Identification of Specific Variations in a Non-Motile Strain of Cyanobacterium Synechocystis sp. PCC 6803 Originated from ATCC 27184 by Whole Genome Resequencing

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Abstract: Cyanobacterium Synechocystis sp. PCC 6803 is a widely used model organism in basic research and biofuel biotechnology application. Here, we report the genomic sequence of chromosome and seven plasmids of a glucose-tolerant, non-motile strain originated from ATCC 27184, GT-G, in use at Guangzhou. Through high-throughput genome re-sequencing and verification by Sanger sequencing, eight novel variants were identified in its chromosome and plasmids. The eight novel variants, especially the five non-silent mutations might have interesting effects on the phenotype of GT-G strains, for example the truncated Sll1895 and Slr0322 protein. These resequencing data provide background information for further research and application based on the GT-G strain and also provide evidence to study the evolution and divergence of Synechocystis 6803 globally.

Keywords: Synechocystis sp. PCC 6803; whole genome resequencing; single nucleotide polymorphism; deletion; insertion

1. Introduction

As the first sequenced photosynthetic organism and with high transformation competency, the freshwater cyanobacteria Synechocystis sp. PCC 6803 was one of the most widely used model
organisms for the research in photosynthesis and stress response, as well as for the biotechnological application of biofuel production [1–5]. The original Berkeley strain of *Synechocystis* sp. PCC 6803 was isolated from freshwater in California [6] and deposited in the Pasteur Culture Collection as PCC 6803 strain and in the American Type Culture Collection as ATCC 27184 strain. A glucose-tolerant (GT) strain was isolated from ATCC 27184 and designated Williams GT strain [7], which later the GT-Kazusa strain was derived from. The chromosome sequences of GT-Kazusa were published as the first *Synechocystis* sp. PCC 6803 genomic sequence [8,9]. In recent years, based on the high-throughput sequencing techniques, several other strains of *Synechocystis* sp. PCC 6803 were sequenced and reported world widely [10–13]. Other than the database errors, unique sequence variations were identified in GT-S, GT-I, PCC-P (positive phototactic), PCC-N (negative phototactic) and PCC-M (Moscow, Russia) strain, as well as the GT-O1 and GT-O2 in New Zealand. It is suggested that strain-specific mutations are likely to be responsible for phenotypic variation, such as pilus biosynthesis and motility. Such widespread genomic variations imply that novel mutations may exist between and within research labs. Recent genomic analysis of stress-evolved *Synechocystis* sp. PCC 6803 strains also revealed interesting information in adaptive evolution and stress response under high temperature or low pH [14,15].

In our lab, a designated wild type strain of *Synechocystis* sp. PCC 6803 was originated from ATCC 27184 and subjected to mutant construction for analyzing the signal transduction in stress response [16–19]. It is glucose-tolerant [17], but its genomic background information was not defined. Thus we re-sequenced and analyzed our own wild type stain GT-G (Guangzhou, China) to provide reference information for future research and to clarify its phylogenetic relationships with various sequenced strains. Our results not only provide background information for further research and application based on GT-G strain, but also provide evidence to study the evolution and divergence of *Synechocystis* 6803 globally.

2. Results and Discussion

2.1. Overview

The glucose tolerant strain originated from ATCC 27184 through routine laboratory culture conditions in our lab was designated GT-G (Guangzhou) and subjected to genomic re-sequencing. More than 8 million short reads (101 bp per read) were obtained from Illumina Hiseq2000 sequencing platform, about 808 Mb high quality data in total. This represents more than 200 folds coverage of the 3.96 Mb *Synechocystis* 6803 chromosome and plasmid genome. Using BWA [20] and VarScan [21,22] software, genomic sequences were constructed and putative variants were identified through mapping reads to the reference sequence of GT-Kazusa chromosome and plasmids. SNPs and indels were identified, while no large structure variation was detected. The putative variants were then verified by Sanger sequencing of the corresponding PCR products. No false-positive variant was found. The genome sequence of GT-G was deposited in the GenBank database under the accession number CP012832.

