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Insights on the genetic repertoire of the coral *Mussismilia braziliensis* endosymbiont *Symbiodinium*

Arthur W. Silva Lima[1] · Luciana Leomil[1,2] · Louisi Oliveira[1,2] · Tooba Varasteh[1,2] · Janelle R. Thompson[3] · Mónica Medina[4] · Cristiane C. Thompson[1,2] · Fabiano L. Thompson[1,2]

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Abstract

Reef-building corals form a symbiotic association with photosynthetic dinoflagellates of the family Symbiodiniaceae. This symbiosis is crucial for the maintenance of coral reefs. In this work, we evaluate the effect of light conditions on the transcriptomic response of *Symbiodinium* CCMR0100 (ITS2 type A4), isolated from the Southwestern Atlantic Ocean endemic *Mussismilia braziliensis*. We obtained a total of 36,224 transcripts (N50 = 1007 bases, mean GC = 55.7%; ~25 Gb of assembled bases). We observed ecologically relevant transcripts encoding i. the complete antioxidant enzymatic system, ii. the recently described algal dimethylsulfoniopropionate (DMSP) lyase, and iii. The Mycosporine-like aminoacids (MAA) biosynthesis pathway. Cultures maintained in dark and light conditions yielded different transcriptomic profiles, and 48 transcripts were differentially expressed between these treatments. Expression of cytochrome P450 was inhibited by light, suggesting that endoplasmic reticulum monooxygenase activity might play a role in light-independent coral bleaching. Light conditions also triggered the induction of transcripts associated to chromatin condensation and mitosis, consistent with the light dependent progression of Symbiodiniaceae cell cycle. The repression of transcripts associated to the phosphatidylinositol (PI) signaling pathwaysuggests this pathway shall be related to light-induced morphological changes in Symbiodiniaceae cell.

Keywords Symbiodiniaceae · *Symbiodinium* · Transcriptome · Mycosporine-like aminoacids (MAA) · Differential expression

1 Introduction

Reef-building corals are mixotrophic organisms that rely on their endosymbiotic photosynthetic dinoflagellates for energy production (Muller-Parker et al. 2005; Baker 2003). Initially described as a single species, these coral endosymbionts are now recognized as members of the diverse family Symbiodiniaceae (LaJeunesse et al. 2018). Symbiodiniaceae species in the *Symbiodinium, Breviolium, Cladocopium* and *Durusdinium* genera are commonly found in association with corals (Baker 2003; LaJeunesse et al. 2018). Despite this evolutionary diversification, recent climate change has led to increased coral bleaching (Carpenter et al. 2008), a stress symptom in which dinoflagellate cells are expelled or digested by the coral host (Weis 2008). Coral bleaching is caused by an excessive production of reactive oxygen species (ROS) in Symbiodiniaceae, triggered by the synergistic effect of high seawater temperature and irradiance (Warner et al. 1999; Lesser 2006; Roberty et al. 2014).

The *Symbiodinium* genus (formerly clade A) is comprised by symbiotic, opportunistic and free-living species (Hansen and Daugbjerg 2009; LaJeunesse et al. 2015; Lajeunesse et al. 2018). Species in the *Symbiodinium* genus are highly prevalent in culture collections (Santos et al. 2001; Silva-Lima et al. 2015) and dominate free living Symbiodiniaceae communities in oceanic water samples (Yamashita and Koike

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✉ Arthur W. Silva Lima
arthurwlima@gmail.com

✉ Fabiano L. Thompson
fabianothompson1@gmail.com

[1] Laboratório de Microbiologia, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Av Carlos Chagas Filho 373, CCS, Sala A3-202, Rio de Janeiro CEP: 21.941-599, Brazil

[2] Sage/Coppe, Centro de Gestão Tecnológica–CT2, Universidade Federal do Rio de Janeiro, Rua Moniz de Aragão, no.360 - Bloco 2, Ilha do Fundão - Cidade Universitária CEP: 21.941-972, Brazil

[3] Singapore-MIT Alliance for Research and Technology, Center for Environmental Sensing and Modeling, Singapore, Singapore

[4] Pennsylvania State University, 324 Mueller Laboratory, University Park, PA, USA

2013; Decelle et al. 2018). In culture conditions, the Symbiodiniaceae exhibit a two-stage cell cycle with a coccoid and a mastigote (motile) stage. Cell division typically occurs in dark conditions, with cells in the coccoid stage, while the occurrence of mastigote cells is higher at the onset of light exposure (Fitt and Trench 1983, Wang et al. 2008, Sorek et al. 2004, Fujise et al. 2018). Light exposure also has an effect on the organization of the cytoskeleton (Villanueva et al. 2015) and of the extracellular matrix structure (Xiang et al. 2015).

The *Symbiodinium* genus has a pantropical distribution and is often associated to shallow waters, but some species can also be observed in deep reefs (Santos and LaJeunesse 2006; Silva-Lima et al. 2015; Picciani et al. 2016; LaJeunesse et al. 2018). Naturally occurring in high and low-irradiance habitats, some *Symbiodinium* species are among the most resistant Symbiodiniaceae to coral bleaching (Rowan et al. 1997; Baker 2001; Grottoli et al. 2014; Díaz-Almeyda et al. 2017). Adaptations proposed to explain the higher occurrence of *Symbiodinium* spp. in high-irradiance habitats include the differential dissipation of light energy from photosynthetic pigments (Reynolds et al. 2008), lipid composition of thylakoid membranes (Tchernov et al. 2004; Díaz-Almeyda et al. 2011), de novo synthesis of chloroplast proteins (Takahashi et al. 2013), increased ROS scavenging (McGinty et al. 2012; Roberty et al. 2016) and the production of mycosporine-like aminoacids (MAA) (Banaszak et al. 2000).

