Title

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Journal

New Zealand Journal of Botany, 57(2)

ISSN

0028-825X

Author

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Publication Date

2019-04-03

DOI

10.1080/0028825x.2018.1551805

Peer reviewed

HHS Public Access

Author manuscript

. Author manuscript; available in PMC 2020 January 01.

Published in final edited form as:

. 2019 ; 57(2): 70–75. doi:10.1080/0028825X.2018.1551805.

The international journeys and aliases of *Synechococcus elongatus*

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Abstract

This perspective provides a historical account of the isolation and nomenclature of the cyanobacterial strains currently known as *Synechococcus elongatus*. The story focuses on an isolate from the San Francisco Bay area of California (Pasteur Culture Collection PCC 7942) that has, for decades, been the genetic model for this species, and its close relative isolated from Waller Creek in Texas (PCC 6301, also known as the University of Texas at Austin Culture Collection of Algae UTEX 625). Until recently, these strains have been the only representatives of the species. A new wild isolate, UTEX 3055, is distinctly different from the prior reference strains. *S. elongatus* strains have been widely used by labs around the world to discover fundamental cellular processes and to engineer cyanobacteria to generate useful products. The review clarifies relationships among strains that carry different names, and explains how names that appear in the literature have changed over the years.

Keywords

Anacystis nidulans; cyanobacteria; genetic transformation; Synechococcus elongatus; Thermosynechococcus elongatus

A globe-trotting transformer

The cyanobacterium currently known as *Synechococcus elongatus* PCC 7942 was originally isolated by students of an algology course taught by Kenneth W. Floyd at California State University, San Francisco. The students sampled natural California aquatic habitats and isolated bacteria-free algae cultures. Sergey V. Shestakov of Moscow State University received several freshwater samples of blue-green algae, as cyanobacteria were known at the time, from Dr. Floyd in 1973 while attending the XIII International Congress of Genetics, held at the University of California at Berkeley. Among these strains was one isolate, termed R-2, which was shown by Galina Grigorieva in the Shestakov lab to be highly transformable (Grigorieva 1976). Her approach used chromosomal DNA from erythromycin- and streptomycin-resistant mutants of this strain and another cyanobacterium in their lab, *Anacystis nidulans* 602 (originally obtained from a culture collection of Leningrad State

An abbreviated telling of this history appears in the review article “A hard day’s night: cyanobacteria in diel cycles” (Welkie et al. 2019).

University). Based on the high-frequency cross-transformation of antibiotic resistance between the two strains, Griogorieva and Shestakov concluded that R-2 is closely related to *A. nidulans* 602, and the new strain became known for many years as *A. nidulans* R2.

In 1976 Kees (C.A.M.J.J.) van den Hondel, in the lab group of Gerard van Arkel at the University of Utrecht, was new to the field of cyanobacteria and was eager to find an exciting project. He had the opportunity to ask advice from Dr. Shestakov, while both waited at a train station between conferences in Dundee and Oxford. Dr. Shestakov recommended searching for molecular evidence of the genetic transformation in cyanobacteria that his lab had observed, and particularly analyzing strains for plasmids that could serve as the basis of cloning vectors. Dr. van den Hondel first requested strains of *A. nidulans* from Rosmarie Rippka, Director of the Pasteur Culture Collection (PCC) in Paris, and attempted to transform them with plasmids from *Escherichia coli*, but was unsuccessful. He decided that he should obtain the *A. nidulans* strains that Grigorieva and Shestakov had already shown to be transformable, and took advantage of the XIV International Congress of Genetics held in Moscow in 1978 to visit the Shestakov lab. Dr. Shestakov provided both *A. nidulans* strains, but the 602 sample was contaminated, so van den Hondel focused on *A. nidulans* R2. He was able to isolate mutants in which the transposon Tn*901* had jumped into a small endogenous plasmid of *A. nidulans* R2, enabling the transformation of the wild type to ampicillin resistance, recovery of the plasmids, and molecular characterization by restriction mapping and heteroduplex formation with the native plasmid. This demonstration laid the foundation for recombinant DNA-based molecular genetics research in cyanobacteria (van den Hondel et al. 1980). Drs. van den Hondel and van Arkel deposited the strain with the PCC, where it was the 42nd accession of 1979 (hence, PCC 7942).

