Characterization of a New Cyanobacterial Cell Factory Strain for Saudi Arabia Application

Yi Mei Ng[1], Noor Azlin Mokhtar[1], Suhaiza Ahmad Jamhor[1], John Archer[1]

[1]Computational Bioscience Research Center, King Abdullah University of Science and Technology, PO Box 4700, Thuwal Jeddah, 23955-6900, Saudi Arabia;
Email : john.archer@kaust.edu.sa

Introduction

The use of photosynthetic cells as platform for bio-manufacturing is gaining increasing interest as it promises to deliver a sustainable, carbon neutral production system for biomass-derived chemicals, fuels and foods. Saudi Arabia, in particular, is an excellent place for the deployment of photosynthetic cell factory as algal biotechnology leverages the country's abundant sunlight, availability of large CO_2 point source emissions and access to Red Sea and Gulf waters (38 – 40 PSU). Currently a few model picocyanobacterial strain have been extensively studied for biofuel and biochemical production, but these strains are limited to mild temperature, light and salinity conditions thus cannot operate under Saudi Arabian climate. Therefore, in order to develop cyanobacterial cell factory applications for the Arabian Peninsula, there is a pressing need to discover and develop strains that can thrive under extremely warm temperatures, high insolation and high salinity. In our study, a native unicellular *Synechococcus* sp. RSCCF101 strain isolated from the central Red Sea has been identified as a potential cell factory candidate. Here we present physiological and genomic characterization of *Synechococcus* sp. RSCCF101 to support its development as a new robust marine cell factory strain.

Results

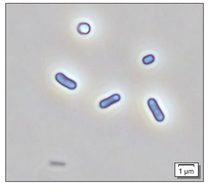

Figure 1: Strain RSCCF101 is a unicellular cyanobacterium strain isolated from the Red Sea. Cells are green, oval, unicellular, dividing by equivalent binary fission to form daughter cells. Mature cells are 0.90 - 1.08 µm long and 0.77 - 0.80 µm in diameter. Scale bar = 1µm. Image visualized under 1000x phase contrast using Olympus CX41 microscope.

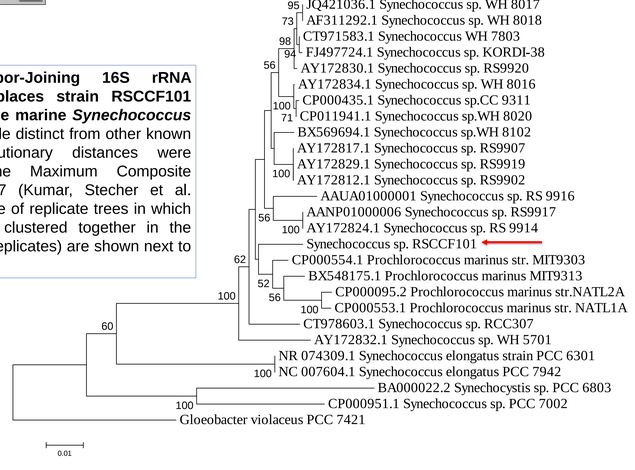

Figure 2: Neighbor-Joining 16S rRNA phylogenetic tree places strain RSCCF101 (red arrow) within the marine *Synechococcus* cluster 5 but in a clade distinct from other known species**.** The evolutionary distances were computed using the Maximum Composite Likelihood in MEGA7 (Kumar, Stecher et al. 2016). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches.

Acknowledgement
This work is supported by KAUST Center Competitive Funding FCC/1/1976. The work was carried out in Microbial Technology Laboratory (PI JACA), King Abdullah University of Science and Technology (KAUST). We thank KAUST Coastal and Marine Research Laboratory for support in marine sampling expeditions, KAUST Bioscience Core Lab for genome sequencing work, KAUST Computational Bioscience Research Center and KAUST IT for computational analysis support.

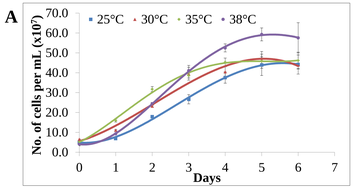

Figure 3: *Synechococcus* sp. RSCCF101 is a thermo-and halotolerant cyanobacterial strain. Panel A) At a constant 100 µmol photons m^{-2} s^{-1}, 35 PSU salinity, RSCCF101 exhibited a robust growth profile between 25°C and 38°C. The maximum growth rate was 0.929 day^{-1} at 38°C. Panel B) At a constant 30°C, 100 µmol photons m^{-2} s^{-1}, RSCCF101 grew efficiently over the salinity range of 10 PSU to 40 PSU and remained viable at 50 PSU and 60 PSU. Cultures were grown in Multicultivator MC-1000-OD (PSI). The standard error was calculated from 4 biological replicates.