The production of MAA, UV-absorbing molecules, is an important energy dissipation mechanism in marine organisms (Rosic and Dove 2011). MAA concentrations are often higher in shallow waters, where its production is induced by UV radiation, but also by photosynthetic active radiation (Oren and Gunde-Cimerman 2007). Besides its photoprotection role, MAAs might have diverse functions in marine organisms (Oren and Gunde-Cimerman 2007), including ROS scavenging properties, leading to a potential role alleviating photo-oxidative damage on coral bleaching (Yakovleva et al. 2004; Gao and Garcia-Pichel 2011). Both the production of MAA and the genes encoding for MAA biosynthesis have been observed in *Symbiodinium* (Banaszak et al. 2000; Shoguchi et al. 2018). Contrastingly, production of MAAs have not been observed in other Symbiodiniaceae genera, including *Brevolium*, *Cladocopium* and *Fugacium* (Banaszak et al. 2000). Moreover, genomic analysis indicates that the biosynthesis pathway is absent or eroded in non-MAA producing genera (Liu et al. 2018; Shoguchi et al. 2018).

RNA-seq analysis indicated that light conditions induce the transcripts associated to chromatin condensation and cellular differentiation (Xiang et al. 2015). Cellular differentiation and chromatin condensation are also affected by thermal stress (Levin et al. 2016; Gierz et al. 2017). Thermal stress induces the transcription of molecular chaperones and ROS scavangers in bleaching-resistant Symbiodiniaceae (Baumgarten et al. 2013; Levin et al. 2016; Gierz et al. 2017). Interestingly, thermal stress has been associated to cell cycle arrestment in Symbiodiniaceae, both in culture (Mclenon and diTullio 2012; Fujise et al. 2018) and in hospite (Dubousquet et al. 2016).

Recently, genome sequences have been developed for Caribbean and the Pacific Symbiodiniaceae taxa (Shoguchi et al. 2013; Lin et al. 2015; Aranda et al. 2016; González-Pech et al. 2017; Shoguchi et al. 2018). However, there is no information on the genetic repertoire of Symbiodiniaceae from the Southwestern Atlantic Ocean (SAO). Coral reefs in the SAO are characterized by restricted gene flow and a high level of endemism (Nunes et al. 2009). Moreover, water turbidity is high compared to other reef systems worldwide (Suggett et al. 2012), and corals have a relatively low bleaching susceptibility (Teixeira et al. 2019).

In this study, we present the first transcriptomic analysis of *Symbiodinium* CCMR0100 (ITS2 type A4) from the SAO and evaluate the hypothesis that dark and light conditions modulate transcriptomic response of cultured *Symbiodinium*. Our aim was to provide a transcriptomic profile of *Symbiodinium* CCMR0100 and, identify transcriptional processes associated with the effect of light on the physiology of this Symbiodiniaceae strain. *Symbiodinium A4* is a widespread, generalist symbiont found from tidal pools to down to 20 m deep reefs in the Vitoria Trindade Sea Mounts (Santos and LaJeunesse 2006; Silva-Lima et al. 2015; Picciani et al. 2016). *Symbiodinium A4* is associated with a wide range of hosts (LaJeunesse 2001), and was found to be resistant to thermal bleaching, both in culture (Díaz-Almeyda et al. 2017) and in association with *Porites divaricata* (Grottoli et al. 2014).

2 Material and methods

2.1 Culture origin and maintenance

Symbiodinium CCMR0100 was isolated from *Mussismilia braziliensis* corals, the main coral reef builder of the Abrolhos reefs, in the Southwest Atlantic Ocean. Detailed isolation methods, culture conditions and isolate identification were described previously (Silva-Lima et al. 2015). Briefly, isolation was performed by single-cell sorting on a flow cytometer and the resulting clonal culture identified by ITS2 sequencing. Before the experiments, culture was maintained on sterile F/2 media at 24 °C and 70 umol m^{-2} s^{-1}, with a 14/10 dark/light cycle (Keller et al. 1987). Artificial illumination was provided by fluorescent lamps, yielding light in the visible range spectra. Originally described as isolate 043D10, the strain is now deposited in the microorganism culture collection at UFRJ, under accession number CCMR0100.

2.2 Transcriptome experiment

For the transcriptome experiment, a 200 ml batch culture of *Symbiodinium* CCMR0100 was maintained in late exponential growth by weekly renovation of three quarters of the culture media. Transcriptomic analysis was performed with densities of 2.5×10^5 cells/ml and cells were maintained at 24 °C throughout the experiment. At the onset of the experiment, the batch culture was divided in four 40 ml aliquots and exposed to two different treatments, with two replicates per treatment. A dark treatment where replicates were sampled after 24 h without illumination ('Dark'), and a treatment where replicates were sampled after 6 h of illumination at 70 umol m^{-2} s^{-1} ('Light'). All samples were collected 24 h after the aliquots were prepared, at 14:00 (Fig. S1; supplementary material). For both treatments, a mix of antibiotics was used to control for bacterial growth two days before sampling cells (Polne-Fuller 1991; Silva-Lima et al. 2015).

