The Little Green Plant That Could: Duckweed as a Renewable and Sustainable Biofuel Feedstock

Philomena Chu, Nikolai Borisjuk, Eric Lam
Department of Plant Biology & Pathology | Rutgers, The State University of New Jersey

Renewable and Sustainable Fuel Solutions for the 21st Century
A NSF IGERT Project at Rutgers University

Introduction

Currently, 71% of the US petroleum supply supports the transportation sector. The majority of this industry is derived from petroleum (94%) with only 4% from renewable energy [1]. As energy demand increases, alternatives must be sought to replace the non-renewable resources.

The development of renewable and sustainable biofuels will aid in overcoming these daunting economic and environmental challenges. Corn, the main biofuel feedstock in the United States today, has serious drawbacks as an energy source; its high-maintenance cultivation requires significant amounts of fertilizer and freshwater while competing with food crops for arable land. In contrast, the small aquatic plant duckweed, comprising 37 species over 5 genera, has emerged as a highly promising biofuel feedstock. Its composition—high starch (up to 75% dry weight) [2] and low lignin (~5%) [3]—is ideal for ethanol production. Furthermore, duckweed does not compete with land crops, has a doubling rate ranging from ~24 hours to 3 days [4], and is easily harvestable. We are conducting pilot-scale studies to assess the potential of growing duckweed on wastewater for biofuel production.

Optimization of this approach involves screening duckweed plants to find a strain that is best suited for each purpose. These efforts will lay the groundwork for future experiments, providing the much needed insight into basic duckweed biology for environmental and energy applications.

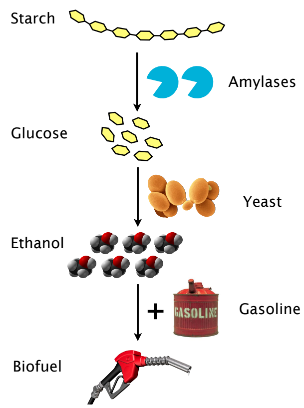
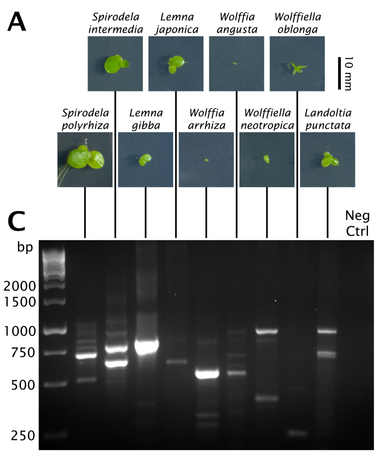


Fig. 1. The process of biofuel production.

Genotyping duckweed



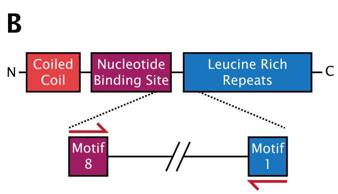


Fig. 2. Genotyping approach. (A) Duckweed species. (B) Structure of NBS-LRR gene. Primers (red arrows) anneal to conserved motifs within the indicated domains. The intervening region is of variable length. (C) Gel of PCR-amplified fragments using primers from (B). Species show unique banding patterns.

Duckweeds are relatively easy to distinguish at the genus level but their discrimination proves more challenging at the lower taxonomic levels; many species, and especially strains, are morphologically similar or identical (Fig. 2A) but have varying physiological characteristics. To be able to characterize each strain for its potential use in future applications, we first need an efficient and robust technique to identify individuals.

We employ a molecular method called genotyping, which exploits the allelic variation in duckweed strains. Highly variable regions of DNA, flanked by conserved sequences, are amplified by polymerase chain reaction (PCR). Our target region is located in Nucleotide Binding Site-Leucine Rich Repeat (NBS-LRR) genes, a sub-class of the disease resistance genes (Fig. 2B). Amplification of this region produces a species-specific banding pattern (Fig. 2C). Further work will be performed to determine if a strain-specific pattern exists. To discern finer differences among the NBS-LRR alleles, the PCR products will be analyzed by capillary electrophoresis, which measures the length (in base pairs) of each fragment. Our goal is to be able to genotype each duckweed strain from unique and reproducible banding patterns.

