_isotig12422    gi|123963072|gb|ABM77828.1|
33.78      148  93   4    34   474  105  248  6e-14       79.3
ORF_isotig12422    gi|331218212|ref|XP_003321784.1|    41.98       81   47
0    196  438  150  230  7e-14       81.3 ORF_isotig12422
gi|550281002|ref|WP_022607401.1|    35.62       146  84   5    49   483  99
235  9e-14      78.6 ORF_isotig12422    gi|800135199|dbj|GAO34503.1|
36.55      145  88   3    40   474  90   230  2e-13       77.4
ORF_isotig12422    gi|331218210|ref|XP_003321783.1|    42.31       78   45
0    202  435  277  354  2e-13       79.3 ORF_isotig12422
gi|336447064|gb|AEI36370.1|    41.89       74   41   1    253  474  6    77
3e-13      73.9 ORF_isotig12422    gi|909606181|gb|KNE96553.1|    38.20
89   55   0    202  468  220  308  3e-13       79.7 ORF_isotig12422
gi|831778908|ref|XP_012755554.1|    37.84       111  64   3    181  513  166
271  3e-13      79.3 ORF_isotig12422    gi|290993528|ref|XP_002679385.1|
38.82      85   52   0    202  456  145  229  4e-13       76.6
ORF_isotig12422    gi|675852512|ref|XP_009011816.1|    38.74       111  66
1    211  543  206  314  6e-13       78.6 ORF_isotig12422
gi|676386555|ref|XP_009036556.1|    41.76       91   52   1    205  474  152
242  7e-13      76.3 ORF_isotig12422    gi|504984674|ref|WP_015171776.1|
37.90      124  75   2    145  513  111  233  9e-13       77.8
ORF_isotig12422    gi|568027492|gb|ETM01446.1|    37.00       100  60   2
175  474  59   155  1e-12      75.1 ORF_isotig12422
gi|999977806|gb|KXJ16980.1|    41.77       79   44   1    238  474  430  506
1e-12      77.8 ORF_isotig12422    gi|999977806|gb|KXJ16980.1|    32.61
138  67   5    142  492  138  270  5e-12      75.9 ORF_isotig12422
gi|909134564|gb|KNE60758.1|    33.61       119  76   2    145  501  24   139
1e-12      75.5 ORF_isotig12422    gi|586633751|gb|EWS64201.1|    39.18
97   55   1    199  489  31   123  1e-12      73.2 ORF_isotig12422
gi|999977804|gb|KXJ16978.1|    34.65       101  58   2    190  474  152  250
1e-12      75.9 ORF_isotig12422    gi|737401579|ref|WP_035382755.1|
35.33      150  89   4    37   480  86   229  2e-12      75.1
ORF_isotig12422    gi|685951299|ref|XP_009269521.1|    38.54       96   57
2    145  432  127  220  2e-12      77.0 ORF_isotig12422
gi|524911978|ref|XP_005110845.1|    35.87       92   59   0    145  420  116
207  2e-12      77.0 ORF_isotig12422    gi|198417053|ref|XP_002128372.1|
33.07      127  84   1    145  525  110  235  2e-12       76.6
ORF_isotig12422    gi|219124481|ref|XP_002182531.1|    30.63       160  83
3    16   456  393  537  3e-12      76.6 ORF_isotig12422
gi|909134565|gb|KNE60759.1|    33.61       119  76   2    145  501  93   208
3e-12      75.1 ORF_isotig12422    gi|909134566|gb|KNE60760.1|    33.61
119  76   2    145  501  119  234  4e-12      75.5 ORF_isotig12422
gi|727439853|ref|XP_010501307.1|    37.63       93   58   0    142  420  108
200  4e-12      76.6 ORF_isotig12422    gi|727439853|ref|XP_010501307.1|
37.63      93   58   0    142  420  313  405  4e-12       76.6
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145  501  119  234  5e-12      75.9 ORF_isotig12422
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gi|564587188|gb|AHB86971.1|    41.56    77   45   0    190  420  124  200
6e-12    75.9  ORF_isotig12422    gi|223995009|ref|XP_002287188.1|
51.67    60   29   0    253  432  144  203  7e-12    72.8
ORF_isotig12422    gi|505014688|ref|WP_015201790.1|    38.83    103  58
3    169  474  118  216  7e-12    75.1  ORF_isotig12422
gi|953489559|emb|CEG46925.1|    36.54    104  63   2    163  474  265  365
7e-12    75.1  ORF_isotig12422    gi|731346801|ref|XP_010684647.1|
41.77    79   46   0    202  438  75   153  8e-12    75.1
ORF_isotig12422    gi|983147566|gb|KWU45526.1|    39.39    99   57   2
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gi|731346799|ref|XP_010684646.1|    41.77    79   46   0    202  438  129
207  9e-12    75.1  ORF_isotig12422    gi|490267384|ref|WP_004163951.1|
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ORF_isotig12422    gi|727603973|ref|XP_010475030.1|    37.78    90   56
0    151  420  111  200  1e-11    74.7  ORF_isotig12422
gi|752845383|ref|WP_041474394.1|    29.45    146  91   2    28   438  102
244  1e-11    73.2  ORF_isotig12422    gi|727569811|ref|XP_010457419.1|
37.78    90   56   0    151  420  111  200  1e-11    74.7
ORF_isotig12422    gi|312171048|emb|CBX79307.1|    29.45    146  91   2
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gi|470239215|ref|XP_004351373.1|    35.14    111  66   2    190  519  475
580  1e-11    75.1  ORF_isotig12422    gi|490258075|ref|WP_004155296.1|
29.45    146  91   2    28   438  102  244  1e-11    72.8
ORF_isotig12422    gi|310766388|gb|ADP11338.1|    29.45    146  91   2
28   438  115  257  1e-11    73.2  ORF_isotig12422
gi|219111005|ref|XP_002177254.1|    34.75    118  65   3    145  474  108
221  1e-11    72.4  ORF_isotig12422    gi|478729310|emb|CCP05801.1|
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ORF_isotig12422    gi|635369185|emb|CCI42660.1|    34.38    128  80   4
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gi|752845037|ref|WP_041474048.1|    29.45    146  91   2    28   438  102
244  1e-11    72.8  ORF_isotig12422    gi|817516937|ref|WP_046572938.1|
37.34    158  94   4    1    474  126  278  1e-11    73.2
ORF_isotig12422    gi|490272265|ref|WP_004168384.1|    29.45    146  91
2    28   438  102  244  1e-11    72.8  ORF_isotig12422
gi|224965368|emb|CAX56900.1|    29.45    146  91   2    28   438  115  257
1e-11    72.8  ORF_isotig12422    gi|297848618|ref|XP_002892190.1|
