Bioprospecting for Microalgae

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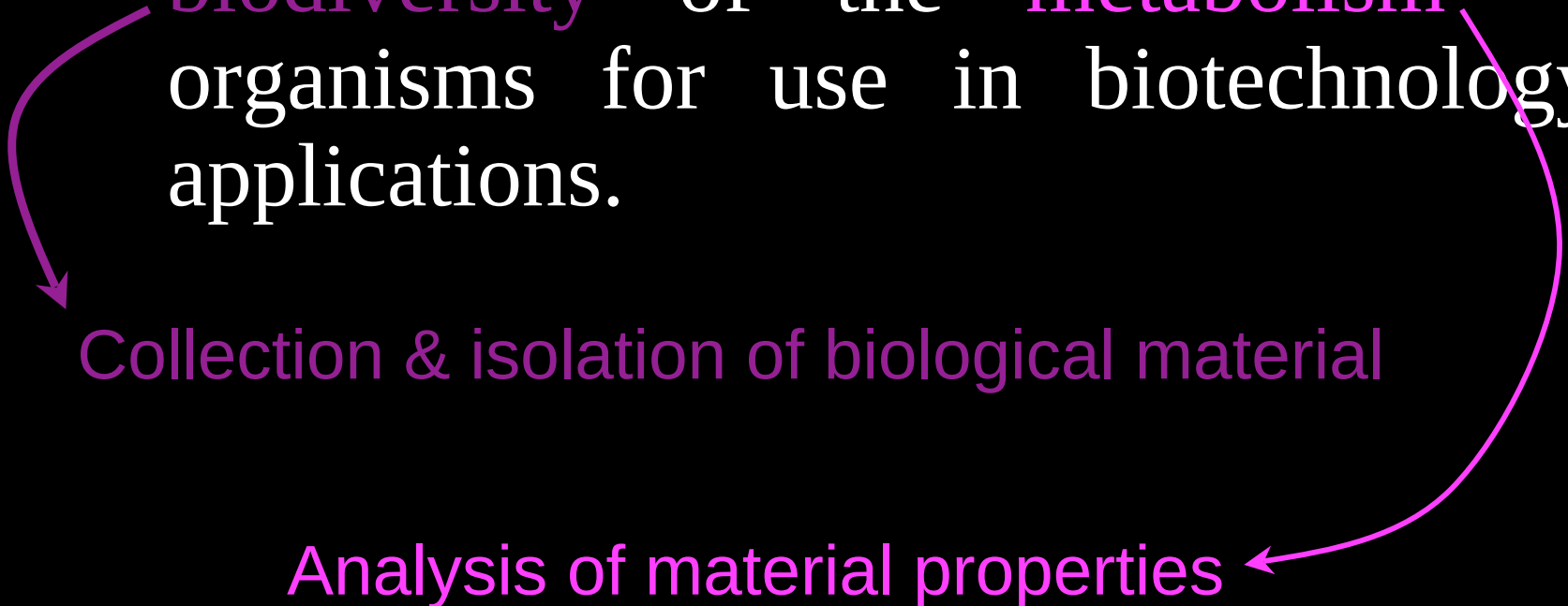
Presentation Outline

- Bioprospecting
- Culture Collection Overview
- Sampling for new microalgae
- Isolation
- Identification
- Challenges

Bioprospecting

The term refers to the process of exploring biodiversity of the metabolism of organisms for use in biotechnology applications.



Microalgae Collection Efforts in the US

In the USA microalgae collection efforts with establishment of culture collections date back to the early 1920's.

Major Collections

UTEX, Texas http://www.utex.org with ~3000 strains
Originally established by Dr. Starr at Indiana University in 1953.

CCMP, Maine http://ccmp.bigelow.org/ with ~ 2400 strains
Location, Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, ME.
Originally established by Dr. Provasoli at Yale University and Dr. Guillard at Woods Hole Oceanographic Institution.