In total, 40 SNPs and indels were identified and verified in GT-G strain, 34 in chromosome and six in plasmids (Table 1). Among these, 32 variations were previously reported, including the 21 database
errors of GT-Kazusa reported previously [10] (Table 2). Excluding the errors of database, among the 19 mutations of GT-G, 10 mutations are shared with PCC-M, nine are shared with PCC-N and PCC-P, six are shared with GT-O1 and GT-O2, five are shared with GT-I, and three are shared with GT-S [10–13].

2.2. Chromosome Variations Shared with Other Strains

Mutation #1 implies that the 102 base pair deletion in \textit{slr1084} is specific to the GT-Kazusa and GT-S strains (Table 2) [11,12]. Mutation #2 implies that GT-G originated from ATCC 27184 before the 154 base pairs deletion appeared upstream and within \textit{slr2031}. However, GT-G shares with the other glucose tolerant, non-motile strains the 1 bp insertion in \textit{sll1574/5 (spkA)} gene, as checked and confirmed by PCR (Supplemental Table S1). The \textit{spkA} gene was essential for motility and pilus biosynthesis [23,24] and its mutation might partly explain the non-motility in GT strain. Mutation #3 occurs in the non-coding region between \textit{infA} and \textit{adk} gene, 12 bp upstream of the transcriptional start site of \textit{infA} gene [25]. This variation was also identified in PCC-P, PCC-N and PCC-M strain [11,12]. It changes the putative $-10$ element from “TGTGAT” to “TATGAT”. Thus it might have an effect on the transcription of \textit{infA} gene, which encodes translation initiation factor IF-1.

It was reported that re-sequencing and mapping might fail to detect large indels, but report SNPs in the target region instead [11,13]. However in this study, several large indels are successfully called by mapping and confirmed by PCR and Sanger sequencing. Three 1.2 kb deletions in GT-G represent that the \textit{ISY203b} (#6), \textit{ISY203e} (#11), and \textit{ISY203g} (#34) transposases insertion does not appear in GT-G, thus suggesting that they are specifically present in GT-Kazusa and/or GT-S [10–12].

The 1 bp deletion in \textit{slr0162 (pilC, #14)} is a variation common to all the reported PCC strains and GT strains except for GT-Kasuza [10], which suggests that the 1 bp insertion in \textit{slr0162} was specific in GT-Kazusa (Table 2). This insertion caused a frameshift mutation in \textit{pilC} gene and resulted in a truncated PilC protein, which might contribute to the lack of motility in GT-Kasuza [26]. Four novel variants in the chromosome unique to the GT-G strain are identified and verified as #8, #15, #16, and #17, which will be discussed in detail later. Two SNPs, mutations #27 and #29 are shared between GT-G strain and all PCC strains, suggesting their close relationship. They result in a silent mutation in PleD like protein coding gene \textit{slr0302} and an amino acid change in a putative transposase ISY100v3 coding gene \textit{ssr1176}, respectively.

2.3. Variations in Plasmids

Sequencing data cover all the seven plasmids and identify six mutations in three plasmids (#35–#40), which are all successfully verified by Sanger sequencing of PCR product. Of the six mutations in plasmid, four are unique to GT-G strain (#36, #37, #38, #40) and will be discussed in the next section. The 1.2 kb deletion in plasmid pSYSM (#35) represents the ISY203j transposase missing in GT-G, which was also reported in PCC-M [12]. The SNP in \textit{ssr6089} of plasmid pSYSX (#39) results in a N37S change in the hypothetical protein, and is shared with GT-O1 and GT-O2 strains [13].
Table 1. Location and effects of SNPs and indels identified in GT-G compared with the nucleotide sequence of GT-Kazusa in the database. Specific variants identified in GT-G are highlighted in bold. The errors of database are in grey. SNP, insertions, deletions and intergenic region are labeled as S, I, D and IGR respectively.