Louis A. Sherman from the University of Missouri visited the van Arkel lab in 1980 while on sabbatical at the University of Leiden to learn recombinant DNA techniques. A leader in photosynthetic physiology and biophysics in cyanobacteria, Dr. Sherman was excited about the prospect of having a genetically tractable strain in which to use genetic approaches to reveal the biochemical components behind the fluorescence signals of the Z scheme. He obtained PCC 7942, establishing a hub of research on the strain in the USA. Two new graduate students, Cris Kuhlemeier in the van Arkel group at Utrecht, and Susan Golden with Lou Sherman in Missouri, built their dissertation projects in the early 1980s on creating cloning vectors and developing the genetic system for *A. nidulans* R2. They were joined by others around the world who developed this, and other genetically tractable, cyanobacterial species as genetic models (Williams and Szalay 1983; Chauvat et al. 1983; Wolk et al. 1984]; Buzby et al. 1985; Vermaas et al. 1986).

The name game, siblings, and “kissing cousins”

In the mid-1980s the methods of molecular phylogeny were developing sufficiently to warrant re-evaluation of the taxonomic structure of the cyanobacteria, including their nomenclature. The accessions of unicellular cyanobacteria in various culture collections around the world that had been known as *A. nidulans* became standardized as members of the genus *Synechococcus*, but for several years did not possess a species name. Publications during that time generally referred to the strains by genus and culture collection number.

A second edition of Bergey's Manual of Systematic Bacteriology was published in 2001, with Volume One addressing the Archaea and the deeply branching and phototropic bacteria, including cyanobacteria. In it Drs. Rippka, Michael Herdman, Richard Castenholz, and John Waterbury described the *Synechococcus* clade, including the Cluster 1.1 typified by PCC 6301, and proposed as the species *Synechococcus elongatus*: a designation approved by the Botanical Code of Nomenclature (Herdman et al. 2001). By this time the close relationship of PCC 6301, first isolated by W.A. Kratz from Waller Creek in Austin, Texas (Kratz and Myers 1955), and PCC 7942 from California, was well established (Golden et al. 1989). Hence, *A. nidulans* R2 had a new legitimate species name: *S. elongatus* PCC 7942.

The close relatives of *S. elongatus* PCC 7942 are known by many names, but all of them are accessions of the same original Kratz isolate from Waller Creek, Texas. One such accession was the foundational strain of the PCC: number 6301, first deposited by Mary B. Allen when the collection was initiated at the Berkeley lab of Roger Stanier and later renamed as PCC when the group moved to the Pasteur Institute in Paris (PCC 6301: https://brclims.pasteur.fr/crbip_catalogue/faces/ficheCatalogue_S.xhtml). Other aliases include TX20 (van Baalen 1967), *Synechococcus leopoliensis* UTEX 625 (UTEX.org), and *Synechococcus leopoliensis* CCAP 1405/1 (CCAP.ac.uk). A recent revival of an archived sample of UTEX 625 by the lab of Himadri Pakrasi yielded a fast-growing strain, redeposited as UTEX 2973, whose genome is closely related to, but not identical to, the sequence expected for UTEX 625 (approximately 1600 single nucleotide polymorphisms or insertions/deletions)(Yu et al. 2015). Surprisingly, the UTEX 2973 genome is very close to that of PCC 7942, which was collected 1500 miles from the UTEX 625 Kratz isolate from which UTEX 2973 emerged (Yu et al. 2015). Although the origin of UTEX 2973 is bewildering, it may represent an independent strain from Waller Creek that was lurking in the UTEX 625 Kratz sample all along, and was selected only under the conditions used by the Pakrasi lab. The close genetic of relationship among all of these isolates, collected in California and Texas, is remarkable, as only 55 single nucleotide polymorphisms separate PCC 7942 and UTEX 2973 (Yu et al. 2015).