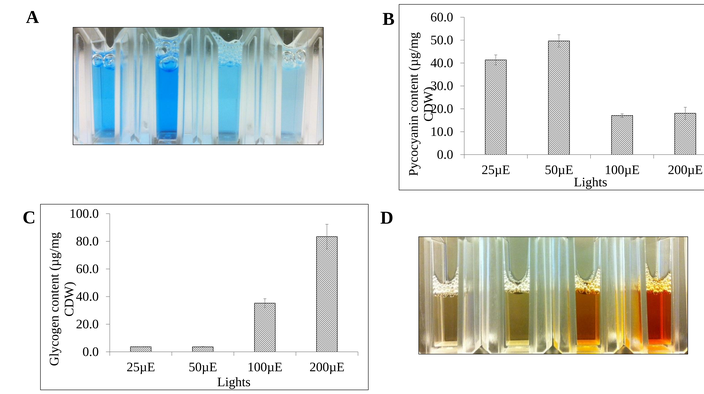

Figure 4: *Synechococcus* sp. RSCCF101 is a phycocyanin and glycogen rich cyanobacterial strain. Panel (A/B) RSCCF101 produced highest phycocyanin amount (49.7 µg/mg) when grown under light 50 µmol photons m^{-2} s^{-1} and Panel (C/D) highest glycogen content (83.4 µg/mg) when grown under light 200 µmol photons m^{-2} s^{-1}). Phycocyanin was extracted by lysozyme digestion in 0.1M TrisHCL-EDTA buffer, pH 7.5, while glycogen was extracted by bead beating in distilled water and assayed using ABCAM glycogen assay kit. Absorbance was measured at 620nm (phycocyanin) and 450nm (glycogen).

References

Aikawa, S., et al. (2014). "Glycogen production for biofuels by the euryhaline cyanobacteria Synechococcus sp. strain PCC 7002 from an oceanic environment." Biotechnology for Biofuels **7**(1): 88.

Chakdar, H. and S. Pabbi (2016). Cyanobacterial Phycobilins: Production, Purification, and Regulation. Frontier Discoveries and Innovations in Interdisciplinary Microbiology. P. Shukla. New Delhi, Springer India**:** 45-69.

Nielsen, J., et al. (2017). "Building a bio-based industry in the Middle East through harnessing the potential of the Red Sea biodiversity." Appl Microbiol Biotechnol **101**(12): 4837-4851.

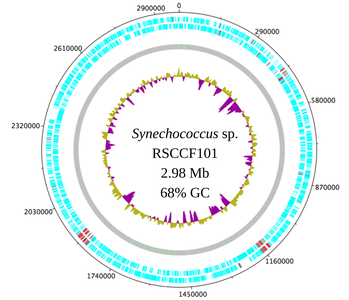

Figure 5: The *Synechococcus* sp. RSCCF101 genome has a compact of size 2.98Mb and high GC content 68%. The position of the mobile elements (putative genomic islands) is indicated in red on the map. Image was obtained using DNA Plotter in Artemis. RSCCF101 genome was sequenced by Illumina's HiSeq 2500 System and PacBio's SMRT Sequencer and reads were assembled using SPAdes Genome Assembler pipeline (Bankevich, Nurk et al. 2012). Genome annotation was performed in RAST (Rapid Annotation using Subsystem Technology) (Aziz, Bartels et al. 2008).

Figure 6: Genes that involve in glycogen biosynthesis are present in the *Synechococcus* sp. RSCCF101 genome. *glgC*, glucose-1-phosphate adenylyltransferase; *glgA*, glycogen synthase; *glgB*, 1,4-alpha-glucan branching enzyme; *glgP*, glycogen phosphorylase.

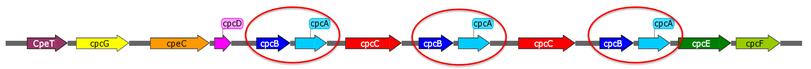

Figure 7: The phycocyanin (*cpcBA*), phycocyanin-linkers (*cpcG*, *cpeC*/*cpcC*) and lyases (*cpeT, cpcEF*) genes are found in a cluster in the *Synechococcus* sp. RSCCF101 genome. The genome consisted of three *cpcBA* genes, circled in red. Gene image was obtained using Snapgene View software.

Summary

- Strain **RSCCF101** is a candidate marine cyanobacterial cell factory strain **isolated from the central Red Sea**. It has an ovoid structure of about 1 µm length and 0.8 µm diameter (Figure 1) and 16S rRNA phylogenetic tree analysis places **RSCCF101 within the *Synechococcus* cluster** (Figure 2).
- The physiological profile showed that *Synechococcus* sp. RSCCF101 is **both thermo- and halotolerant** where it is able to grow efficiently at **temperature up to 38°C and salinity between 10 PSU and 40 PSU**, while remained viable at 50 PSU and 60 PSU (Figure 3A and 3B). These are desirable characteristics of a cell factory candidate as the chassis strain has to be able to survive the warm climate and intense insolation in Saudi Arabia (Nielsen, Archer et al. 2017).
- *Synechococcus* sp. RSCCF101 is capable of producing **high amount of phycocyanin** under low light condition **and glycogen** under high light condition (Figure 4A to 4D). Phycocyanin is a valuable product for bioassay, bioimaging, pharmaceutical, food and cosmetics industry application (Chakdar and Pabbi 2016) while glycogen can be used as biofuel feedstock (Aikawa, Nishida et al. 2014).
- **Whole genome was successfully constructed for *Synechococcus* sp. RSCCF101** (Figure 5), from which a complete gene set involving in **glycogen biosynthesis** was identified (Figure 6). In addition, the genome contains **three sets of *cpcBA*** which encodes the alpha and beta subunits of phycocyanin (Figure 7).
- Future work will include a more detailed genomic and transcriptomic profiling of RSCCF101 to establish the understanding of the physiological and molecular traits for synthetic biology purposes.