| Event | Effect | Locus | Gene Name | Product |
|-------|--------|-------|-----------|---------|
| **# M Start End Size Nucl Change Ref→mut AA Change Result** | **Chromosome** | **Gene Name** | **Product** |
| 1 | I | 386410 386411 | 102 | - - - | 34 additional AAs | slr1084 | - | Hypothetical protein |
| 2 | I | 781625 781626 | 154 | - - - | 5'extension of reading frame | IGR slr2030-slr2031 | - | - |
| 3 | S | 831647 831647 | 1 | C→T - - | Possible effect on infA promoter | IGR adk-infA | - | - |
| 4 | S | 943495 943495 | 1 | G→A GTC→ATC V→I | AA ‘change | slr1834 | paa | P700 apoprotein subunit Ia |
| 5 | S | 1012958 1012958 | 1 | G→T - - | - | - | - |
| 6 | D | 1200294 1201476 | 1183 | - - - | ISY203b missing | sll1780 | - | Transposase |
| 7 | S | 1364187 1364187 | 1 | A→G TTG→CTG L→L | -silent- | sll0838 | pyrF | Orotidine 5’ monophosphate decarboxylase |
| 8 | I | 1765792 1765793 | 1 | *→T AAT→AAA N→K | Frameshift | sll1995 | - | Hypothetical protein |
| 9 | S | 1819782 1819782 | 1 | A→G TCT→TCC S→S | -silent- | sll1867 | psbA3 | Photosystem II D1 protein |
| 10 | S | 1819788 1819788 | 1 | A→G CTT→CTC L→L | -silent- | sll1867 | psbA3 | Photosystem II D1 protein |
| 11 | D | 2048412 2049594 | 1183 | - - - | ISY203e missing | sll1635 | - | Transposase |
| 12 | S | 2092571 2092571 | 1 | A→T TTA→TAA L→* | New stop codon | sll0422 | - | Asparaginase |
| 13 | S | 2198893 2198893 | 1 | T→C TTA→TGG L→L | -silent- | sll0142 | - | Probable cation efflux system protein |
| 14 | D | 2204584 2204584 | 1 | G→* GGT→GTT G→V | Frameshift | srr0162 | gapF,pilC | A part of pilC, pilin biogenesis protein, required for twitching motility |
| 15 | S | 2235441 2235441 | 1 | A→G GGT→GGC G→G | -silent- | sll1851 | - | Hypothetical protein, no conserved domains |
| 16 | S | 2272418 2272418 | 1 | C→A CCC→ACC P→T | AA change | srr0322 | pilL-C | Homologous to the C-terminal of CheA-like protein, essential for motility, thick pili biosynthesis and transformation competency. |
| 17 | D | 2272927 2273907 | 981 | - - - | delete 327 AAs | srr0322 | pilL-C | As above |
| 18 | S | 2301721 2301721 | 1 | A→G AAG→GAG K→E | AA change | srr0168 | - | Hypothetical protein, no conserved domains |
| 19 | I | 2350285 2350286 | 1 | *→A - - | - | IGR sml0001-slr0363 | - | - |
Table 1. Cont.