Algal cells were collected by centrifugation (900 x g for 5 min), the supernatant was discarded and the pellet was instantly frozen in liquid nitrogen. Total RNA was extracted with TRIzol (Invitrogen) and purified on columns (Qiagen RNeasy mini kit), according to the manufacturer's instructions. cDNA was synthesized from poly-A mRNA with a SMARTer PCR synthesis kit (Clontech) and cDNA libraries were prepared using NexteraXT (Illumina). Libraries were quantified using 7500 Real Time PCR (Applied Biosystems) and KAPA Library Quantification Kit (Kapa Biosystems) and the fragment size distribution was checked using 2100 Bioanalyzer (Agilent) and High Sensitivity DNA Kit (Agilent). Paired-end sequencing (2 × 250 bp) was performed on MiSeq (Illumina).

Pairs of reads were merged with *PEAR* (Zhang et al. 2014), and *cutadapt* was used to remove either SMARTer or Illumina adapter sequences (Martin 2011). Sequences with ambiguous bases and low mean quality (phred <25) were discarded while poly-A/T and low quality tails were trimmed with *prinseq* (Schmieder and Edwards 2011). The resulting high quality reads from the replicates were combined in a single "cross-assemble" with *Trinity* (Grabherr et al. 2011). Ribosomal RNA reads were filtered with bowtie2 (Langmead and Salzberg 2012), mapping against the truncated SSU/LSU Silva database, version 119 (Quast et al. 2013). Blastn analysis (e-value 10e-3) of the reads mapping the SILVA database indicated the occurrence of bacterial rRNA fragments, from the families Rhodospirillaceae (Alphaproteobacteria), Flammeovirgacea (Cytophagales) and Flavobacteriales (Flavobacteria), indicating bacterial DNA contamination. To remove residual bacterial DNA fragments, assembled contigs were retained only if they presented less than 65% amino acid identity to bacterial sequences and more than 80% nucleotide identity to available Symbiodiniaceae mRNA databases (*Cladocopium C3* [Leggat et al. 2007], *Breviolium minutum* – *B1* [Bayer et al. 2012, Shoguchi et al. 2013], *Symbiodinium microadriacticum* – *A1* [Bayer et al. 2012, Voolstra et al. 2009]). Completeness of the final *Symbiodinium* CCMR0100 transcriptome assembly was assessed by BUSCO analysis, with the eukaryotic set of single copy genes (Simão et al. 2015). Uniqueness of the assembled mRNA contigs were evaluated with cdhit-est (Fu et al. 2012), and high quality mRNA reads were mapped back to the final *Symbiodinium CCMR0100* transcriptome with *bowtie2* (Table S1).

2.3 Identification of transcripts involved in MAA production, antioxidant activity, and transcripts with site-specific transcription factors

The assembled transcriptome was annotated by BLASTx searches against the Uniprot (SwissProt, TrEMBL) and NCBI-nr databases, with an e-value threshold of 10e-5. In case of homology with multiple databases, transcript annotation followed the priority order, based on the curation level of each database: SwissProt – TrEMBL – NCBI-nr. Gene ontology (GO) assignments were performed by local mapping with the UniProt-GOA database (ftp://ftp.uniprot.org/pub/databases/uniprot/current_release/knowledgebase/idmapping/).

Transcripts associated to the MAA biosynthesis pathway were identified by blastx analysis with the proteins described in Shoguchi et al. (2018), with an e-value cutoff of 1e-10. These genes code for the enzymes dimethyl 4-deoxygadusol (DDG), *O*-methyltransferase (*O*-MT), ATP-grasp and a D-alanine-D-Alanine ligase-like protein (D-ala). A fusion of DDG and O-MT genes is observed in dinoflagellates (Waller et al. 2006; Shoguchi et al. 2018). Results were further validated by Uniprot annotations (Table 1). Differences in transcription levels of these transcripts between Dark and Light treatments (see below, *Differential expression analysis*) are also presented in Table 1.

Identification of contigs with sequence specific transcription factor (TF) domains was based on Pfam models compiled in Ryu et al. (2011) (Table S2). We followed the approach of Bayer et al. (2012) for quantification of TF domains: sequences were counted only once if multiple isoforms of the gene carried the same domain or if a sequence contained repetitions of the same domain. Transcripts involved in the antioxidant response were identified with HMMER searches against the list of Pfam models described in Bayer et al. (2012) (Table S3), including genes in the enzymatic antioxidant system (superoxide dismutases: PF00080.18, PF00081.20, PF02777.16, and PF09055.9; catalase: PF00199.13; and peroxidase: PF00141.21) and genes associated with redox-signalling systems (thioredoxin: PF00085.18 and glutaredoxins: PF00462.22 and PF04399.11). Given the potential importance of DMSP in oxidative stress (Sunda et al.