HCC, Hawaii http://0-cmmed.hawaii.edu.pugwash.lib.warwick.ac.uk/research/HICC/index.html
Less than 200 strains remaining from the collection efforts of the Aquatic Species Program and about 170 Cyanobacteria from the Mitsui collection from Florida.

Microalgae Collection Efforts

CCME, Oregon - ? strains

University of Oregon Culture Collection of Microorganisms from Extreme Environments. Created in 1999 by the consolidation of the collections of Dr. Friedmann of Florida State University and Dr. Castenholz of the University of Oregon. (NASA-Ames center affiliation)

Carolina Biological Supply Company, North Carolina http://www.carolina.com/ with ~ 50 strains

Founded in 1927 by Dr. Thomas E. Powell, Jr.

ATCC, Virginia http://www.atcc.org/ - ? strains

Established in 1925 by a committee of scientists.

Bioprospecting of Microalgae for Biofuels

Previous work on strain isolation:

- Aquatic Species Program.

Examples for other more recent efforts:

- Florida Institute of Technology researchers will enrich, isolate, screen and select algal strains with high oil content (Jan 22nd 2008 press release).
- South Australian Research & Development Initiative (SARDI) within the Biofuels Research Program Objective (see website).

Bioprospecting of Microalgae for Biofuels

Goal: About 150 strains of green algae & diatoms.
Current collection efforts are independent of potential later use in outdoor pond systems or in photobioreactors.

Strategy

Dual spacial and temporal sampling approach

Various locations with a number of different sampling sites.

Sample from the same site at different times during the year.

Sampling for Novel Microalgae Strains

- Habitats
  - > Freshwater
    - Ponds
    - Lakes
    - Creeks
    - Rivers
  - > Brakish
    - Estuaries
  - > Marine
    - Beaches
  - > Hypersaline
    - Salt Lakes
    - Lagunes
- Locations
  - > New York
  - > Salton Sea
  - > Great Salt Lake