37.78    90   56   0    151  420  111  200  2e-11    74.3
ORF_isotig12422    gi|15219610|ref|NP_171894.1|    37.78    90   56   0
151  420  110  199  2e-11    74.3  ORF_isotig12422
gi|397645345|gb|EJK76788.1|    42.22    90   47   1    241  495  150  239
2e-11    74.3  ORF_isotig12422    gi|551588562|ref|XP_005778952.1|
38.38    99   58   2    178  474  103  198  2e-11    71.6
ORF_isotig12422    gi|301112447|ref|XP_002997994.1|    37.00    100  60
2    175  474  308  404  2e-11    73.9  ORF_isotig12422
gi|729701043|emb|CEJ02028.1|    42.11    76   44   0    205  432  163  238
2e-11    73.9  ORF_isotig12422    gi|754352951|ref|XP_011270847.1|
41.24    97   50   2    184  456  336  431  2e-11    73.9
ORF_isotig12422    gi|562746587|ref|WP_023653709.1|    30.00    140  86
2    28   420  102  238  2e-11    72.0  ORF_isotig12422
gi|922346013|ref|XP_013449921.1|    36.56    93   59   0    190  468  125
217  2e-11    73.9  ORF_isotig12422    gi|729710313|emb|CEI93301.1|
42.11    76   44   0    205  432  66   141  2e-11    73.6
ORF_isotig12422    gi|215983522|gb|ACJ71777.1|    37.08    89   56   0
202  468  128  216  2e-11    71.6  ORF_isotig12422
gi|303281270|ref|XP_003059927.1|    32.22    180  93   7    10   474  57
232  2e-11    71.6  ORF_isotig12422    gi|224001048|ref|XP_002290196.1|
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32.69   104   62   2   145   435   122   224   3e-11   71.6
ORF_isotig12422   gi|552916579|gb|ESA01781.1| 42.67   75   43   0
208   432   124   198   3e-11   71.2 ORF_isotig12422
gi|727146284|emb|CEG71516.1| 42.11   76   44   0   205   432   139   214
3e-11   73.6 ORF_isotig12422   gi|66803020|ref|XP_635353.1| 33.64
110   68   3   190   519   252   356   3e-11   73.9 ORF_isotig12422
gi|727146285|emb|CEG71517.1| 42.11   76   44   0   205   432   163   238
3e-11   73.6 ORF_isotig12422   gi|675213985|ref|XP_008915877.1|
37.00   100   60   2   175   474   303   399   3e-11   73.6
ORF_isotig12422   gi|570996289|gb|ETP52930.1| 37.00   100   60   2
175   474   302   398   3e-11   73.6 ORF_isotig12422
gi|570335908|gb|ETO83856.1| 37.00   100   60   2   175   474   302   398
3e-11   73.6 ORF_isotig12422   gi|568057987|gb|ETM54635.1| 37.00
100   60   2   175   474   303   399   3e-11   73.6 ORF_isotig12422
gi|444891677|gb|AGE13358.1| 51.67   60   29   0   253   432   230   289
3e-11   72.8 ORF_isotig12422   gi|570959955|gb|ETP24934.1| 37.00
100   60   2   175   474   303   399   3e-11   73.2 ORF_isotig12422
gi|330794008|ref|XP_003285073.1| 40.26   77   46   0   190   420   160
236   3e-11   73.6 ORF_isotig12422   gi|388497750|gb|AFK36941.1|
41.10   73   43   0   202   420   128   200   3e-11   73.6
ORF_isotig12422   gi|122202937|sp|Q2QKL5.1|PCS3_LOTJA   41.10   73
43   0   202   420   128   200   4e-11   73.2 ORF_isotig12422
gi|147719992|sp|Q2TE74.2|PCS2_LOTJA   41.10   73   43   0   202   420
128   200   4e-11   73.2 ORF_isotig12422   gi|50659119|gb|AAT80341.1|
41.10   73   43   0   202   420   123   195   4e-11   73.2
ORF_isotig12422   gi|568027478|gb|ETM01432.1| 35.29   102   63   2
175   480   59   157   4e-11   70.5 ORF_isotig12422
gi|593799054|ref|XP_007162565.1| 42.86   77   44   0   190   420   125
201   4e-11   73.2 ORF_isotig12422   gi|902234844|gb|KNA23610.1|
38.71   93   51   2   202   474   129   217   4e-11   73.2
ORF_isotig12422   gi|281202856|gb|EFA77058.1| 37.66   77   48   0
190   420   144   220   4e-11   71.2 ORF_isotig12422
gi|758352771|dbj|GAN05107.1| 33.94   109   57   2   253   534   238   346
4e-11   73.6 ORF_isotig12422   gi|570959958|gb|ETP24937.1| 35.29
102   63   2   175   480   85   183   4e-11   70.9 ORF_isotig12422
gi|518404331|ref|WP_019574538.1| 45.95   74   38   1   253   474   182
253   5e-11   71.2 ORF_isotig12422   gi|325516458|gb|ADZ24787.1|
36.67   90   57   0   151   420   111   200   5e-11   72.8
ORF_isotig12422   gi|590715531|ref|XP_007050222.1| 36.56   93   56
2   145   420   110   200   5e-11   72.8 ORF_isotig12422
gi|970660098|gb|KUF97679.1| 37.00   100   60   2   175   474   303   399
6e-11   72.8 ORF_isotig12422   gi|654738300|ref|WP_028194554.1|
36.71   158   95   4   1   474   126   278   6e-11   71.2
ORF_isotig12422   gi|736937357|ref|WP_034934372.1| 32.41   108   71
1   145   468   148   253   7e-11   70.9 ORF_isotig12422
gi|397628930|gb|EJK69115.1| 35.71   112   71   1   166   498   271   382
7e-11   72.4 ORF_isotig12422   gi|737586499|ref|WP_035557439.1|
36.71   158   95   4   1   474   139   291   7e-11   71.2
ORF_isotig12422   gi|357575758|gb|AET85547.1| 32.69   104   62   2
145   435   122   224   8e-11   72.4 ORF_isotig12422
gi|599364119|ref|XP_007405638.1| 40.26   77   46   0   202   432   159
235   8e-11   70.5 ORF_isotig12422   gi|568057990|gb|ETM54638.1|
35.29   102   63   2   175   480   161   259   8e-11   71.2
ORF_isotig12422   gi|823127937|ref|XP_012440935.1| 40.26   77   46
0   190   420   124   200   8e-11   72.4 ORF_isotig12422
gi|823127935|ref|XP_012440927.1| 40.26   77   46   0   190   420   124
200   8e-11   72.4 ORF_isotig12422   gi|907093431|gb|KNC99293.1|

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45.31      64   35   0    241   432   201   264   8e-11      72.4
ORF_isotig12422      gi|661892612|emb|CDP03770.1|   35.51      107  62   2
145   465   110   209   8e-11      72.4 ORF_isotig12422
gi|823127939|ref|XP_012440942.1|   40.26      77   46   0    190   420   62
138   8e-11      72.0 ORF_isotig12422      gi|566032216|gb|ETI55127.1|
35.29      102  63   2    175   480   161   259   9e-11      71.2
ORF_isotig12422      gi|4322421|gb|AAD16046.1|      39.73      73   44   0
202   420   128   200   9e-11      72.0 ORF_isotig12422