| #  | M | Start   | End     | Size | Nucl change | Ref→mut | AA change | Result   | Locus    | Gene name                | Product                      |
|----|---|---------|---------|------|-------------|---------|-----------|----------|----------|--------------------------|------------------------------|
| 20 | I | 2360245 | 2360246 | 1    | *→C         | GCG→GCC | A→A       | Frameshift| slr0364/slr0366 | -                       | Hypothetical protein, no conserved domains |
| 21 | D | 2409244 | 2409244 | 1    | C→*         | GGA→GAT | G→D       | Frameshift| slr00762    | -                       | Hypothetical protein, no conserved domains |
| 22 | D | 2419399 | 2419399 | 1    | T→*         | AAT→ATG | N→M       | Frameshift| sll0751(ycf22);sll0752 | ycf22                    | Hypothetical protein YCF22    |
| 23 | I | 2544044 | 2544045 | 1    | *→C         | AGG→GAG | R→E       | Frameshift| ssl0787;ssl0788 | -                       | Hypothetical protein, no conserved domains |
| 24 | S | 2602717 | 2602717 | 1    | C→A         | CAC→CAA | H→Q       | AA change | sfr0468     | -                       | Hypothetical protein, no conserved domains |
| 25 | S | 2602734 | 2602734 | 1    | T→A         | ATT→AAT | I→N       | AA change | sfr0468     | -                       | Hypothetical protein, no conserved domains |
| 26 | S | 2748897 | 2748897 | 1    | C→T         | -       | -         | -         | IGR sfr0210-sfr0332 | -                       | -                           |
| 27 | S | 3014665 | 3014665 | 1    | T→C         | ACT→ACC | T→T       | -silent-  | slr0302     | pleD                     | PleD-like protein            |
| 28 | S | 3096187 | 3096187 | 1    | T→C         | ATA→ACA | I→T       | AA change | ssl1175(transposase) | -                       | Located in a mobile element(ISY100v1) |
| 29 | S | 3098707 | 3098707 | 1    | T→C         | TGT→CTG | C→R       | AA change | ssl1176(transposase) | -                       | Located in a mobile element(ISY100v3) |
| 30 | S | 3110189 | 3110189 | 1    | G→A         | -       | -         | -         | IGR sll0665-sll0666 | -                       | Located in a mobile element(ISY523) |
| 31 | S | 3110343 | 3110343 | 1    | G→T         | CCA→CAA | P→Q       | AA change | sll0665     | -                       | Transposase                 |
| 32 | S | 3142651 | 3142651 | 1    | A→G         | CTT→CTC | L→L       | -silent-  | sll0045     | spsA                    | Sucrose phosphate synthase   |
| 33 | S | 3260996 | 3260996 | 1    | C→*         | -       | -         | -         | IGR sll0528-sll0529 | -                       | -                           |
| 34 | D | 3400331 | 3401513 | 1183 | -           | -       | -         | -         | ISY203j missing | sll1474     | -                       | Transposase                 |
| 35 | D | 117269  | 118451  | 1183 | -           | -       | -         | -         | ISY203j missing | sll5131 | -                       | Transposase                 |
| 36 | S | 4241    | 4241    | 1    | C→G         | ATC→ATG | I→M       | AA change | srl6004     | -                       | Hypothetical protein, no conserved domains |
| 37 | S | 4253    | 4253    | 1    | C→T         | CCC→CCT | P→P       | -silent-  | srl6004     | -                       | Hypothetical protein, no conserved domains |
| 38 | S | 4295    | 4295    | 1    | T→C         | TCT→TCC | S→S       | -silent-  | srl6004     | -                       | Hypothetical protein, no conserved domains |
| 39 | S | 82405   | 82405   | 1    | A→G         | AAC→AGC | N→S       | AA change | srx6089     | -                       | Hypothetical protein, no conserved domains |
| 40 | D | 1211    | 1211    | 1    | A→*         | CAG→CGG | Q→R       | Frameshift| MYO_820     | -                       | Hypothetical protein, no conserved domains |
Table 2. Comparison of SNPs and indels identified in GT-G with sequences from other reported strains. The errors of database are in grey. NI<sup>a</sup>: not investigated.