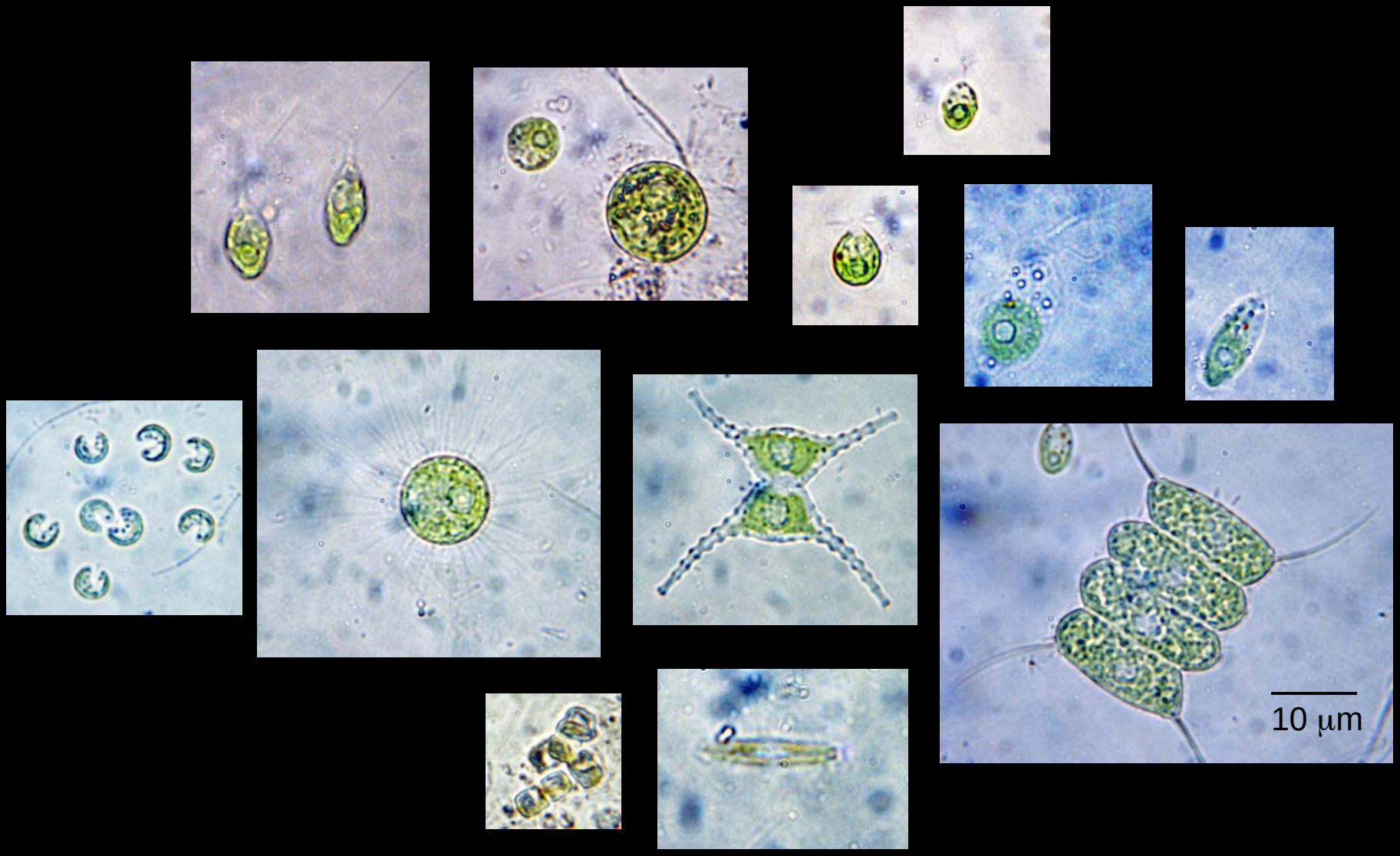
Sampling Location Brooklyn, NY

Sampling Location Salton Sea, CA

Sampling Location Great Salt Lake, Utah

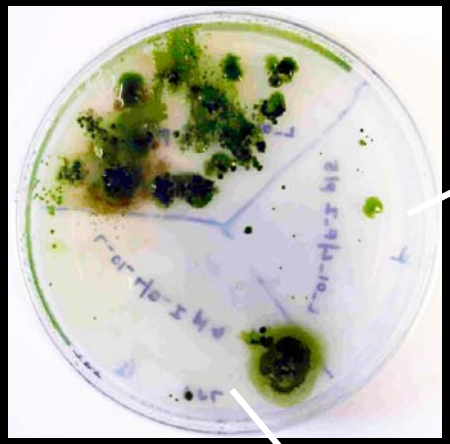
Sampling

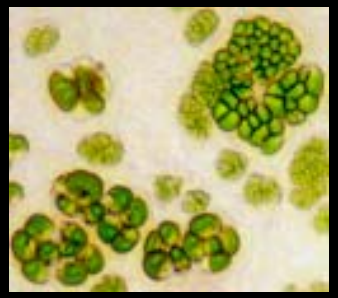
Example for Biodiversity in Freshwater