Table 1 Full MAAs biosynthesis pathway was observed in *Symbiodinium* CCMR0100 transcriptome. Functional annotation (gene name, description and accession number) derived from best hit at the Uniprot or NCBI nr databases. Fusion of the DDG and O-MT genes in dinoflagellates is evidenced by the observed annotation

Transcript_id	Gene	Description	Acc Number	logFC	p value
comp441_c0	ATP-grasp	Uncharacterized protein	K7W7U9 *	1.97	0.89
comp4981_c0	D-ala	Dapdiamide A synthase	E2JA31	2.39	0.74
comp15193_c0	D-ala	Alanine--anticapsin ligase	P39641	1.39	0.81
comp6287_c0	D-ala	Uncharacterized protein y4rH	P55641	0.91	0.83
comp6242_c0	D-ala	Argininosuccinate lyase 2	Q981V0	0.40	1.00
comp5318_c0	D-ala	UDP-N-acetylmuramate--L-alanine ligase	B6IRG1	–	–
comp5549_c0	D-ala	D-alanine--D-alanine ligase	A5FGN3	–	–
comp30976_c0	D-ala	Dapdiamide A synthase	E2JA31	–	–
comp3496_c0	DDG, O-mt	Catechol O-methyltransferase	Q5H879	0.58	0.99
comp6235_c0	DDG, O-mt	3-dehydroquinate synthase	A4J3A3	0.46	0.98
comp8250_c0	DDG, O-mt	Branched-chain-amino-acid aminotransferase	P54689	0.32	1.00
comp8483_c0	DDG, O-mt	Pentafunctional AROM polypeptide	A3LSZ2	0.26	1.00
comp3098_c0	DDG, O-mt	3-dehydroquinate synthase	Q2RMW0	–	–
comp3184_c0	DDG, O-mt	Catechol O-methyltransferase	A7MBI7	–	–
comp7371_c0	DDG, O-mt	Chloroplast 3-dehydroquinate synthase/ O-methyltransferase fusion	Q15BR2 *	–	–
comp7767_c0	DDG, O-mt	Chloroplast 3-dehydroquinate synthase/ O-methyltransferase fusion	Q15BR2 *	–	–
comp9581_c0	DDG, O-mt	Chloroplast 3-dehydroquinate synthase	Q15BR0 *	–	–
comp10417_c0	DDG, O-mt	3-hydroxyanthranilate 3,4-dioxygenase	Q4UT95	–	–
comp11298_c0	DDG, O-mt	Peptide chain release factor 3	Q47CH1	–	–
comp12362_c0	DDG, O-mt	Chloroplast 3-dehydroquinate synthase/ O-methyltransferase fusion	Q15BR2 *	–	–
comp12919_c0	DDG, O-mt	Chloroplast 3-dehydroquinate synthase/ O-methyltransferase fusion	Q15BR2 *	–	–
comp13440_c0	DDG, O-mt	3-dehydroquinate synthase	K9YA00 *	–	–
comp21784_c0	DDG, O-mt	Catechol O-methyltransferase	Q5H879	–	–
comp31004_c0	DDG, O-mt	3-dehydroquinate synthase	A6LEZ7	–	–
comp47405_c0	DDG, O-mt	Putative O-methyltransferase YrrM	O32036	–	–
comp59503_c0	DDG, O-mt	3-dehydroquinate synthase	A9MMD9	–	–

DDG and O-mt Accession numbers for the NCBI nr database are marked by '*'. *LogFC* - Change in expression in the Light compared with the Dark treatment (log-transformed, base 2); *p value* - adjusted for differential expression analysis, after false-discovery rate correction. Transcripts with less than fifteen reads mapping back to assembled contig were excluded from DE analysis, and are indicated by an '-' on DE results

2002), we further included the hmm model for bacterial DMSP-lyases (PF16867). Additionally, transcripts that were annotated (BLASTx at the SwissProt database) as the recently described algal DMSP-lyase gene were included in the comparison (Alcolombri et al. 2015). Nucleotide contigs from the transcriptome were translated into amino acids in the 6 frames with Transeq (EMBOSS) and HMMER searches were carried out with an e-value threshold of 10e-6 (Eddy 2003).

2.4 Transcript expression levels

The final *Symbiodinium* CCMR0100 mRNA pool was mapped back to the transcriptome with *bowtie,* and RSEM was used to quantify the expression levels of each transcript, resolving ambiguously-mapping reads (Li and Dewey 2011). The set of 50 most expressed contigs was selected based on the geometric mean of TPM (Transcripts per million) levels among replicates, representing a single *Symbiodinium* CCMR0100 transcriptomic profile for both Light and Dark samples. Clustering of the samples was analyzed with a dendogram of ln-transformed TPM values, with distance based in *Pearson* correlations between each pair of samples (R Core Team 2016).

2.5 Differential expression analysis

Analysis of differentially expressed (DE) genes between the Dark and Light treatments were conducted using edgeR software (Robinson et al. 2010). To avoid the possible influence of residual DNA sequences on DE results,

reads mapping at bacterial contigs were discarded, and reads with a GC content lower than 45% were filtered out of the *DE* analysis. This prevented the occurrence of unannotated bacterial sequences but also of sequences from *Symbiodinium* chloroplast and mitochondrial genomes. Massive transfer of genes to the nuclear genome led to a lower GC content and to an extremely reduced gene content in the chloropolast (14 genes, GC: 36.8% Barbrook et al. 2014; Mungpakdee et al. 2014) and mitochondrial genomes in Symbiodiniaceae (3 genes, GC: 35.7%, Shoguchi et al. 2015). The varying number of mitochondria per cell made this conservative approach necessary to avoid artifacts in DE gene calling. The final mRNA pool was used to quantify *Symbiodinium* CCMR0100 contig expression levels with RSEM, and contigs with a minimum support of 15 reads were selected for analysis of differential gene expression. Treatments were compared after TMM normalization and DE genes were called at a 0.05 significance level and a 10% false-discovery rate with *Benjamini-Hochberg* correction (Robinson et al. 2010). GO enrichment analysis on differentially expressed transcripts was performed with Fisher's exact test at a 0.05 significance level and with the 'weight01' algorithm to account for the GO topology, using the bioconductor package topGO (Alexa and Rahnenfuhrer 2010). Enriched GO terms were retained if at least 2 transcripts were found among the DE transcripts.

