Accomplishments Report (2010-2014)
Western Regional Biomass Research Center

Agricultural Research Service
U.S. Department of Agriculture

This report summarizes the accomplishments of the Agricultural Research Service's Western Regional Biomass Research Center (WRBRC) focusing on feedstock development, feedstock production, and conversion and co-product utilization. A key purpose of the report is to provide information for an external assessment of the WRBRC's performance over the past four years (2010-2014).

Overview

In 2010, four regional USDA Biomass Research Centers were established to coordinate USDA-ARS intramural research to help accelerate the establishment of commercial biofuel supply chains based on agricultural feedstocks. The WRBRC is a network of existing ARS facilities and scientists located in Arizona, California, Colorado, Hawaii, Nevada, New Mexico, and Utah.

Research Units Reporting

Plant Physiology and Genetics Research Unit – Maricopa, AZ
Crop Improvement and Utilization Research Unit – Albany, CA
Bioproduct Chemistry and Engineering Research Unit – Albany, CA
Genomics and Gene Discovery Research Unit – Albany, CA
Tropical Plant Genetics Resources and Disease Research Unit – Hilo, HI

Accomplishments

Feedstock Development

Using next-generation sequencing technologies for linkage mapping in switchgrass, ARS scientists in Albany, CA have increased the density of markers and enabled direct links with the draft switchgrass genome. This work used an existing linkage mapping population; however, the techniques can be applied to controlled crosses or natural populations as well in order to link traits to specific genetic loci. Additional efforts by scientists in Albany are underway to apply genome editing and insertional mutagenesis technologies to grasses. Genome editing techniques use site specific nucleases to induce mutations at a target sequence and will allow hypothesis about function to be tested genetically for virtually any sequence target. Application to cellulosic energy crops will provide a new biotechnological approach to modify traits in a relatively benign fashion without introducing non-native sequences that some find objectionable. Insertional mutagenesis in the model grass Brachypodium has thus far generated over 32,000 sequence-tagged T-DNA insertions that are being made available to the plant research community as 20,000 individual lines for screening purposes. These lines can be studied to test predictions about the function of the disrupted genes (Bragg et al., 2012; Li et al., 2014). CRIS Project 5325-21000-017

California almond growers generate a $4B/yr industry by producing roughly 100% of the US domestic market, but they are looking for new commercial outlets for almond byproducts (shells and hulls). Markets are tight for shells due to the rapid growth in almond production and the drought-accelerated decline of the dairy industry take up the shells for cattle bedding and hulls as a feed component for dairy cattle. In collaboration with and with financial support from the Almond Hullers & Processors Association, ARS scientists proposed new uses for almond byproducts. Together they completed a State-wide sampling and analysis program to determine sugars and other components in almond hulls and shells based on their locality, variety, harvesting/storage conditions and age. Results showed that hulls contain up to 32% free (readily fermentable) sugars which was readily converted into ethanol for biofuels use, but could also have food and/or nutraceuticals applications. Highest yields for hull-derived sugars proved to be from the nonpareil almonds, the market leader (Offeman et al, 2014; Gong et al., 2011; and Wood et al., 2013). CRIS Project 5325-41000-049-00

In the process of photosynthesis, plants convert light into chemical energy. The energy produced by photosynthesis is then used to synthesize the carbon compounds that are harvested for food, fuel, fiber or other natural products. Consequently, photosynthesis determines the overall yield of the plant. ARS scientists at the US Arid Land Agricultural Research Center in Maricopa, AZ, uncovered important new information about the control of Rubisco, the rate-determining enzyme in photosynthesis, by its regulatory companion, Rubisco activase. The research showed that changes in the regulatory properties of Rubisco activase affected the rate at which photosynthesis turned on when light was increased. These findings suggest a new strategy for increasing photosynthetic performance in certain variable light environments based on altering the regulatory properties of Rubisco activase (Henderson et al., 2013; Carmo Silva and Salvucci, 2013). CRIS Project 5347-21000-012-00