| # | Event | Strains Reported in Literatures and This Work |
|---|-------|---------------------------------------------|
|   |       | GT-Kazusa [10,11] | GT-S [10] | GT-I [11] | GT-O1 [13] | GT-O2 [13] | GT-G [11] | PCC-P [11] | PCC-N [11] | PCC-M [12] |
| 1 | I     | - | - | √ | √ | √ | √ | √ | √ | √ |
| 2 | I     | - | - | - | - | - | √ | √ | √ | √ |
| 3 | S     | - | - | - | - | - | √ | √ | √ | √ |
| 4 | S     | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| 5 | S     | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| 6 | D     | - | - | √ | √ | √ | √ | √ | - | - |
| 7 | S     | √ | √ | √ | √ | √ | √ | √ | - | - |
| 8 | I     | - | - | - | - | - | √ | - | - | - |
| 9 | S     | √ | √ | - | - | - | √ | - | - | - |
|10 | S     | √ | √ | - | √ | √ | √ | - | - | - |
|11 | D     | - | - | √ | √ | √ | √ | √ | - | - |
|12 | S     | √ | √ | √ | - | √ | √ | - | - | - |
|13 | S     | √ | √ | √ | √ | √ | √ | - | - | - |
|14 | D     | - | √ | √ | √ | √ | √ | - | - | - |
|15 | S     | - | - | - | - | - | √ | - | - | - |
|16 | S     | - | - | - | - | - | √ | - | - | - |
|17 | D     | - | - | - | - | - | √ | - | - | - |
|18 | S     | √ | √ | √ | √ | √ | √ | - | - | - |
|19 | I     | √ | √ | √ | √ | √ | √ | - | - | - |
|20 | I     | √ | √ | √ | √ | √ | √ | - | - | - |
|21 | D     | √ | √ | √ | √ | √ | √ | - | - | - |
|22 | D     | √ | √ | √ | √ | √ | √ | - | - | - |
|23 | I     | √ | √ | √ | √ | √ | √ | - | - | - |
|24 | S     | √ | √ | √ | √ | √ | √ | - | - | - |
|25 | S     | √ | √ | √ | √ | √ | √ | - | - | - |
|26 | S     | √ | √ | √ | √ | √ | √ | - | - | - |
|27 | S     | - | - | - | - | - | √ | - | - | - |
|28 | S     | √ | √ | √ | √ | √ | √ | - | - | - |
|29 | S     | - | - | - | - | - | √ | - | - | - |
|30 | S     | √ | √ | - | √ | √ | - | - | - | - |
|31 | S     | √ | √ | - | √ | √ | - | - | - | - |
|32 | S     | √ | √ | - | √ | √ | - | - | - | - |
|33 | D     | √ | √ | √ | √ | √ | - | - | - | - |
|34 | D     | - | √ | √ | √ | √ | √ | - | - | - |
|35 | D     | NP | NI | NI | - | - | √ | NI | NI | √ |
|36 | S     | NI | NI | NI | - | - | √ | NI | NI | - |
|37 | S     | NI | NI | NI | - | - | √ | NI | NI | - |
|38 | S     | NI | NI | NI | - | - | √ | NI | NI | - |
|39 | S     | NI | NI | NI | √ | √ | √ | NI | NI | - |
|40 | D     | NI | NI | NI | - | - | √ | NI | NI | - |
2.4. Novel Variations in GT-G

Among the eight GT-G specific mutations identified here, five are SNP, two are deletion, and one is insertion, all of which locate in the open reading frame. Three SNPs (#15, #37 and #38) are silent mutations, while the other mutations cause amino acid change or frameshift.

The 1 bp insertion in sll1895 gene (#8) leads to frameshift and results in a truncated Sll1895 protein (Figure 1a). The 696 amino-acids long Sll1895 protein in GT-Kazusa is predicted to contain several functional domains, such as FHA (Forkhead-associated domain for phosphopeptide recognition), GGDEF (diguanylate cyclase domain), and EAL (candidate for a diguanylate phosphodiesterase function). It was suggested to contribute to signal transduction according to its conserved domain [27] and Sll1895 protein was found upregulated by hexane in a proteomic analysis [28]. The 377 amino acids-long truncated Sll1895 in GT-G strain lose EAL domain and part of the GGDEF domain, which may result in a non-functional protein (Figure 1a).