gi|571445940|ref|XP_006576950.1|   38.96      77   47   0    190   420   61
137   9e-11      72.0 ORF_isotig12422      gi|662152692|ref|WP_030100516.1|
36.71      158  95   4    1    474   126   278   9e-11      70.9
ORF_isotig12422      gi|823127931|ref|XP_012440911.1|   40.26      77   46
0    190   420   124   200   9e-11      72.0 ORF_isotig12422
gi|908401434|ref|XP_013070252.1|   38.46      104  58   2    202   513   130
227   9e-11      72.0 ORF_isotig12422      gi|763743549|gb|KJB11048.1|
40.26      77   46   0    190   420   124   200   1e-10      72.0
ORF_isotig12422      gi|734345461|gb|KHN10759.1|   38.96      77   47   0
190   420   123   199   1e-10      72.0 ORF_isotig12422
gi|965670943|dbj|BAT85550.1|   33.93      112  72   1    85   420   92   201
1e-10      72.0 ORF_isotig12422      gi|823127933|ref|XP_012440917.1|
40.26      77   46   0    190   420   124   200   1e-10      72.0
ORF_isotig12422      gi|325186679|emb|CCA21228.1|   37.04      108  65   3
151   474   144   248   1e-10      70.9 ORF_isotig12422
gi|675213991|ref|XP_008915880.1|   35.29      102  63   2    175   480   161
259   1e-10      70.9 ORF_isotig12422      gi|950949688|ref|XP_014495217.1|
33.93      112  72   1    85   420   92   201   1e-10      72.0
ORF_isotig12422      gi|570335911|gb|ETO83859.1|   35.29      102  63   2
175   480   161   259   1e-10      70.9 ORF_isotig12422
gi|763743550|gb|KJB11049.1|   40.26      77   46   0    190   420   124   200
1e-10      72.0 ORF_isotig12422      gi|570996278|gb|ETP52920.1|   35.29
102  63   2    175   480   161   259   1e-10      70.9 ORF_isotig12422
gi|947119173|gb|KRH67422.1|   38.96      77   47   0    190   420   88   164
1e-10      71.6 ORF_isotig12422      gi|356505041|ref|XP_003521301.1|
38.96      77   47   0    190   420   123   199   1e-10      71.6
ORF_isotig12422      gi|167537519|ref|XP_001750428.1|   31.69      142  95
2    37   456   105   246   1e-10      70.1 ORF_isotig12422
gi|727647674|ref|XP_010494405.1|   37.08      89   56   0    202   468   128
216   1e-10      71.6 ORF_isotig12422      gi|675852508|ref|XP_009011814.1|
34.43      122  70   3    208   561   173   288   1e-10      71.6
ORF_isotig12422      gi|674249977|gb|KFK42742.1|   35.56      90   58   0
151   420   111   200   1e-10      71.6 ORF_isotig12422
gi|402586327|gb|EJW80265.1|   39.24      79   48   0    202   438   68   146
1e-10      69.3 ORF_isotig12422      gi|567185115|ref|XP_006403146.1|
38.20      89   55   0    202   468   128   216   1e-10      71.6
ORF_isotig12422      gi|449443293|ref|XP_004139414.1|   35.71      98   59
2    202   495   53   146   1e-10      68.2 ORF_isotig12422
gi|551561391|ref|XP_005767549.1|   40.00      100  60   0    211   510   140
239   1e-10      69.7 ORF_isotig12422      gi|290980155|ref|XP_002672798.1|
33.90      118  67   3    145   474   153   267   1e-10      71.6
ORF_isotig12422      gi|284466089|gb|ACL00594.3|   39.73      73   44   0
202   420   128   200   2e-10      71.2 ORF_isotig12422
gi|549049394|emb|CCX11465.1|   35.51      107  65   3    145   462   127   230
2e-10      71.2 ORF_isotig12422      gi|21104518|dbj|BAB93120.1|   34.26
108  70   1    145   468   110   216   2e-10      71.2 ORF_isotig12422
gi|694541843|ref|XP_009497159.1|   37.50      96   59   1    145   432   48
142   2e-10      71.2 ORF_isotig12422      gi|493073505|ref|WP_006122095.1|
31.52      92   63   0    145   420   148   239   2e-10      69.7
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ORF_isotig12422      gi|814540591|emb|CEQ42050.1|   37.80      82   51   0
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gi|748749862|ref|WP_040007977.1|   44.59      74   39   2    253  474  171
242  2e-10      69.7 ORF_isotig12422      gi|870854053|gb|KMT05872.1|
41.10     73   43   0    202  420  104  176  2e-10      71.2
ORF_isotig12422      gi|870854052|gb|KMT05871.1|   41.10      73   43   0
202  420  129  201  2e-10      71.2 ORF_isotig12422
gi|870854054|gb|KMT05873.1|   41.10      73   43   0    202  420  145  217
2e-10      71.2 ORF_isotig12422      gi|970651949|gb|KUF90697.1|   35.29
102  63   2    175  480  161  259  2e-10      70.5 ORF_isotig12422
gi|902234842|gb|KNA23608.1|   41.10      73   43   0    202  420  129  201
2e-10      71.2 ORF_isotig12422      gi|588263371|ref|XP_006960829.1|
36.36     77   49   0    202  432  160  236  2e-10      70.1
ORF_isotig12422      gi|15240084|ref|NP_199220.1|   39.73      73   44   0
202  420  128  200  2e-10      71.2 ORF_isotig12422
gi|18254401|gb|AAL66747.1|AF461180_1   39.73      73   44   0    202  420
128  200  2e-10      71.2 ORF_isotig12422
gi|694541835|ref|XP_009497157.1|   37.50      96   59   1    145  432  315
409  2e-10      71.6 ORF_isotig12422
gi|7229390|gb|AAF42805.1|AF162689_1   39.73      73   44   0    202  420
128  200  2e-10      71.2 ORF_isotig12422      gi|870854055|gb|KMT05874.1|
41.10     73   43   0    202  420  129  201  2e-10      71.2
ORF_isotig12422      gi|4768281|gb|AAD29446.1|AF085231_2   37.08      89
56   0    202  468  145  233  2e-10      71.2 ORF_isotig12422
gi|729349445|ref|XP_010542900.1|   35.96      89   57   0    202  468  128
216  2e-10      71.2 ORF_isotig12422      gi|384491545|gb|EIE82741.1|
29.94     157  97   2    208  639  134  290  2e-10      70.9
ORF_isotig12422      gi|671683790|emb|CDS12824.1|   41.56      77   45   0
202  432  206  282  2e-10      71.2 ORF_isotig12422
gi|731346805|ref|XP_010684649.1|   41.10      73   43   0    202  420  181
253  2e-10      71.2 ORF_isotig12422      gi|731346807|ref|XP_010684650.1|
41.10     73   43   0    202  420  181  253  2e-10      71.2