Isolation of Novel Strains

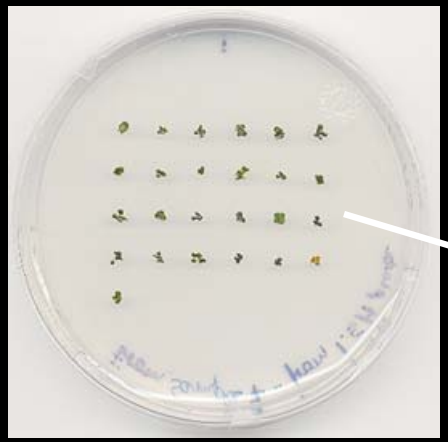
2. Direct plating

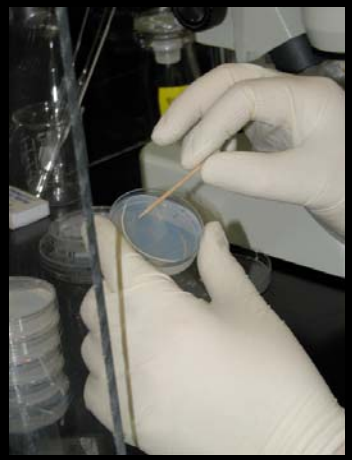




1.Enrichment in liquid media

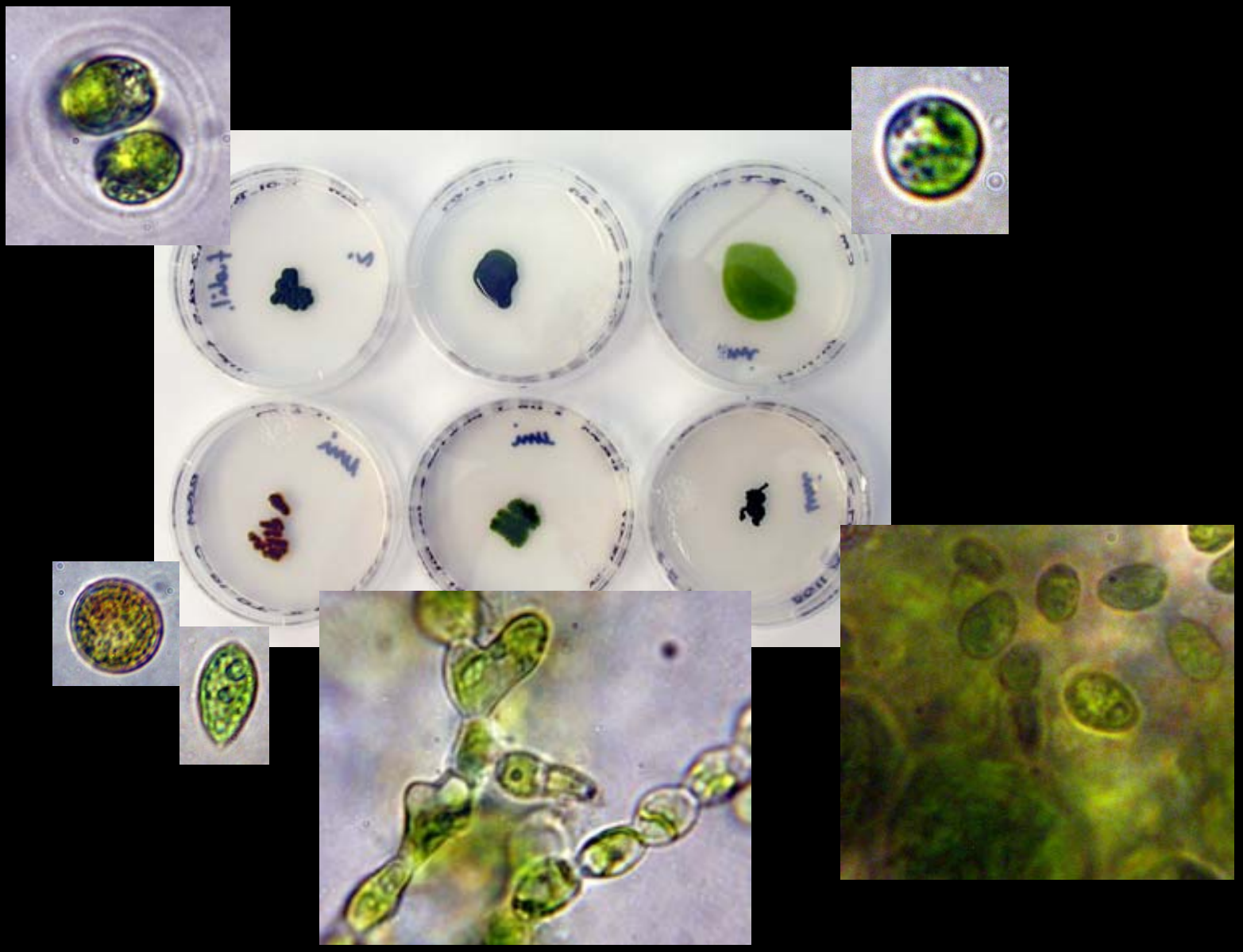
transfer to index plates