Hawaii imports over 85% of its food, and depends on imported petroleum to meet 89% of its energy needs. The overall goal of this project is to produce biofuel from heterotrophic algae that use waste products from agriculture systems or regional plant material that are weedy and widespread, based on the concept of zero-waste. Thirty-five percent of all papaya brought to the packinghouse is discarded as waste. Hawaii now produces about 20 million pounds of papaya waste a year. Using papaya waste for biofuels and feed adds value to the papaya crop. Using this approach, the farmer gains a new revenue stream that will increase their income and allow them to raise more papaya. This would increase their fresh sales and increase the amount of fuel and obtained from papaya waste. Algae strains (*Chlorella prototechoides*) were adapted to grow on papaya fruit puree by our cooperator BioTork, and used in the Daniel K. Inouye Pacific Basin Agricultural Research Center (PBARC) laboratory in experiments to develop conditions to grow algae and maximize algae oil production for biofuel and algae meal for animal feed. ARS Scientists from Hilo, HI have made significant progress in using papaya as a feedstock for algae under laboratory conditions and are getting ready to move towards the mini-pilot plant scale. CRIS Project 5320-21000-015-00D

Given the competition for the water resources in Hawaii and across the Pacific Basin islands, sustainable production of bioenergy feedstocks will be driven by management strategies that optimize water use efficiency, enhance feedstock yields, while minimizing environmental impacts. ARS scientists and partners were able to obtain crucial model parameterization data for evaluating water use efficiency and potential bioenergy cropping systems' impacts on ecosystems services; soil organic carbon, greenhouse gas fluxes (CO_2, N_2O and CH_4 and related soil properties (moisture, temperature, pH, nutrient contents). Simulations to compare the productivity and global warming potentials (GWPs) of sugarcane (a crop with a high water-demand) and banagrass (a relatively drought tolerant bioenergy crop) at three irrigation levels are being conducted on the HC&S sugarcane lands in Maui, Hawaii. The goal is to develop a unified modeling framework for assessing the dependability of biomass supplies for use in the production of biofuels for the Navy. Funding was provided by the Office of Naval Research. Set to begin in 2014, scientists will adapt and apply the generated technologies to the southern USA, a region which has the perfect agroclimate for optimizing bioenergy feedstock production. CRIS Project 5320-21000-015-15R

Anaerobic digesters utilizing biofuel that could be adapted to the farm scale level in Hawaii would help to alleviate fossil fuel import dependence and rising energy costs. Anaerobic digesters are ideal for utilizing waste streams because the output of this process results in production of energy, fertilizer, and value added products. Albizia is an invasive tree throughout the Pacific islands that would be an abundant feedstock source. Collaborative research with the USDA Forestry and the University of Hawaii at Hilo has used high-resolution satellite imagery and geospatial and remote sensing software to map the current spread of albizia in the Hilo and Puna districts on the island of Hawaii. This albizia detection technology will be extended to measure albizia stands throughout the state of Hawaii. We will utilize albizia as a potential feedstock and identify additional feedstocks that selected microbes will efficiently digest feedstock combinations under anaerobic conditions. Albizia and agricultural sweet potato and taro waste products were sent to the Ohio State University for biochemical analysis to determine methane potential under anaerobic conditions. CRIS Project 5320-21000-015-00D

Feedstock Production

Torrefaction is a thermomechanical process involving heating and compression of wood and ag-residues (under low-oxygen conditions), resulting in a readily burnable fuel: "biocoal". Western-based utility companies have utilized biocoal to generate electricity, but are not willing to pay the higher prices. To offset the cost of transporting biomass, ARS Scientists and their CRADA partner, Renewable Fuel Technology, built a mobile torrefaction unit mounted on an 18-wheel flat-bed truck, and tested it on various biomass sources available in the West (see http://renewablefueltech.wordpress.com/). In research funded through a significant grant from the CDFA, this team compared torrefied products and energy balances for wood chips, olive grove trimmings, grape pomace, olive pomace, apple pomace, tomato pomace, almond shells, and walnut shells. In a different approached, this team showed that torrefied biomass can significantly improved the mechanical properties of plastics; when added at 2-5% composite-filler, torrefied almond hulls improved the heat-softening temperature of packaging plastics by up to 24°C. These composites were sent to FDS, one of the leading ag-packagers in the Western US and prototype flower pots were made. Their current flower pots become soft during very hot summer days and these composites would eliminate this softening behavior (Chiou et al., 2012; Chiou et al., 2014). CRIS Project 5325-41000-056-00