ORF_isotig12422      gi|13928024|emb|CAC37692.1|   36.26      91   56   1
202  474  128  216  2e-10      70.9 ORF_isotig12422
gi|470508086|ref|XP_004367696.1|   40.00      75   45   0    196  420  108
182  2e-10      70.9 ORF_isotig12422      gi|902234843|gb|KNA23609.1|
41.10     73   43   0    202  420  129  201  2e-10      70.9
ORF_isotig12422      gi|685326730|ref|XP_009101739.1|   36.26      91   56
1    202  474  128  216  2e-10      70.9 ORF_isotig12422
gi|443301362|gb|AGC82138.1|   39.73      73   44   0    202  420  129  201
2e-10      70.9 ORF_isotig12422      gi|520899875|ref|WP_020322885.1|
31.31     99   68   0    145  441  148  246  2e-10      69.3
ORF_isotig12422      gi|731346810|ref|XP_010684651.1|   41.10      73   43
0    202  420  120  192  2e-10      70.9 ORF_isotig12422
gi|731346803|ref|XP_010684648.1|   41.10      73   43   0    202  420  181
253  2e-10      70.9 ORF_isotig12422      gi|301128435|gb|ADK61091.1|
41.10     73   43   0    202  420  128  200  2e-10      70.9
ORF_isotig12422      gi|297794999|ref|XP_002865384.1|   39.73      73   44
0    202  420  128  200  3e-10      70.9 ORF_isotig12422
gi|923860545|ref|XP_013706438.1|   36.26      91   56   1    202  474  185
273  3e-10      70.9 ORF_isotig12422      gi|565432669|ref|XP_006280379.1|
34.86     109  68   2    145  468  110  216  3e-10      70.5
ORF_isotig12422      gi|170594708|ref|XP_001902100.1|   39.24      79   48
0    202  438  144  222  3e-10      70.5 ORF_isotig12422
gi|702451810|ref|XP_010025777.1|   39.19      74   45   0    199  420  144
217  3e-10      68.9 ORF_isotig12422      gi|284468345|gb|ABW98498.2|
39.73     73   44   0    202  420  128  200  3e-10      70.5
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ORF_isotig12422     gi|511010013|gb|EPB91244.1|     45.00     60   33   0
253 432 233 292 3e-10     70.9 ORF_isotig12422
gi|301112441|ref|XP_002997991.1|     37.80     82   50   1   175  420  161
241 3e-10     69.7 ORF_isotig12422     gi|928498313|gb|KPD18813.1|
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ORF_isotig12422     gi|672829171|gb|KFH74060.1|     38.53     109  56   4
190 504 488 589 3e-10     70.5 ORF_isotig12422
gi|629091683|gb|KCW57678.1|     39.19     74   45   0   199  420  127  200
3e-10     68.9 ORF_isotig12422     gi|567155800|ref|XP_006418181.1|
35.56     90   58   0   151  420  75   164 4e-10     70.1
ORF_isotig12422     gi|494729398|ref|WP_007465264.1|     44.00     75   42
0   208 432 171  245 4e-10     68.9 ORF_isotig12422
gi|296417443|ref|XP_002838367.1|     33.03     109  71   2   145  468  110
217 4e-10     70.1 ORF_isotig12422     gi|567155803|ref|XP_006418182.1|
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ORF_isotig12422     gi|727621889|ref|XP_010481782.1|     37.08     89   56
0   202 468 128  216 4e-10     70.1 ORF_isotig12422
gi|21104516|dbj|BAB93119.1|     33.02     106  71   0   151  468  111  216
4e-10     70.1 ORF_isotig12422     gi|219129430|ref|XP_002184892.1|
41.56     77   45   0   190  420  594  670 4e-10     70.5
ORF_isotig12422     gi|727535400|ref|XP_010441944.1|     37.08     89   56
0   202 468 128  216 4e-10     70.1 ORF_isotig12422
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4e-10     70.1 ORF_isotig12422     gi|502110105|ref|XP_004493800.1|
42.25     71   41   0   208 420  129  199 4e-10     70.1
ORF_isotig12422     gi|743862914|ref|XP_010943667.1|     39.73     73   44
0   202 420 42   114 4e-10     70.1 ORF_isotig12422
gi|410994522|gb|AFV96147.1|     34.83     89   58   0   202  468  43   131
4e-10     66.6 ORF_isotig12422     gi|747088495|ref|XP_011091839.1|
38.96     77   47   0   190  420  124  200 5e-10     70.1
ORF_isotig12422     gi|695439924|ref|XP_009532833.1|     36.94     111  66
3   145 474 310  417 5e-10     70.1 ORF_isotig12422
gi|515351240|ref|WP_016863694.1|     36.36     99   60   2   175  468  120
216 5e-10     69.7 ORF_isotig12422     gi|302789570|ref|XP_002976553.1|
37.65     85   53   0   202 456 128  212 5e-10     70.1
ORF_isotig12422     gi|545355774|ref|XP_005643662.1|     32.69     104  69
1   145 456 83   185 5e-10     67.4 ORF_isotig12422
gi|390364326|ref|XP_003730583.1|     38.06     134  74   5   46   435  76
204 5e-10     67.8 ORF_isotig12422     gi|743862910|ref|XP_010943666.1|
39.73     73   44   0   202 420 66   138 5e-10     69.7
ORF_isotig12422     gi|659070963|ref|XP_008457484.1|     38.36     73   45
0   202 420 233  305 5e-10     69.3 ORF_isotig12422
gi|19114464|ref|NP_593552.1|     28.70     108  76   1   145  468  145  251
6e-10     69.7 ORF_isotig12422     gi|685358931|ref|XP_009114083.1|
39.73     73   44   0   202 420 156  228 6e-10     69.7
ORF_isotig12422     gi|748629167|ref|WP_039887344.1|     33.10     142  91
2   49  474 97   234 6e-10     67.8 ORF_isotig12422
gi|674920166|emb|CDY13130.1|     39.73     73   44   0   202  420  110  182
6e-10     69.7 ORF_isotig12422     gi|302782732|ref|XP_002973139.1|
37.65     85   53   0   202 456 128  212 6e-10     69.7
ORF_isotig12422     gi|758368534|emb|CEP09044.1|     45.00     60   33   0
253 432 234 293 6e-10     69.7 ORF_isotig12422
gi|901821666|gb|KMZ73511.1|     31.52     92   62   1   145  420  110  200
6e-10     69.7 ORF_isotig12422     gi|661175740|emb|CDH61092.1|     40.26
77   46   0   202 432 206 282 7e-10     69.7 ORF_isotig12422
gi|743862902|ref|XP_010943664.1|     39.73     73   44   0   202  420  128
200 7e-10     69.7 ORF_isotig12422     gi|18958247|dbj|BAB85602.1|
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35.16       91    57    1     202   474   128   216   7e-10        69.3
ORF_isotig12422       gi|472585716|gb|EMS23267.1|    34.06       138  64    4