Gene set enrichment analysis was performed in the three sets of transcripts identified (MAA biosynthesis, antioxidant activity and transcription factors) with geneSetTest, with 10.000 permutations (Ritchie et al. 2015). geneSetTest is a competitive approach to identify trends in expression level of a set of transcripts, comparing the distribution of change in expression of the set of genes with the distribution observed in a random set (Simpson et al. 2008).

The assembled transcriptome and raw read files supporting the analysis in this article are available in NCBI BioProject, Accession PRJNA388088. https://www.ncbi.nlm.nih.gov/bioproject/388088

3 Results

The *Symbiodinium* CCMR0100 transcriptome assembly yielded 36,224 transcripts, with a N50 value of 1007 bases and a mean GC content of 55.7% in a total of almost 25 Gb of assembled bases (Table S1). Almost half of these transcripts was annotated with either the SwissProt, TrEMBL or the NCBI-nr database (17,808 transcripts; 49.2%), while 10,847 transcripts were annotated with the SwissProt database (29.9%). Of the 36,224 transcripts, 44 possessed TF domains (0.12%, Table S2). We observed a diverse array of transcripts coding for proteins with antioxidant domains, the most common were peroxidases, glutaredoxin and the superfamily thioredoxin. Despite there were no hits to the catalase domain, two genes were annotated as catalase-peroxidase (katG, Table S3). Two of the four genes with the Sod_Ni domain were annotated as ubiquitins, indicating the occurrence of genes encoding Sod_Ni and ubiquitin domains also in *Symbiodinium* CCMR0100 (Table S3, Bayer et al. 2012). No transcripts with the bacterial type DMSP-lyase domain were observed, but four transcripts homologous to the algal DMSP-lyase were observed (Table S3).

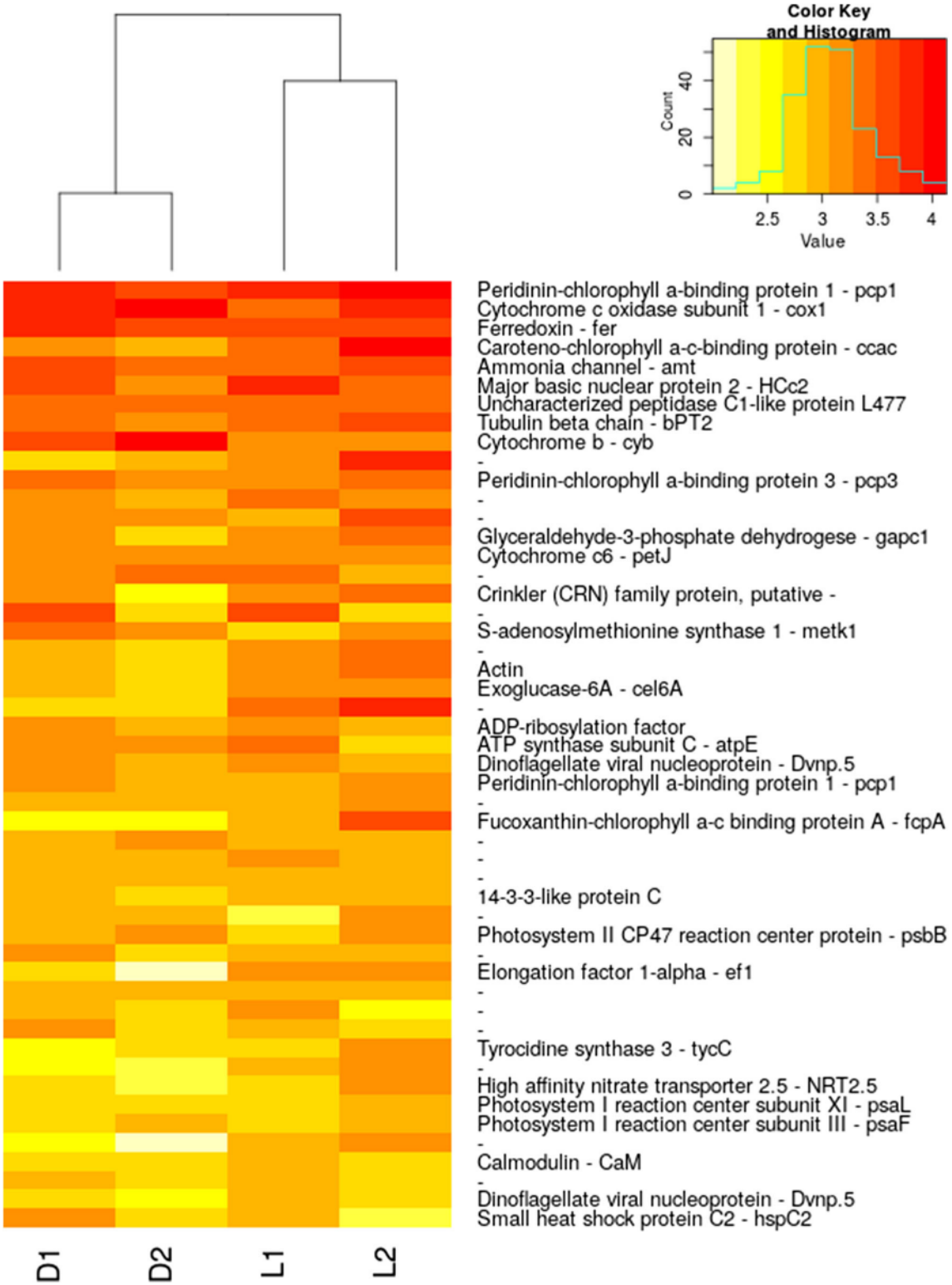
The *Symbiodinium* CCMR0100 transcriptome possesses a complete set of transcripts required for MAA biosynthesis (Table 1). Even with the stringent e-value cutoff of 1e-10, we observed a homolog of the ATP-grasp transcript, 7 homologs of D-ala D-ala ligase and 18 homologs of the DDG-Omt fusion transcript.