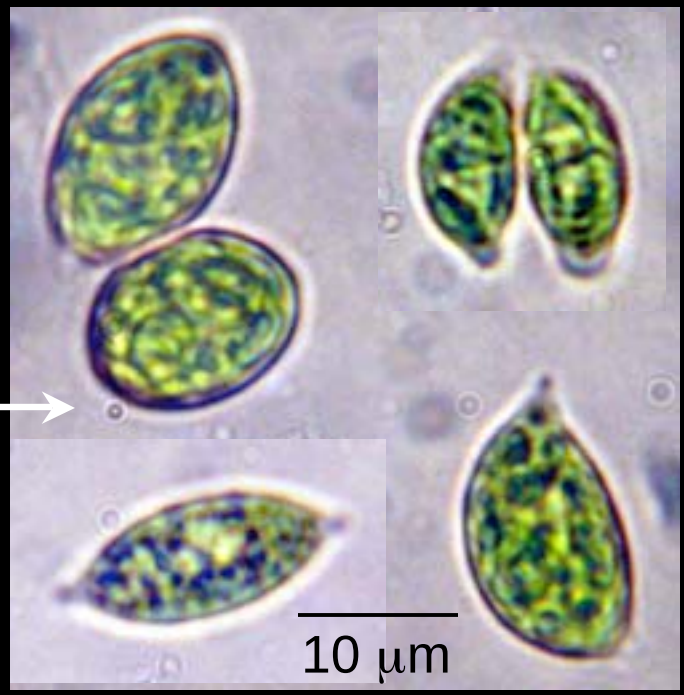
transfer to single plates

Identification of novel Strains?



Identification of Strains

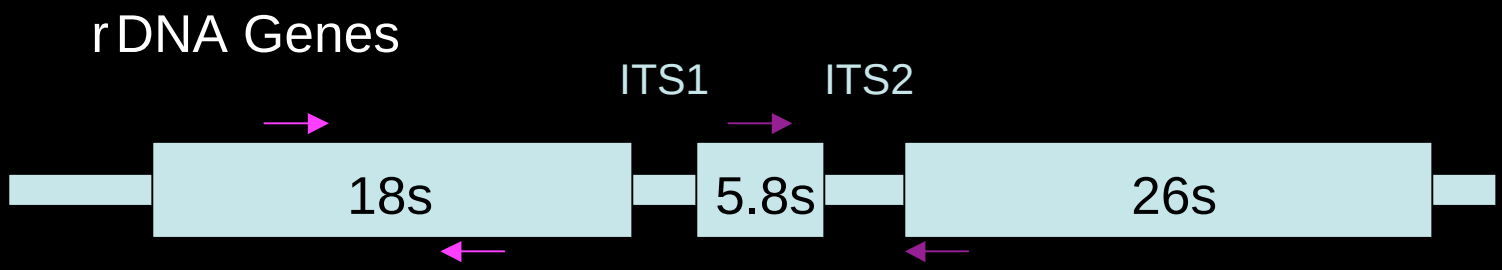
1. Morphology
   - Cell size
   - Cell shape
   - Cell wall
   - Flagella
   - Eye spot
   - Pyrenoid