Duckweed growth on wastewater

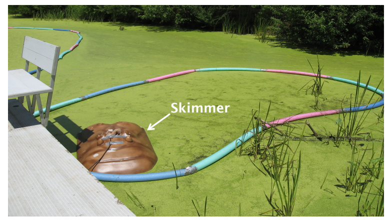
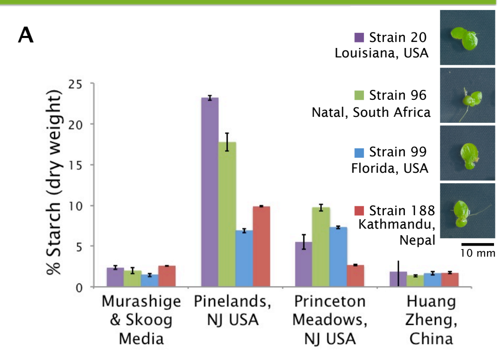


Fig. 3. Duckweed growing within retainment barrier on a fertilizer run-off pond in Columbus, NJ. A motorized pond skimmer (pictured) is used to harvest duckweed.

Excess nutrients in wastewater must be removed prior to disposal to avoid detrimental impacts on waterways. Wastewater is an inexpensive and abundant source of fertilizer that provides duckweed with nitrogen and phosphorous, essential for biomass growth (Fig. 3). In turn, the duckweed cleans the wastewater, which can be recycled. Because duckweed floats on the water's surface, it is easy to harvest. Our aim is to develop duckweed as an environmentally-friendly biofuel feedstock by growing it on agricultural runoff or sewage.



B

Nutrient	MS	PL	PM	HZ
NH_3-N (mg/L)	351	0.3	82	320
P (mg/L)	119	0.5	0.7	18.5

Fig. 4. Duckweed strains of *Spirodela polyrhiza* species accumulate starch to different levels. (A) Starch content of *S. polyrhiza* strains growing on different wastewater sources after 2 weeks. (B) Ammonia-nitrogen (NH_3-N) and phosphate (P) measurements of wastewater sources.

Duckweed strains from different geographical locations vary in their response to environmental factors. For biofuel production, our target is a strain with a high starch content and quick growth rate. We selected fast-growing *Spirodela polyrhiza* strains from the Rutgers Duckweed Stock Cooperative and analyzed their starch content after a 2-week growth period on different wastewater compositions (Fig. 4). We compared accumulation on wastewater from treatment facilities in New Jersey and China as well as a nutrient-rich tissue culture medium (Murashige and Skoog). An inverse correlation between starch accumulation and nutrient load was observed.

Conclusions & Future Work

The development and optimization of duckweed as a renewable and sustainable biofuel feedstock may lead to a cleaner future. Many of duckweed's traits make it a promising candidate for biofuel production: fast growth rate, low lignin, easy harvestability. In addition, the advantages of growing duckweed on wastewater is two-fold. First, duckweed readily uses cheap and abundant wastewater as fertilizer, absorbing the nutrients that would otherwise pollute coastal and freshwater waterways. Second, it can accumulate high levels of starch, a starting material for bioethanol. Because the nutrient load from wastewater treatment plants varies in composition, more screening needs to be conducted to systematically identify optimal strains for each water source, i.e. those with the highest growth rate, target product accumulation and removal of contaminating nutrients. For that purpose, we also need a robust and reliable method of distinguishing duckweeds, where morphological identification is problematic. Duckweed genotyping will allow us to be able to identify and characterize duckweeds at the strain level. Distinguishing strains by their molecular signature will aid future efforts in a duckweed-based fuels landscape.

References

[1] Energy Information Administration, Annual Energy Review, U.S. Department of Energy. 2010.
[2] Reid, MS, & Bieleski, RL. 1970. *Plant Physiol.* 46(4): 609-13.
[3] Chen, Q. et al. 2012. *Energies.* 5, 3019-3032.
[4] Landolt, E. & Kandeler, R. 1987. The family of Lemnaceae, a monographic study. Veroff. Geobot. Inst. ETH, Zurich.