Conversion and Co-Product Utilization

ARS researchers at Albany, CA continue to work with biorefinery operators in the West to develop strategies for optimizing biofuels production. This includes continuing a program with UC Extension Scientists and Chromatin, a sorghum seed company, to assay sorghum as a bioenergy crop. In collaboration with several companies, ARS Scientists from Albany developed a 1 ton/day integrated biorefinery at the Salinas CA, Crazy Horse Landfill that has utilized a variety of biomass feedstocks. This process puts out ethanol, compost material and/or biogas, thus it tests the practicality of integrating ethanol plants with other operations; specifically integrated digesters to produce biogas, and biogas-derived products, including power and bioplastics (Holtman et al., 2010a, 2010b, 2012, 2014a, 2014b; Orts et al., 2010; Offeman et al., 2010, 2014; Robertson et al 2011). CRIS Project 5325-41000-049-00

In collaboration with industry partners, key quality parameters (molecular weight, ash, dirt, and volatile content, others) have been established for guayule natural rubber latex and dried bale rubber derived from guayule grown in the US. Guayule natural rubber latex met the ASTM Category 4 specification for all 11 ASTM physical and chemical methods in ARS documented lot-to-lot testing over all a full year's production. The data demonstrated that guayule latex has consistent physical and chemical properties; a requirement for successful commercialization. Separately, bench-scale process for solvent extraction of bale guayule rubber was scaled to pilot plant (25L and 100L) reactors, to successfully recover kg quantities of solid guayule natural rubber. Physical and chemical characterization of the rubber confirmed the extracted rubber met or exceeded industry standards for Hevea Technically-Specified Rubber (TSR). Guayule latex products, meet ASTM D1076-06 Category 4 standard for latex that is safe for people suffering from Type I latex allergy, and solid bale guayule natural rubber are available for commercial development, and in some cases, are now being used for commercial products (Dong et al, 2013a; Dong et al. 2013b). CRIS Project 5325-21410-020-00D

Metabolic engineering for increased yield in guayule is a key to sustainability of this important domestic natural rubber-producing crop. ARS scientists have created genetically-modified guayule plants expressing key isoprenoid pathway or downregulating competing pathway genes and evaluated phenotypes in the laboratory for 16 transgenes/combinations (HMG-CoA Reductase, Transcription Factors, Farnesyl Pyrophosphate Synthase, Sucrose:sucrose 1-fructosyltransferase, Allene Oxide Synthase, Squalene Synthase and cis-prenyltransferases). Methods have been developed for early detection of the efficacy of metabolic engineering, reducing the time required to assess the impact of genetic manipulation on phenotypes. Transgenic guayule lines expressing six of these constructs have been evaluated in field. Enhanced rubber content in guayule is critical for sustainability of this new industrial crop. These results have identified several strategies that may prove efficacious, and an increase in rubber yield up to 4-fold has been demonstrated at the laboratory scale (Dong et al., 2013a; Dong et al., 2013b; Whalen et al., 2013). CRIS Project 5325-21410-020-00D

Knowledge of genetic sequences is the first step to more effectively engineer biochemical pathways for production of natural rubber. ARS Researchers (Albany CA) published the first genomic sequence information for guayule, a natural rubber-producing crop under developed in the southwestern US. A transcriptome (highly expressed gene sequences) from cold-treated bark tissue high in rubber production revealed sequences for all known rubber-biosynthesis genes, many stress-related genes, and genes of unknown function. The information is a significant step forward in determination of the molecular basis of rubber. Metabolic engineering for increased yield in guayule is a key to sustainability of this important domestic natural rubber-producing crop (Ponciano et al., 2012). CRIS Project 5325-21410-020-00D