3.1 Transcriptome profile and differential expression analysis

The cluster analyses using the set of the 50 most abundant *Symbiodinium* CCMR0100 transcripts indicates that the *Light* and the *Dark* treatments form separate clusters (Fig. 1). The profile of the 50 most expressed transcripts in both *Light* and *Dark* samples is largely dominated by transcripts associated to chloroplast (*atpE, ccac, fcpA, fer, gapc1, HCc2, pcp1, pcp3, petJ, psaF, psaL, psbB*) and mitochondrial proteins (*cox1, cyb*), mostly related to energy generation in electron-transfer chains. Transcripts encoding the cytoskeleton proteins tubulin and actin were highly expressed, as transcripts encoding histone-like nuclear proteins (*HCc2, DNVP.5*). Nitrogen metabolism is represented by the high expression of ammonia and nitrate transporters and transcripts encoding enzymes associated to amino acid synthesis (*metk1, tycC*).

A total of 48 differentially expressed (DE) transcripts (0.1% of total) were observed between the *Light* and *Dark* treatments (Table 2), and 25% of these were annotated (12/48). Enriched GO terms were mostly related to the ER membrane and nuclear organization (Table S4). Five transcripts coding for nucleic acid binding proteins were differentially expressed, and two related to lipid signaling pathway (Table 2).

Gene set enrichment analysis indicate that neither the set of transcripts with antioxidant domains ($p = 0.190$) nor the set of transcripts with TF domains ($p = 0.233$) were associated with light or dark conditions. Conversely, the set of transcripts of the MAA biosynthesis pathway was positively associated with the light condition ($p = 0.011$).


Color Key and Histogram
Count
0
20
40
2.5
3
3.5
4
Value
Peridinin-chlorophyll a-binding protein 1 - pcp1
Cytochrome c oxidase subunit 1 - cox1
Ferredoxin - fer
Caroteno-chlorophyll a-c-binding protein - ccac
Ammonia channel - amt
Major basic nuclear protein 2 - HCc2
Uncharacterized peptidase C1-like protein L477
Tubulin beta chain - bPT2
Cytochrome b - cyb
-
Peridinin-chlorophyll a-binding protein 3 - pcp3
-
-
Glyceraldehyde-3-phosphate dehydrogese - gapc1
Cytochrome c6 - petJ
-
Crinkler (CRN) family protein, putative -
-
S-adenosylmethionine synthase 1 - metk1
-
Actin
Exoglucase-6A - cel6A
-
ADP-ribosylation factor
ATP synthase subunit C - atpE
Dinoflagellate viral nucleoprotein - Dvnp.5
Peridinin-chlorophyll a-binding protein 1 - pcp1
-
Fucoxanthin-chlorophyll a-c binding protein A - fcpA
-
-
-
14-3-3-like protein C
-
Photosystem II CP47 reaction center protein - psbB
-
Elongation factor 1-alpha - ef1
-
-
-
Tyrocidine synthase 3 - tycC
-
High affinity nitrate transporter 2.5 - NRT2.5
Photosystem I reaction center subunit XI - psaL
Photosystem I reaction center subunit III - psaF
-
Calmodulin - CaM
-
Dinoflagellate viral nucleoprotein - Dvnp.5
Small heat shock protein C2 - hspC2
D1
D2
L1
L2

◀ **Fig. 1** Heatmap of the most expressed genes in the *Symbiodinium* CCMR0100 transcriptome. Expression values for the 50 most expressed transcripts, based on the geometric mean of TPM levels of all four samples. Clustering of the samples based on the distance (1-correlation) of ln-transformed TPM values between samples. Transcript description and abbreviations assigned based on Uniprot descriptions. Transcripts without annotation to common databases are marked with '-'. Replicates are assigned by numbers in Light (L1, L2) and Dark (D1, D2) treatments

4 Discussion

4.1 Insights into the biology of *Symbiodinium* from Abrolhos

The *Symbiodinium* CCMR0100 transcriptome presented in this study is the first large scale genomic resource for *Symbiodinaceae* from the *Mussismilia* coral holobiont, endemic in the SAO. Our results are comparable to the usual estimates of gene content in *Symbiodinaceae* (~40,000 genes) (Bayer et al. 2012; Shoguchi et al. 2013; Lin et al. 2015), and revealed the occurrence of 61.3% of eukaryotic single copy orthologs, comparable with available Symbiodiniaceae transcriptomes (Levin et al. 2016). Transcripts encoding characteristics of dinoflagelate genomes as histone-like and the dinoflagellate-viral nuclear proteins (*DVNP*) were highly expressed (Gornik et al. 2012), and Symbiodiniaceae *DVNPs* are also highly expressed in the *M. braziliensis* holobiont (Garcia et al. 2016). The high expression of transcripts encoding for calmodulin and ferredoxin in all samples indicates the importance of iron and calcium homeostasis in *Symbiodinium CCMR0100* basic metabolism.

