

Draft Genome Sequence of Marine Cyanobacterium *Synechococcus* sp. Strain NKBG042902, Which Harbors a Homogeneous Plasmid Available for Metabolic Engineering

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The marine cyanobacterium *Synechococcus* sp. strain NKBG042902 was isolated from coastal areas in Japan. Strain NKBG042902 has four plasmids: pSY8, pSY9, pSY10, and pSY11. Moreover, the hybrid plasmid pUSY02 containing pSY11 and *Escherichia coli* plasmid pUC18 was constructed for this strain. The genetic manipulation technique using pUSY02 was established for this strain and used in metabolic engineering. Here, we report the draft genome sequence of this strain, which has 77 contigs comprising a total length of 3,319,479 bp, with a G+C content of 49.4%.

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Cyanobacteria are photosynthetic prokaryotic microorganisms that are ubiquitous in marine, freshwater, and land systems. Various useful natural compounds have been discovered and produced from cyanobacteria (1). Recently, cyanobacteria have been accepted as a promising candidate for sustainable bioenergy generation because their metabolic pathway can be modified for the production of biofuel compounds through genetic engineering (2, 3). Various biofuel compounds (e.g., 1-butanol, alkane, and ethylene) have successfully been produced from the cyanobacteria *Synechocystis* sp. PCC6803 and *Synechococcus elongates* PCC7942 (4–6), which highlights the possibility of applying the cyanobacteria for biofuel productions.

Synechococcus sp. strain NKBG042902 was isolated as a fastgrowing cyanobacterium from the coastal areas in Nagasaki, Japan (7), and it can survive under a wide range of salt concentrations, from 0 to 5%. A hot-water extract from the NKBG042902 strain showed the ability to promote plantlet formation from somatic embryos of carrot (8). Moreover, strain NKBG042902 has four endogenous plasmids: pSY8, pSY9, pSY10 (2.6 kbp), and pSY11 (2.3 kbp) (9). The hybrid plasmid pUSY02 containing pSY11 and Escherichia coli plasmid pUC18 was constructed for genetic manipulation of this strain and has been successfully applied for the production of eicosapentaenoic acid (EPA) by the expression of the EPA synthesis gene cluster from a marine bacterium, Shewanella putrefaciens strain SCRC-2738 (10). Thus, the strain NKBG042902 can be treated as a useful host for the production of valuable compounds, which are not naturally generated by this strain. Here, we report the draft genome sequence of the marine cyanobacterium Synechococcus sp. strain NKBG042902.

The experimental procedure used in this study was based on a previous report (11). In general, *Synechococcus* sp. NKBG042902 was cultured in BG11 medium (ATCC catalog medium no. 617, supplemented with 3% NaCl) at 26°C under continuous illumination in 100-ml Erlenmeyer flasks placed on a reciprocating

shaker. After 2 weeks of cultivation, the cells were collected and the genomic DNA was extracted using a DNeasy plant minikit (Qiagen). The sequencing of genomic DNA was performed with a Roche/454-GS Junior system, according to the manufacturer's protocol. The reads were assembled by the software Newbler assembler version 2.5 (Roche). The draft genome sequence of strain NKBG042902 consisted of 77 contigs (>500 bp, 22.0-fold average coverage), comprising a total length of 3,319,479 bp, with a G+C content of 49.4%. The resulting contigs were annotated by the software Glimmer 3.02 and BLAST searches against a nonredundant protein sequence database of the National Center for Biotechnology Information. tRNA genes were predicted by the RNAmmer prediction server (12), and rRNA genes were predicted by the software ARAGORN version 1.2.3 (13). The draft genome sequence of the strain includes 3,412 predicted coding regions, 40 tRNA genes, and 4 rRNA genes.

Nucleotide sequence accession numbers. The *Synechococcus* sp. strain NKBG042902 draft genome sequence data have been deposited in DDBJ/EMBL/GenBank under accession no. BAWS00000000. The version described in this paper is version BAWS01000000.

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REFERENCES

- 1. Burja AM, Banaigs B, Abou-Mansour E, Burgess JG, Wright PC. 2001. Marine cyanobacteria—a prolific source of natural products. Tetrahedron 57:9347–9377. http://dx.doi.org/10.1016/S0040-4020(01)00931-0.
- Berla BM, Saha R, Immethun CM, Maranas CD, Moon TS, Pakrasi HB. 2013. Synthetic biology of cyanobacteria: unique challenges and opportunities. Front. Microbiol. 4:246. http://dx.doi.org/10.3389/ fmicb.2013.00246.

- Ducat DC, Way JC, Silver PA. 2011. Engineering cyanobacteria to generate high-value products. Trends Biotechnol. 29:95–103. http:// dx.doi.org/10.1016/j.tibtech.2010.12.003.
- 4. Wang W, Liu X, Lu X. 2013. Engineering cyanobacteria to improve photosynthetic production of alka(e)nes. Biotechnol. Biofuels 6:69. http://dx.doi.org/10.1186/1754-6834-6-69.
- Ungerer J, Tao L, Davis M, Ghirardi M, Maness PC, Yu JP. 2012. Sustained photosynthetic conversion of CO₂ to ethylene in recombinant cyanobacterium *Synechocystis* 6803. Energ. Environ. Sci. 5:8998–9006. http://dx.doi.org/10.1039/c2ee22555g.
- 6. Lan EI, Liao JC. 2011. Metabolic engineering of cyanobacteria for 1-butanol production from carbon dioxide. Metab. Eng. 13:353–363. http://dx.doi.org/10.1016/j.ymben.2011.04.004.
- Matsunaga T, Takeyama H, Nakamura N. 1990. Characterization of cryptic plasmids from marine cyanobacteria and construction of a hybrid plasmid potentially capable of transformation of marine cyanobacterium, *Synechococcus* sp. and its transformation. Appl. Biochem. Biotechnol. 24–25:151–160.
- Wake H, Akasaka A, Umetsu H, Ozeki Y, Shimomura K, Matsunaga T. 1992. Promotion of plantlet formation from somatic embryos of carrot treated with a high-molecular-weight extract from a marine cyanobacte-

rium. Plant Cell Rep. 11:62-65.http://dx.doi.org/10.1128/ genomeA.00954-13.

- Takeyama H, Nakayama H, Matsunaga T. 2000. Salinity-regulated replication of the endogenous plasmid pSY10 from the marine cyanobacterium *Synechococcus* sp. Appl. Biochem. Biotechnol. 84–86:447–453. http://dx.doi.org/10.1385/ABAB:84-86:1-9:447.
- Takeyama H, Takeda D, Yazawa K, Yamada A, Matsunaga T. 1997. Expression of the eicosapentaenoic acid synthesis gene cluster from *Shewanella* sp. in a transgenic marine cyanobacterium, *Synechococcus* sp. Microbiology 143:2725–2731.
- Yoshino T, Honda T, Tanaka M, Tanaka T. 2013. Draft genome sequence of marine cyanobacterium *Synechococcus* sp. strain NKBG15041c. Genome Announc. 1(6):e00954-13. http://dx.doi.org/10.1128/ genomeA.00954-13.
- Lagesen K, Hallin P, Rødland EA, Staerfeldt HH, Rognes T, Ussery DW. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res. 35:3100–3108. http://dx.doi.org/10.1093/ nar/gkm160.
- Laslett D, Canback B. 2004. Aragorn, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. Nucleic Acids Res. 32:11–16. http://dx.doi.org/10.1093/nar/gkh152.