![Figure 1](image)

**Figure 1.** Domain organization of mutated genes coding protein in GT-Kazusa and GT-G. The black box indicates the conserved motif. (a) Domain organization of Sll1895. FHA: Forkhead-associated domain for phosphopeptide recognition; GGDEF: diguanylate cyclase domain; EAL: candidate for a diguanylate phosphodiesterase function. Sll1895 in GT-G loses EAL domain and part of the GGDEF domain; (b) Domain organization of Slr0322. The ATPase domain and CheW like domain are lost in GT-G, which is indicated by the dash lines. The white star indicates the P280T residue change in the histidine kinase domain in GT-G.

A novel large deletion was revealed in GT-G as 981 bp deletion in the middle of slr0322 gene (#17), resulting in 327 amino acids deletion inside the 1095 amino acids long Slr0322 protein (Figure 1b). Slr0322 in GT-Kasuza is a putative two-component hybrid sensor and regulator designated as Hik43, consisting of a histidine kinase domain and two response regulator domains in the N and C terminal respectively [3]. It was also designated Pill-C/CheA since it was homologous to the C-terminal of CheA-like protein and was essential for motility, thick pili biosynthesis, and transformation competency [29]. Slr0322 in GT-Kasuza strain contains the ATPase domain and CheW
like domain between the kinase domain and response regulator domain, but they are lost in GT-G strain due to the deletion (Figure 1b). In addition, SNP in slr0322 (#16) leads to a P280T residue change in the histidine kinase domain of this protein. Such functionally adverse mutations might have an effect on GT-G phenotype. Thus, we examined its surface structure under transmission electron microscope and its motility under lateral illumination. Electron micrographs of negatively stained GT-G cells indicated the deficiency of pilus and no phototactic movement of the GT-G colony was observed under lateral illumination (Figure 2). These phenotypes may be attributed to both the 1 bp insertion in spkA gene and the mutations in slr0322. Further research is needed to characterize the impact of individual mutations in GT-G strain.

Other than the two silent SNPs (#37, #38), SNP #36 in plasmid pSYSX is predicted to result in I64M change in unknown protein Slr6004. One base pair deletion in pCB2.4 (#40) leads to frameshift in hypothetical protein MYO_820 gene.

![Figure 2](image-url)

**Figure 2.** Surface structure and motility of GT-G. (a,b) Electron micrograph of negatively stained GT-G cells; (c) Phototactic movement of colonies of GT-G. The arrow indicated the direction of lateral light and the black line under the colony shows the initial position before lateral illumination.

2.5. Phylogenetic Relationships

Among the Synechocystis strains sequenced and reported so far, the genomic sequence of GT-G is most similar to PCC-M strain, sharing nine chromosome variants and one plasmid variant, though they are different in motile capacity (Table 2, Figure 2). According to our result and the published data, the phylogenetic relationships among various sequenced strains of *Synechocystis* sp. PCC 6803 are summarized in Supplemental Table S1 and visualized in Figure 3. GT-G strain can grow in glucose and cannot move towards light, which are characteristic of GT strains. GT-G strain shares with the other GT strains the 1 bp insertion in sll1574/5 (spkA), which was critical for the motility and pilus biosynthesis [23,24]. However, it doesn’t contain the 154 bp deletion upstream and within slr2031, which makes it different from the other GT strains. GT-G shares with PCC strains SNP ssr1176, SNP slr0302 and SNP before infA, which suggests that GT-G may be the strain closest to the origin of the splitting of the PCC and GT strains.
motile

PCC-N
SNP slr1119
12 bp Del. slr0698 (hik33)
SNP slr1510 (plsX)
SNP slr1962
SNP slr0370 (gabD)
SNPs IGR ssl0105-ssl0606
1 bp Ins. slr0079 (gspE)

PCC-P
SNP slr0698 (hik33)
SNP slr1992
SNP slr0645

45 bp Del. slr1819
1 bp Ins. slr0182
SNP slr1993
SNP slr0698 (hik33)
1 bp Ins. slr1951