Despite the consistent clustering of *Dark* and *Light* replicates, only 0.1% of the total transcripts were differentially expressed (DE) between conditions. Accordingly, the low level of site specific transcription factors in *Symbiodinaceae* genomes was also observed in *Symbiodinium* CCMR0100 (Table S2) (Bayer et al. 2012; Shoguchi et al. 2013; Xiang et al. 2015; Levin et al. 2016). None of the differentially expressed transcripts were associated to photosynthesis (Table 2). The high levels of transcription of photosystem-related genes in dark conditions supports the hypothesis of post-transcriptional regulation of photosynthesis genes in dinoflagellates (Fig. 1, McGinley et al. 2013, Xiang et al. 2015). Interestingly, transcripts related to mitosis and membrane modification were differentially expressed, indicating the effect of light on Symbiodiniaceae cell cycle and cellular differentiation.

4.2 Mycosporine-like aminoacids production in *Symbiodinium* CCMR0100

Our results also indicate that MAAs biosynthesis pathway is present in *Symbiodinium CCMR0100* (*ITS2 A4*), consistent with the high irradiance environment this *Symbiodinium* was isolated from (3-5 M deep, Silva-Lima et al. 2015). The observed association of the MAAs transcript set with *Light* condition was unexpected because illumination in our experiments was provided by fluorescent lamps, typically yielding light in the visible range and negligible amounts of UV radiation. Although it is not clear if the observed changes in expression can elicit the production of MAA in *Symbiodinium* CCMR0100, our results suggests that MAA biosynthesis can be triggered by visible light, reinforcing the hypothesis that MAAs might be multipurpose secondary metabolites (Oren and Gunde-Cimerman 2007). Some MAAs have ROS scavenging properties, indicating a possible physiological role of MAAs in Symbiodiniaceae photo-oxidative stress and coral bleaching (Oren and Gunde-Cimerman 2007; Rosic 2019). Accordingly, mycosporine-glycine is a MAA with ROS scavenging properties (Dunlap and Yamamoto 1995) which is produced by Symbiodiniaceae and other organisms (Yakovleva et al. 2004; Rosic 2019). Differences in the concentration of mycosporine-glycine was correlated with higher thermal tolerance in *Palythoa tuberculosa*, compared with bleaching-susceptible *Stylophora pistillata* (Yakovleva et al. 2004; Gao and Garcia-Pichel 2011).

Moreover, our analysis corroborates the hypothesis that MAA biosynthesis pathway was retained in the *Symbiodinium* genus. MAA production in the family Symbiodiniaceae was suggested to depend on host-symbiont complementarity: selective pressure would be lower in a symbiont inhabiting a MAA producing host than in a symbiont inhabiting a host not able to produce MAA (Shoguchi et al. 2018). However, several Symbiodiniaceae have a free-living stage, and *Symbiodinium* often dominates Symbiodiniaceae free-living communities in oceanic waters (Decelle et al. 2018). Combined with the high diversity of host *Symbiodinium A4* inhabits (LaJeunesse 2001) and higher occurrence in high irradiance environments (Santos and LaJeunesse 2006; Silva-Lima et al. 2015; Picciani et al. 2016), these factors favor the interpretation that MAA production was retained in the *Symbiodinium* genus due to differences in light niche partitioning. The observation that diverse *Symbiodinium* isolates are able to produce MAAs, but no other Symbiodiniaceae genera are, also supports this interpretation (Banaszak et al. 2000). Whether *Mussismilia* spp. are able to produce MAAs themselves (Silveira et al. 2017), and if MAA produced by *Symbiodinium* CCMR0100 could confer UV and photo-oxidative protection for its hosts remains to be determined.

4.3 Effect of light on cell cycle progression in Symbiodiniaceae

The induction of mitosis-related genes *Smc2* and *Lmln* indicates higher chromatin condensation was observed in the

Table 2 Differentially expressed transcripts between *Light* and *Dark* treatments in the *Symbiodinium* CCMR0100 transcriptome

Transcript_id	Inferred Function	Description	LogFC	Acc Number
DNA/RNA metabolism				
comp18355_c0	DNA binding	Alr1392 protein	−8.5	Q8YX26
comp10003_c0	Transcription repressor activity	Heat shock factor-binding protein 1	−3.0	O75506
comp3816_c0	DNA repair	rRNA intron-encoded homing endonuclease	2.5	Q9AY32
comp2198_c1	Chromosome condensation, mitosis	Structural maintenance of chromosomes protein 2 (smc2)	3.0	Q54PK4
comp1820_c0	Chromosome condensation, mitosis	Leishmanolysin-like peptidase (*lmln*)	3.6	Q29AK2
ER / Lipid metabolism				
comp1257_c0	Lipid signalling	Prostaglandin G/H synthase 2 (*pgh2*)	−8.6	O19183
comp20445_c0	Lipid signalling / Electron transfer	Cytochrome P450 4F8 (*Cyp450*)	−8.2	P98187
Others				
comp6076_c0	Calcium homeostasis	Hemolysin-type calcium-binding repeat (2 copies)	−8.6	U5D897
comp16184_c0	Redox reactions	Uncharacterized oxidoreductase C215.11c	−6.1	O94315
comp11897_c0	fatty acid biosynthesis	Putative 4′-phosphopantetheinyl transferase slr0495	−5.9	Q55185
comp5337_c0	Membrane	Transmembrane protein, putative	−5.0	A0A072V3I0
comp6176_c0	Protein kinase	Predicted protein of CLR family	−3.8	A8JA15