Another member of the *S. elongatus* species, more different than any of the previously known isolates are from one another, was recently recovered from Waller Creek by Jerry Brand and David Nobles of UTEX and Ryan Simkovsky of the S. Golden lab at UC San Diego. The new isolate, UTEX 3055, has 38,774 single nucleotide polymorphisms and some plasmid differences relative to PCC 7942 (Yang et al. 2018). Moreover, UTEX 3055 exhibits biofilm formation and phototaxis phenotypes that were likely lost during laboratory domestication of PCC 7942. The likelihood of domestication is supported by evidence that mutants of PCC 7942 can form biofilms (Schatz et al. 2013), and the photoreceptor (PixJ) of PCC 7942 is able to restore phototaxis to a *pixJ* mutant of UTEX 3055 (Yang et al. 2018).

Unfortunately for cyanobacterial fans searching through the literature, *Synechococcus elongatus* had been used as the genus and species name previously for thermophilic cyanobacteria that are quite distant relatives of UTEX 625/PCC 6301 and 7942, clearly belonging to a different genus. Most publications since 2001 refer to those thermophilic strains as *Thermosynechococcus elongatus*.

Claims to fame

S. elongatus (PCC 6301) was the source of cyanobacterial phycobilisomes that Alex Glazer used to characterize the structure and function of these fascinating light-harvesting antennae (Glazer et al. 1983). The DNA photolyase to which the eukaryotic cryptochromes are compared was also purified from *S. elongatus* (Tamada et al. 1997). The facile transformation of PCC 7942 enabled the demonstration in this cyanobacterium, not then possible in chloroplasts, that a specific mutation the *psbA* gene confers resistance to herbicides widely used in photosynthesis studies and agriculture (Golden and Haselkorn 1985). For many years following publication of the complete nucleotide sequence of the genome of *Synechocystis* sp. strain PCC 6803, the first cyanobacterial genome sequence (Kaneko et al. 1996), research surged using that organism and waned for *S. elongatus* PCC 7942. Now that genome sequences are available for many cyanobacteria, including the *S. elongatus* tribe, PCC 7942 has enjoyed a new popularity as a premier strain for metabolic engineering (Kanno et al. 2017), and for the extensive characterization of its robust circadian clock (Cohen and Golden 2015). The isolation of UTEX 2973, being developed as a new bioproduction chassis (Yu et al. 2015), and UTEX 3055, which retains its “wild” phenotypes, promises more discoveries and applications springing from *S. elongatus* in the future (Yang et al. 2018).

Take-home lessons

Many scientists and discoveries with links to *S. elongatus* owe their success to international face-to-face conversations that would be unlikely to arise by electronic communication. Good old-fashioned conferences can provide pivotal interactions that change the course of one’s career. Scientific research is an ecosystem, and the informal exchange of information among colleagues supports the network.

There is a more than 60-year history of research using strains of *S. elongatus*, but the changes in nomenclature that have occurred over the years obscure the relevance of past work for researchers working with these isolates today. Moreover, the overlap of names between the current *S. elongatus* and thermophilic strains that are not close relatives can lead one astray. It pays to ask questions of those who have been in the field many years to uncover work that may boost your own, and to read the materials and methods of papers to ensure that you know what organism is being described: the cyanobacteria currently known as *S. elongatus* will not grow above 45 C.

Acknowledgements

I dedicate this manuscript to Lou Sherman, Kees van den Hondel, and Sergey Shestakov, who made it possible for me to build a career working with the fascinating *S. elongatus* PCC 7942. I thank Rosi Rippka and David Nobles who, along with Lou, Kees, and Sergey, helped me to compile an accurate history of the various isolates of this organism, and aided my research in other ways over the years. The research in the S. Golden lab is supported by a grant from the US National Institutes of Health (R35 GM118290).

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