19    432   107   217   7e-10       69.3 ORF_isotig12422
gi|567902608|ref|XP_006443792.1|    39.73       73    44    0     202   420   128
200   7e-10       69.3 ORF_isotig12422       gi|567902610|ref|XP_006443793.1|
39.73       73    44    0     202   420   128   200   7e-10        69.3
ORF_isotig12422       gi|674877470|emb|CDY54482.1|    35.16       91    57    1
202   474   128   216   7e-10        69.3 ORF_isotig12422
gi|207100019|emb|CAK24968.2|    35.16       91    57    1     202   474   128   216
7e-10       69.3 ORF_isotig12422       gi|672183480|ref|XP_008812020.1|
39.73       73    44    0     202   420   42    114   8e-10        69.3
ORF_isotig12422       gi|641841508|gb|KDO60420.1|    39.73       73    44    0
202   420   128   200   8e-10        69.3 ORF_isotig12422
gi|732757650|gb|KHJ79087.1|    28.97       107   73    1     202   513   7     113
8e-10       65.5 ORF_isotig12422       gi|1005176715|gb|KYF56577.1|    44.59
74    37    1     253   474   150   219   8e-10        68.6 ORF_isotig12422
gi|659071562|ref|XP_008460630.1|    39.02       82    44    1     202   429   235
316   8e-10        68.6 ORF_isotig12422       gi|333458192|gb|EGK86811.1|
33.10       142   91    2     49    474   127   264   8e-10        68.2
ORF_isotig12422       gi|255577926|ref|XP_002529835.1|    33.33       90    60
0     151   420   111   200   8e-10        69.3 ORF_isotig12422
gi|694415952|ref|XP_009336118.1|    38.20       89    55    0     202   468   128
216   8e-10        69.3 ORF_isotig12422       gi|720020389|ref|XP_010262388.1|
36.36       77    49    0     190   420   88    164   9e-10        69.3
ORF_isotig12422       gi|694415950|ref|XP_009336117.1|    38.20       89    55
0     202   468   140   228   9e-10        69.3 ORF_isotig12422
gi|720020382|ref|XP_010262386.1|    36.36       77    49    0     190   420   124
200   9e-10        69.3 ORF_isotig12422       gi|723802071|ref|NP_001289777.1|
36.36       77    49    0     190   420   124   200   9e-10        69.3
ORF_isotig12422       gi|915281149|ref|XP_013305271.1|    29.52       105   73
1     145   456   117   221   9e-10        68.2 ORF_isotig12422
gi|255964729|gb|ACU44656.1|    34.44       90    59    0     151   420   111   200
9e-10       69.3 ORF_isotig12422       gi|923847316|ref|XP_013702842.1|
33.02       106   71    0     151   468   111   216   9e-10        68.6
ORF_isotig12422       gi|672183478|ref|XP_008812019.1|    39.73       73    44
0     202   420   128   200   9e-10        69.3 ORF_isotig12422
gi|720020386|ref|XP_010262387.1|    36.36       77    49    0     190   420   124
200   9e-10        69.3 ORF_isotig12422       gi|46949222|gb|AAT07467.1|
38.36       73    45    0     202   420   128   200   9e-10        68.9
ORF_isotig12422       gi|760447765|ref|XP_011400951.1|    34.44       90    59
0     151   420   131   220   9e-10        68.9 ORF_isotig12422
gi|363413051|gb|AEW23125.1|    38.36       73    45    0     202   420   128   200
9e-10       68.9 ORF_isotig12422       gi|1000945507|ref|XP_015581239.1|
33.33       90    60    0     151   420   111   200   9e-10        68.9
ORF_isotig12422       gi|410994560|gb|AFV96166.1|    38.81       67    41    0
202   402   103   169   1e-09        66.2 ORF_isotig12422
gi|923762135|ref|XP_013677346.1|    35.16       91    57    1     202   474   184
272   1e-09       68.9 ORF_isotig12422       gi|922533447|ref|XP_013598130.1|
35.16       91    57    1     202   474   184   272   1e-09        68.9
ORF_isotig12422       gi|220683835|gb|ACL80669.1|    39.73       73    44    0
202   420   128   200   1e-09       68.9 ORF_isotig12422
gi|676382929|ref|XP_009034743.1|    42.47       73    42    0     214   432   142
214   1e-09       66.6 ORF_isotig12422       gi|923885023|ref|XP_013714028.1|
39.73       73    44    0     202   420   128   200   1e-09        68.6
ORF_isotig12422       gi|764527045|ref|XP_011458022.1|    35.56       90    58
0     199   468   131   220   1e-09       68.9 ORF_isotig12422
gi|922563714|ref|XP_013610130.1|    39.73       73    44    0     202   420   130
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202  1e-09     68.6 ORF_isotig12422     gi|693266154|gb|AIS24729.1|
41.10     73   41   1    256  474  188  258  1e-09     68.6
ORF_isotig12422     gi|53760453|gb|AAU93349.1|     34.44     90   59   0
151  420  111  200  1e-09     68.6 ORF_isotig12422
gi|873224759|emb|CEM25435.1|  34.62     104  65   2    199  507  172  273
1e-09     68.9 ORF_isotig12422     gi|813208513|dbj|GAO50417.1|  37.65
85   53   0    181  435  229  313  1e-09     68.6 ORF_isotig12422
gi|470240016|ref|XP_004351977.1|  32.14     112  73   2    169  501  143
252  1e-09     68.6 ORF_isotig12422     gi|657981623|ref|XP_008382838.1|
37.08     89   56   0    202  468  119  207  1e-09     68.6
ORF_isotig12422     gi|674895889|emb|CDY36967.1|  39.73     73   44   0
202  420  128  200  2e-09     68.2 ORF_isotig12422
gi|802717229|ref|XP_012085275.1|  33.33     90   60   0    151  420  111
200  2e-09     68.6 ORF_isotig12422     gi|449445620|ref|XP_004140570.1|
33.62     116  69   2    202  549  128  235  2e-09     68.6
ORF_isotig12422     gi|470107265|ref|XP_004289969.1|  35.56     90   58
0    199  468  127  216  2e-09     68.6 ORF_isotig12422
gi|302843401|ref|XP_002953242.1|  37.65     85   53   0    181  435  125
209  2e-09     66.6 ORF_isotig12422     gi|922548834|ref|XP_013602398.1|
33.02     106  71   0    151  468  111  216  2e-09     68.2
ORF_isotig12422     gi|657961954|ref|XP_008372572.1|  37.08     89   56
0    202  468  107  195  2e-09     68.2 ORF_isotig12422
gi|966405784|ref|WP_058449190.1|  46.67     60   32   0    253  432  119