LogFC - Change in expression in the Light compared with the *Dark* treatment (log-transformed, base 2). Positive values of *LogFC* indicate an induction of the transcript in *Light*, while negative values indicate repression. Gene descriptions and accession numbers were retrieved from the best hit to the *Swissprot* (preferred) or NCBI database. Transcript inferred function deduced from the *Swissprot* description, assigned GO term and/or literature review

Light treatment, suggesting that the progression of the cell cycle shall depend on light. This result is consistent with cytological observations of the cell cycle in Symbiodiniaceae, with completed cellular division and higher motility at the onset of light (Fitt and Trench 1983, Wang et al. 2008, Xiang et al. 2015, Fujise et al. 2018). As both treatments were analyzed at the same time, DE transcripts observed in this study cannot be attributed to circadian regulation (Sorek et al. 2004), suggesting light might serve as a direct trigger for cellular differentiation in Symbiodiniaceae. Associated to higher chromatin condensation, we observed an inhibition of Cytochrome P450-4F8 (Cyp450) and Prostaglandin G/H synthase in *Light* conditions, indicating the regulation of the phosphatidylinositol (PI) signaling pathway, a conserved pathway in Symbiodiniaceae (Rosic et al. 2015). PI signaling pathway is associated to changes in cellular membrane system (Balla 2013) and might be associated to observed changes in Symbiodiniaceae external membranes in light and dark conditions (Xiang et al. 2015). In *Brevolium* SSB01, induction of transcription of chromatin condensation regulators was accompanied by modifications in the dinoflagellate extracellular matrix and by changes in transcripts associated to cell adhesion (Xiang et al. 2015). Accordingly, in our study, inhibition of PI signaling pathway is tied with a drastic inhibition of transcripts for Hemolysin-type protein (Table 2), an extracellular calcium binding protein, reinforcing the importance of Ca^{2+} homeostasis in Symbiodinaceae cells (Weston et al. 2015; Parkinson et al. 2016).

Thermal stress influences the transcription of meiosis and cellular differentiation genes (Levin et al. 2016; Gierz et al. 2017). Symbiodiniaceae cell cycle arrestment under heat stress has been observed in culture (McLenon and DiTullio 2012; Fujise et al. 2018) and *in hospite* (Dubousquet et al. 2016). In the context of photooxidative coral bleaching, the observed inhibition of *CytP450* in *Light* deserves further attention (Table 2). *CytP450* is involved in endoplasmic reticulum (ER) monooxygenase reactions, a potential source of ROS in eukaryotic cells (Lesser 2006). Based on its detoxification activity, a protective effect for *CytP450* in Symbiodiniaceae under thermal stress has been suggested (Rosic et al. 2010). Previous studies observed an induction of *CytP450* in thermally stressed corals (Voolstra et al. 2009) and cultured Symbiodiniaceae (Rosic et al. 2010; Levin et al. 2016). However, an acute heat shock or a prolonged exposure to thermal stress caused repression of *CytP450* (Rosic et al. 2010). In these studies, thermal stress was evaluated in light conditions, in which *CytP450* is strongly repressed. Given that coral bleaching also occurs in the dark, when there is no activity of photochemical reactions (Suggett et al. 2008; Hill et al. 2009; Saragosti et al. 2010; Tolleter et al. 2013), our results suggest that *CytP450* and ER monooxygenase activity can be sources of ROS in these conditions.

5 Conclusion

Our transcriptomic analysis of *Symbiodinium CMR0100,* symbiont of the endemic coral *M. braziliensis,* hints to a diverse gene expression repertoire, including the production of MAAs. MAA biosynthesis in *Symbiodinium* CCMR0100

supports the hypothesis that high irradiance was a selective pressure retaining this pathway in *Symbiodinium.* Future studies shall evaluate the depth distribution of *Symbiodinium* A4 in SAO and MAA production on different host, to investigate whether MAA production from *Symbiodinium* might confer UV and photo-oxidative protection to its hosts.

Our results also shows that light exposure also has an effect on biological processes previously associated to thermal stress on Symbiodiniaceae, as chromatin condensation and *cytP450* monooxigenase activity. These results indicates that caution is required on interpretation of changes on expression of these molecular markers in thermal stress conditions.

Authors' contributions AWSL conceived the study design, RNA extractions, the bioinformatic analysis and drafted the manuscript.

LSO and LL performed the transcriptome sequencing and prepared the MiSeq sequencing libraries.

JT participated in the discussion of the results and the draft manuscript, and the acquisition of funding.

MM, TV, and CLT participated in the discussion of the results and the draft manuscript.

FLT participated in the acquisition of funding, conceived the study design, discussion of the results and draft of the manuscript.

All authors read and approved the final manuscript.

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Conflict of interest The authors declare they have no conflict of interest.

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