PCC-M
SNP slr0242
SNP slr1609
SNP slr1898
SNP slr1564
SNP slr0334
1 bp Del. slr1496
SNP slr1564
SNP slr0753
1 bp Del. slr1951

ATCC27184
SNP slr1865
SNP slr1983
SNP slr0222

BERKELEY
R. Kunisawa, 1968; Isolation from fresh water, Oakland, California

Figure 3. Visualization of phylogenetic relationships among various sequenced stains of *Synechocystis* sp. PCC 6803. The presence of identified SNPs and indels are indicated along the branches. The variants in plasmid are in italic. Putative insertions, deletions, and intergenic regions are labeled as Ins, Del, and IGR respectively. Modified after Trautmann *et al.*, Kanesaki *et al.* and Morris *et al.* [11–13].

3. Experimental Section

3.1. Strain and DNA Extraction

The GT-G strain of *Synechocystis* sp. PCC 6803 was derived from ATCC 27184. It was cultured in BG11 medium with 20 mM HEPES-NaOH (pH 7.5) at 29 °C, and illuminated with 30 μE·m⁻²·s⁻¹.
The cells of mid-logarithmic phase (OD730 = 1.0) were harvested by centrifugation at 5000× g for 5 min. Total DNA was extracted using the extraction kit (Dongs heng, Guangzhou, China) according to the manufacturer’s instructions.

3.2. Sequencing Methods and Data Analysis

The DNA was randomly fragmented by ultrasonication. Gel size selection, adaptor ligation, and amplification resulted in sequencing libraries of DNA clusters at around 300 bp. Paired-end sequencing was performed at the Illumina HiSeq 2000 platform. The high quality sequencing data were mapped to the GT-Kasuza reference sequences using BWA software [20]. The accession numbers of reference sequences are chromosome, BA000022; pSYSM, AP004310; pSYSX, AP006585; pSYSA, AP004311; pSYSG, AP004312; pCC5.2, CP003272; pCA2.4, CP003270 and pCB2.4, CP003271. SNPs and indels were detected by VarScan software [21,22]. Large structure variation was detected by Break Dancer software [30].

3.3. Mutation Verification

All the putative SNPs and indels were verified through Sanger sequencing the PCR products, which covered the variation site. Annotation information was obtained from Cyanobase [31]. The reported error of database not called by software was checked by Sanger sequencing.

3.4. Electron Microscopy and Motility Assay

The electron microscopy and phototactic assay were performed as previously described [29]. Briefly, the cell surface structures were examined after staining with 0.8% (w/v) phosphotungstic acid (pH 7.0) under transmission electron microscope (1200EX, JEOL, Tokyo, Japan). Phototactic movement was observed on 0.8% (w/v) agar under lateral illumination.

4. Conclusions

Re-sequencing of the GT-G strain of Synechocystis 6803 identified eight novel variants, which are likely to affect gene function. Mutations found in GT-G strain indicate that it is divergent from ATCC 27184 after the 1 bp insertion in spkA, and before the 154 bp deletion upstream and within slr2031. Agreement with previously reported error of database and the successful verification of variations by Sanger sequencing indicate the effectiveness and powerfulness of re-sequencing at about 200-fold coverage. Our data highlight the specific variants in the GT-G strain originated from ATCC 27184 and provide background information for future research based on GT-G strain. It also provides further evidence to identify the evolution and divergence of Synechocystis 6803 globally.

Supplementary Materials

Supplementary materials can be found at http://www.mdpi.com/1422-0067/16/10/24081/s1.
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Author Contributions

Qinglong Ding, Yuling Wang, and Gu Chen designed and performed the experiments; Qinglong Ding, Yuling Wang, and Gu Chen analyzed the data and wrote the manuscript; Dong Wei contributed reagents, materials, and analysis tools.

Conflicts of Interest

The authors declare no conflict of interest.

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