Identification of Strains

2. Molecular Markers

A. Nuclear



B. Plastidal

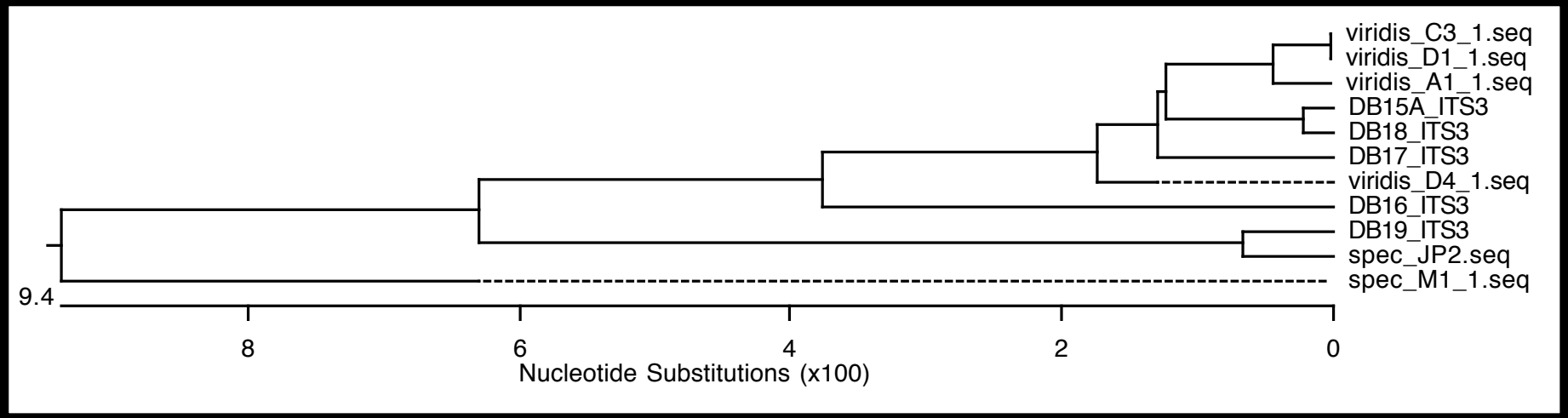
C. Mitochondrial

Phylogenetic Analysis based on partial 18s rDNA

Scenedesmaceae sp. Tow 9/21 P-1w 18S ribosomal RNA gene, partial sequence
Scenedesmus deserticola isolate BCP-HAF2-VF10 18S ribosomal RNA gene, partial sequence
Scenedesmus deserticola isolate BCP-YPGChar 18S ribosomal RNA gene, partial sequence
Scenedesmus deserticola isolate BCP-SNI-2 18S ribosomal RNA gene, partial sequence
Scenedesmus deserticola isolate BCP-EM2-VF3 18S ribosomal RNA gene, partial sequence
Scenedesmus deserticola isolate BCP-EM2-VF30 18S ribosomal RNA gene, partial sequence
Scenedesmus bajacalifornicus isolate BCP-MX7-VF7 18S ribosomal RNA gene, partial sequence
Scenedesmus bajacalifornicus isolate BCP-LG2-VF34 18S ribosomal RNA gene, partial sequence
Scenedesmus sp. LG2VF16 18S small subunit ribosomal RNA gene, partial sequence
Scenedesmus obliquus 18S rRNA gene, strain UTEX 1450
Scenedesmus acutus 18S rRNA gene, strain MPI
Scenedesmus ovalternus 18S rRNA gene
Tetradesmus wisconsinensis gene for 18S rRNA, complete sequence
Scenedesmus pectinatus var. distendus gene for 18S rRNA, complete sequence
Scenedesmus obtusus gene for 18S rRNA, complete sequence
Scenedesmus acuminatus gene for 18S rRNA, complete sequence

Phylogenetic Analysis based on rDNA ITS2

Diversity of *Dunaliella* species at the Great Salt Lake



Summary

- About 150 strains are unialgal.
- About 60 strains potentially unialgal.
- A large number of strains is at the stage of two to three strains per plate.
- A large number of enrichment cultures exist that await further analysis.
- Current efforts focus on strains identification.

Future work - Screening of Strains for Metabolites

- Lipid Production
  - Nile Red screening
- Hydrogen Production
  - Plate essay
- Carbohydrate Production
- Hydrocarbon Production

Challenges in Strain Isolation

1. Labor Intensive

2. Time consuming

3. Permits for Sampling

Challenges in Bioprospecting

1. Intellectual Property rights of original owners need to be protected.

2. A policy for strain distribution is necessary.

3. Material Transfer Agreements protecting the original owners and intermediaries (collector/isolator) are mandatory.

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