178  2e-09     65.9 ORF_isotig12422     gi|657981621|ref|XP_008382837.1|
37.08     89   56   0    202  468  128  216  2e-09     68.2
ORF_isotig12422     gi|113594574|dbj|BAF18448.1|  32.41     108  67   1
190  513  125  226  2e-09     67.0 ORF_isotig12422
gi|731346789|ref|XP_010684640.1|  33.61     119  76   2    145  501  24
139  2e-09     68.2 ORF_isotig12422     gi|676430709|ref|XP_009046015.1|
31.25     96   66   0    145  432  116  211  2e-09     66.6
ORF_isotig12422     gi|657961958|ref|XP_008372575.1|  37.08     89   56
0    202  468  108  196  2e-09     68.2 ORF_isotig12422
gi|729447939|ref|XP_010522567.1|  37.97     79   49   0    202  438  128
206  2e-09     68.2 ORF_isotig12422     gi|923864531|ref|XP_013707638.1|
35.96     89   57   0    202  468  128  216  2e-09     68.2
ORF_isotig12422     gi|922429656|ref|XP_013620881.1|  35.96     89   57
0    202  468  128  216  2e-09     68.2 ORF_isotig12422
gi|294877972|ref|XP_002768219.1|  37.36     91   53   2    208  474  132
220  2e-09     67.8 ORF_isotig12422     gi|922429654|ref|XP_013620880.1|
35.96     89   57   0    202  468  128  216  2e-09     68.2
ORF_isotig12422     gi|685383195|ref|XP_009123930.1|  35.96     89   57
0    202  468  128  216  2e-09     68.2 ORF_isotig12422
gi|985450624|ref|XP_015386247.1|  38.36     73   45   0    202  420  92
164  2e-09     68.2 ORF_isotig12422     gi|731346783|ref|XP_010684637.1|
33.61     119  76   2    145  501  110  225  2e-09     68.2
ORF_isotig12422     gi|312861917|gb|ADR10438.1|  37.50     72   45   0
205  420  129  200  2e-09     68.2 ORF_isotig12422
gi|224004956|ref|XP_002296129.1|  41.56     77   45   0    202  432  205
281  2e-09     67.8 ORF_isotig12422     gi|891585491|ref|XP_013022696.1|
27.88     104  74   1    145  456  146  248  2e-09     67.8
ORF_isotig12422     gi|985450620|ref|XP_015386245.1|  38.36     73   45
0    202  420  128  200  2e-09     68.2 ORF_isotig12422
gi|731346787|ref|XP_010684639.1|  33.61     119  76   2    145  501  110
225  2e-09     67.8 ORF_isotig12422     gi|522072901|ref|WP_020584110.1|
34.69     98   61   1    190  483  101  195  2e-09     65.5
ORF_isotig12422     gi|641841507|gb|KDO60419.1|  38.36     73   45   0
202  420  126  198  2e-09     67.8 ORF_isotig12422
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gi|674936482|emb|CDX96939.1|    35.96      89   57    0    202  468  128  216
2e-09    67.8 ORF_isotig12422    gi|922867416|gb|KOO34056.1|    33.63
113  73   1    100  432  125  237  2e-09    66.6 ORF_isotig12422
gi|551535405|ref|XP_005756498.1|    49.18      61   31    0    250  432  227
287  2e-09    67.4 ORF_isotig12422    gi|567902606|ref|XP_006443791.1|
38.36     73   45    0    202  420  128  200  2e-09    67.8
ORF_isotig12422    gi|676493293|ref|XP_009066193.1|    35.06     77   50
0    190  420  119  195  3e-09    65.9 ORF_isotig12422
gi|42742267|gb|AAS45236.1|    34.83     89   58    0    202  468  128  216
3e-09    67.8 ORF_isotig12422    gi|312072379|ref|XP_003139039.1|
36.84     76   48    0    211  438  140  215  3e-09    65.9
ORF_isotig12422    gi|407312484|gb|AFU06381.1|    32.26     124  75   3
145  513  110  225  3e-09    67.8 ORF_isotig12422
gi|657961548|ref|XP_008372367.1|    37.66     77   48    0    202  432  128
204  3e-09    67.8 ORF_isotig12422    gi|536747464|gb|ERB64445.1|
34.62     78   51    0    187  420  175  252  3e-09    66.6
ORF_isotig12422    gi|473798304|gb|EMS46513.1|    32.41     108  67   1
190  513  38   139  3e-09    67.8 ORF_isotig12422
gi|573946675|ref|XP_006655670.1|    31.40     121  77   1    151  513  113
227  3e-09    67.8 ORF_isotig12422    gi|740321886|ref|WP_038159030.1|
34.62     78   51    0    187  420  175  252  3e-09    66.6
ORF_isotig12422    gi|970053982|ref|XP_015088621.1|    33.33     90   60
0    151  420  111  200  3e-09    67.8 ORF_isotig12422
gi|757790478|ref|WP_043008233.1|    34.62     78   51    0    187  420  175
252  3e-09    66.6 ORF_isotig12422    gi|529161423|gb|AGS56990.1|
37.08     89   56    0    202  468  128  216  3e-09    67.8
ORF_isotig12422    gi|565364522|ref|XP_006348973.1|    36.36     77   49
0    190  420  69   145  3e-09    67.4 ORF_isotig12422
gi|28569702|emb|CAD68108.1|    33.33     90   60    0    151  420  75   164
3e-09    67.8 ORF_isotig12422    gi|972777756|ref|NP_001305597.1|
33.33     90   60    0    151  420  75   164  3e-09    67.8
ORF_isotig12422    gi|444891679|gb|AGE13359.1|    41.56     77   45   0
202  432  205  281  3e-09    67.4 ORF_isotig12422
gi|313483709|gb|ADR51683.1|    34.07     91   58   1    202  474  92   180
3e-09    67.8 ORF_isotig12422    gi|313483707|gb|ADR51682.1|    34.07
91   58   1    202  474  92   180  3e-09    67.8 ORF_isotig12422
gi|567185112|ref|XP_006403145.1|    35.96     89   57    0    202  468  128
216  3e-09    67.8 ORF_isotig12422    gi|568215457|ref|NP_001275308.1|
33.33     90   60    0    151  420  111  200  3e-09    67.8
ORF_isotig12422    gi|373405317|gb|AEY68568.1|    36.26     91   56   1
202  474  128  216  3e-09    67.8 ORF_isotig12422
gi|930111143|gb|KPH26997.1|    34.62     78   51    0    187  420  175  252
3e-09    66.2 ORF_isotig12422    gi|313483702|gb|ADR51680.1|    34.07
91   58   1    202  474  92   180  3e-09    67.4 ORF_isotig12422
gi|740725877|ref|WP_038511163.1|    34.62     78   51    0    187  420  175
252  3e-09    66.2 ORF_isotig12422    gi|460404089|ref|XP_004247517.1|
33.33     90   60    0    151  420  111  200  3e-09    67.8
ORF_isotig12422    gi|443690998|gb|ELT92982.1|    29.92     127  85   2
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gi|518106286|ref|WP_019276494.1|    34.62     78   51    0    187  420  175
252  3e-09    66.2 ORF_isotig12422    gi|24963931|gb|AAG22095.3|
31.11     90   62    0    151  420  25   114  3e-09    67.4
ORF_isotig12422    gi|494020115|ref|WP_006962411.1|    34.62     78   51
0    187  420  175  252  3e-09    66.2 ORF_isotig12422
gi|970053984|ref|XP_015088622.1|    33.33     90   60    0    151  420  111
200  3e-09    67.4 ORF_isotig12422    gi|723731779|ref|XP_010326652.1|
36.36     77   49    0    190  420  124  200  3e-09    67.4
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ORF_isotig12422        gi|659070971|ref|XP_008457528.1|    33.33      93     61
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200   3e-09       67.4 ORF_isotig12422        gi|685369694|ref|XP_009118681.1|
33.02      106   71     0     151   468   111   216   4e-09       67.4
ORF_isotig12422        gi|674900498|emb|CDY32521.1|    33.02      106   71     0
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gi|743869433|ref|XP_010905811.1|    35.37      82     51     1     175   420   35
114   4e-09       67.0 ORF_isotig12422        gi|923703471|ref|XP_013660298.1|
33.02      106   71     0     151   468   111   216   4e-09       67.4
ORF_isotig12422        gi|166798217|gb|ABY89660.1|    34.44      90     59     0
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gi|590715534|ref|XP_007050223.1|    33.33      90     60     0     151   420   113
202   4e-09       67.4 ORF_isotig12422        gi|218197390|gb|EEC79817.1|
32.41      108   67     1     190   513   38     139   4e-09       67.0
ORF_isotig12422        gi|747072284|ref|XP_011083048.1|    36.71      79     50
0     202   438   128   206   4e-09       67.4 ORF_isotig12422
gi|939611289|gb|KPV73315.1|    47.46      59     31     0     256   432   161   219
4e-09       67.0 ORF_isotig12422        gi|695064415|ref|XP_009420754.1|
36.99      73     46     0     202   420   128   200   4e-09       67.0
ORF_isotig12422        gi|685369692|ref|XP_009118680.1|    33.02      106   71
0     151   468   111   216   4e-09       67.0


ORF_comp2951_c3_seq1        gi|501733845|ref|WP_012629148.1|    39.31      145
81     2     115   549   44     181   5e-23       103 ORF_comp2951_c3_seq1
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181   1e-22       102 ORF_comp2951_c3_seq1 gi|740234212|ref|WP_038075400.1|
41.10      146   77     4     115   546   36     174   2e-22       101
ORF_comp2951_c3_seq1        gi|648291774|ref|WP_026072923.1|    39.44      142
78     2     121   546   46     179   4e-22       100 ORF_comp2951_c3_seq1
gi|501377640|ref|WP_012409206.1|    37.93      145   82     4     115   546   41
178   9e-22       100 ORF_comp2951_c3_seq1 gi|505020123|ref|WP_015207225.1|
37.93      145   82     3     115   546   44     181   9e-21       97.4
ORF_comp2951_c3_seq1        gi|738534441|ref|WP_036482850.1|    35.42      144
90     2     118   546   46     187   1e-20       97.1 ORF_comp2951_c3_seq1
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181   1e-20       97.1 ORF_comp2951_c3_seq1
gi|522054471|ref|WP_020565680.1|    35.81      148   76     3     121   546   54
188   2e-20       96.7 ORF_comp2951_c3_seq1
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gi|515385832|ref|WP_016877723.1|    38.19      144   79     4     121   546   46
181   3e-20       95.5 ORF_comp2951_c3_seq1        gi|689678301|emb|CEF48871.1|
37.93      145   77     3     121   546   40     174   4e-20       95.5
ORF_comp2951_c3_seq1        gi|656719681|gb|KEF42411.1|    38.78      147   80
4     112   546   22     160   4e-20       95.1 ORF_comp2951_c3_seq1
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174   6e-20       94.7 ORF_comp2951_c3_seq1
gi|763312982|ref|WP_044171432.1|    37.32      142   82     2     121   546   38
172   7e-20       94.7 ORF_comp2951_c3_seq1
gi|826018244|ref|WP_047165229.1|    37.93      145   80     3     115   546   33
168   1e-19       94.4 ORF_comp2951_c3_seq1
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gi|567902610|ref|XP_006443793.1|    30.20        202   119   6     115   702   22
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Horizontal gene transfer of phytochelatin synthases from bacteria to extremophilic green algae; Microbial Ecology; Olsson S*, Penacho V, Puente-Sánchez F, Díaz S, Aguilera A; * corresponding author, Department of Agricultural Sciences, P.O. Box 27, 00014 University of Helsinki, Finland, sanna.olsson@helsinki.fi

Online resource 5 Introns in the predicted PCS genes from C. acidophila and D. acidophila. There are three spliceosomal introns in CaPCS2, four in CaPCS1 and three in DaPCS1. The introns are either the most common GT-AG splice site or variations of it.

Gene	Intron 5' end	Intron 3' end
CaPCS2	GAG | GTGAGG	TAG | G
	CAG | GTGTGT	TAN | G
	CAA | GTAAGG	TAG | G
CaPCS1	AAG | GTAAGC	CAG | G
	CAG | GTACTT	CAG | C
	CAG | GTGATG	CAG | A
	CAG | GTGAGA	TAG | G
DaPCS1	CTG | GTGGGT	GAG | G
	CAG | GCGAGC	CAG | A
	CAA | GTGCGG	CAG | G

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* corresponding author, Department of Agricultural Sciences, P.O. Box 27,
00014 University of Helsinki, Finland, sanna.olsson@helsinki.fi.

Online Resource 6 Isoforms of component comp17619_c0 coding for the CaPCS1
gene in Chlamydomonas acidophila

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Horizontal gene transfer of phytochelatin synthases from bacteria to extremophilic green algae; Microbial Ecology;

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* corresponding author, Department of Agricultural Sciences, P.O. Box 27, 00014 University of Helsinki, Finland, sanna.olsson@helsinki.fi.

Online Resource 7 Isoforms of component comp11852_c0 coding for the CaPCS2 gene in Chlamydomonas acidophila

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ATCTTGGTGGGTACCAGTTTCAGAGCTGTTTGCAGCAGTCCTTCCAAGAGATCCGTCAGATGTAGAAGATCCAG
GAAGGG
GCCTCGTGTTTGTCTGGGCTCAAACATCAGATGGGACACTGCAACCTTCCTTAGGAAGGGAGTTCTTCTCATCA
GGGCTG
GACGATGGCATGCCTACTTATGACTTTTGCGATGATGTGCACTCAGCATTTGGTCTTGAGGTGCTGTCCTTTTG
GCTGGG
ATTGGGATATTGTGCTTGCCTCTGTTGTTGTTTATGTCAACTGGAGTTATCAAGGTCAGCTACCAGGCACCCTC
TTGGGG
GATCGTTGGAATAAGAAACTTCTGGGCTAGGGACCTGAGATGTTCAATCAAAGTGCAGGGGCATAAACAGATTG
CTACCG
AATTCCATCAAGACCAGCAGCGAAGAGCAGGCGCTGACTATGAGTTATCTGCAACATCATTTGATGACACTGAA
TTATCA
CCACTGTGCGCTGTTAGATGAACTGAAGCGGTTCTTCTGATTCCTTTGCATGTCAGCTTTTAACGTTCTTGACA
AAACTG
TTCCTGATGTCATCATGCGTGCCCTCGGTTTTTACATGAGCACAAGCTCTTCGAATCATCAATGTAAAGAGTAC
ATTGAA

TAGCATAGATAGGAAAACTCACAAAAATTGAACATATTTTTTGGATAATCAGGCTATCGAGAGCAACTGGTGGA
AAAAGT
TATACATACTATCTCTGTCTCAATACAACAACATTTTTATGAAGAAACAGAAGCCTTGTAAACATAGAAAGCAG
GAAATG
TTCATCATCTCCAATTAATTCTGAGGTCTGATGTAGCAAGCTTCACCAAAGAGGTAAGAACATGCAGTTCCAAG
GATTCT
GTTCTGACCACTCTCCTGCTCAGGTTTTGATTTTCATGGCCTCTGCCACTGGAGAAGCGACAGCTCCGTTGTTA
TTGCTG
GTAGCGTTCAGCTTGTTTGACACCTCATCAATGGAGGCGTTGAGCTGTGCAATCTTATCAAACAGATCCTGTTT
GGTCTG
TTCAGGGTCCTTCACCTCATCCTCAAGAAATCTTGGAAGTTTCAGGCGTTGATCATCGCCCAAGAGCGCTTTAG
AGATCT
GCAGAGAACAAAATAAAACCGCATGGTTCACACACCGCGGGAAAGAACGGCACATGGAATGAATGACATGGCAT
GTTAAT
AAGCACAGTTAAATAAATCTCCATGTTGAGGTATCTCAATTTCAAGATTATGATGTAGGTAACTGCGTTTCAGC
AGTTAT
TCAAAAAATTAGTAGGCCTACATGGCTATTGCACATGTGTATGTGATGCCTTTGCAATTCATCATACCTGCGGG
GCAAAT
ATGAATCCCAGAGTACCGAAAACAACACCCCCAAGGACAAATCCAGAGACGAAGCTGTCACTTCGGTCACTATT
CTCAGC
TCTAGCTACGATATTCCTCTCTTTTCTTAACGTTCTTGTCACAGGAGTATGCTGTATCGGAGAGCTTTCGCGAC
AATGGA
AAGATCCCGAACATAAGTTCCCTGTCCTGCTTGTTAACATTCTTGAACACTACTATGCGTATCGTTAATCAGGA
GGACTC CGTGTAAGCGTTAAAGGGCAATCATGCAGCCACGTAAG

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Online Resource 8 PCR products amplified in *Dunaliella acidophila* strain RT5 using gradient annealing run on 1% agarose gel (1X TBE). The gel electrophoresis shows amplification of products sized 1198 bp and 542 bp and several non-specific amplification products. The intensity of the non-specific bands depends on the used annealing temperature and PCR program. Wells 1: Ladder 1kb (Promega), marker sizes from 250 kb to 10000 kb, well2: annealing 52-55°C. Well 3: annealing 55-60°C.

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Online Resource 9 Phylogram based on the novel phytochelatin synthase genes and the most similar genes obtained with a BLASTx search against NCBI's databases. The tree represents the majority consensus of trees sampled after stationarity in the Bayesian analysis. PP values from the Bayesian inference > 0.50 are indicated above each branch. The amino acid alignment was 383 aa long.

Taxon	Classification
Trichodesmium erythraeum	Cyanobacteria
Synechococcus sp2	Cyanobacteria
Leptolyngbya sp	Cyanobacteria
Coleofasciculus chthonoplastes	Cyanobacteria
Moorea producens	
Cyanothece sp1	Cyanobacteria
Cyanothece sp3	
Cyanothece sp2	Cyanobacteria
Microcoleus vaginatus	Cyanobacteria
Chamaesiphon minutus	Cyanobacteria
Crinalium epipsammum	
Chroococcidiopsis thermalis	
Calothrix sp	
Nostoc PCC 7107	
Anabaena variabilis	Cyanobacteria
Anabaena sp	
Cylindrospermum stagnale	
Nostoc punctiforme	
Prochlorococcus marinus	
Synechococcus sp1	Cyanobacteria
Cyanobium gracile	
Methylosinus trichosporium	
Burkholderia oklahomensis	Bacteria
Burkholderia pseudomallei1	
Burkholderia pseudomallei2	
Aureococcus anophagefferens2	Chromalveolata
Burkholderia xenovorans	Bacteria
CaPCS2	
DaPCS1	Green algae
DaPCS1	
Monosiga brevicollis	Choanoflagellate
Coxiella burnetii	Bacteria
Francisella sp2	Bacteria
Saccharophagus degradans	Bacteria
Strongylocentrotus purpuratus	Metazoa
Thalassiosira oceanica	Chromalveolata
Polynucleobacter necessarius	Bacteria
Rhodobacterales bacterium	Bacteria
Thalassosira profundimaris	Diatom
Shewanella sediminis	
Pantoea stewartii	
Erwinia amylovora	Bacteria
Erwinia pyrifoliae	
Aureococcus anophagefferens1	
Phaeodactylum tricornutum	Chromalveolata
Thalassiosira pseudonana	
Rhizopus delemar	Fungi
Melampsora laricipopulina	Fungi
Puccinia graminis	
Naegleria gruberi2	Excavata
Schizosaccharomyces japonicus	Fungi
Cyanidioschyzon merolae	Red algae
Schistosoma mansoni	Metazoa
Albugo laibachii	
Phytophthora infestans	Fungi
Phytophthora sojae	
Nematostella vectensis3	
Nematostella vectensis1	
Nematostella vectensis2	
Naegleria gruberi1	Metazoa
Brugia malayi	
Wuchereria bancrofti	
Caenorhabditis elegans1	
Caenorhabditis remanei	
Volvox carteri1	
Chlorella variabilis	
Coccomyxa subellipsoidea	Green algae
CaPCS1	
Chlamydomonas reinhardtii	
Volvox carteri2	
Pteris vittata	
Selaginella moellendorffii	
Lotus japonicus LjPCS3	
Lotus japonicus LjPCS2 7R	
Ceratophyllum demersum	Streptophyta
Salix	
Lathyrus sativus	
Lotus japonicus	
Avicennia germinans	
Arabidopsis thaliana	Land plants
Hirschfeldia incana	
Aegilops tauschi	
Oryza sativa2	
Cynodon dactylon	
Oryza sativa1	
Hordeum vulgare	
Triticum aestivum	
Triticum urartu	