Guayule is a US native crop under commercial development in the SW USA for production of natural rubber and bioenergy. Increasing the yield of natural rubber, using the tools of biotechnology, could significantly impact the economic sustainability of this new crop. ARS scientists in Albany, CA developed a successful method for chloroplast transformation of guayule, for the first time providing a new method to insert important rubber pathway genes. Chloroplast transformation is especially attractive in that it allows high levels of foreign proteins to be produced while preventing escape of foreign genes, since guayule pollen contains no chloroplasts. Genetically-modified guayule with high levels of natural rubber and low environmental impact could be developed with this technology (Kumar et al, 2012; McMahan et al., 2014). CRIS Project 5325-21410-020-00D

ARS researchers in Albany, CA, in collaboration with tire industry manufacturers and the National Renewable Energy Laboratory, conducted a Life Cycle Analysis comparing petroleum-based and bio-based material usage in tire manufacturing. The analysis estimated the potential impact of biobased materials technology on reducing oil dependency related to 1) raw material manufacture, 2) gasoline savings from improved fuel efficiency, 3) net energy savings associated in making raw materials, and 4) the potential impact on greenhouse gas emissions. The use of natural rubber in all tire components was premised. The analysis effectively demonstrated the positive contributions the US tire industry could make toward the goal of decreasing petroleum dependency by converting from petroleum based materials to non-petroleum based materials (Ray et al., 2010; Kim and McMahan, 2010). CRIS Project 5325-21410-020-00D

Expansion of cultivation of guayule, a natural rubber-producing crop under development in the US southwest, would be significantly enhanced by demonstration of the suitability of use of guayule rubber in modern tires. ARS Scientists (Albany CA, and Maricopa AZ), in collaboration with multiple partners, developed a detailed program to close key technology gaps in feedstock development, agronomics, extraction process development, and design, construction, and testing of a commercial passenger tire based on guayule rubber. The knowledge gained will inform future public and private domestic rubber research and development toward a more sustainable path (Sanchez et al., 2014a, 2014b). USDA-NIFA/DOE Biomass Research and Development Initiative Grant No. 2012-10006-19391, Securing the future of natural rubber – An American tire and bioenergy platform from guayule

Kazak dandelion (*Taraxacum kok-saghyz*) roots have the potential of being a new source of natural rubber, inulin, and biomass for bioenergy in the USA and presently, there is a high level of industrial interest. ARS scientists at Albany, CA and Pullman, WA, in collaboration with scientists from Kazakhstan, mounted a 2008 Kazak dandelion collecting expedition in the high river valleys in southeastern Kazakhstan. Collections were made from 22 different populations have been deposited in the National Germplasm Collection. Increased Kazak dandelion genetic diversity in the USDA-ARS germplasm collection will now allow greater germplasm selection, from the former two viable accessions, to more than 20 different accessions. Over 400 seed packets have been distributed to individuals and organizations in 6 countries (US, Canada, China, Korea, Germany and France). Russian dandelion from this collection is growing in test plots in Virginia, Ohio, Washington, Nevada, California, Europe, and other locations. Half of the original collected material was transferred to the Vavilov Institute of Research (VIR), St. Petersburg, Russia, which will contribute to the worldwide availability of *T. kok-saghyz*. The VIR collection will be able to grow from having no viable accessions, to more than 20 different accessions. Following deposition of Kazak dandelion germplasm from expedition, characterization of plant phenotypes was completed. Individual line phenotypes (plant biomass, root morphology, rubber and inulin stored in the root tissue) were developed from 2010, 2011, and 2012 harvests of greenhouse and field plots in partnership with the ARS-NPGS Western Regional Plant Introduction Station and the University of Nevada-Reno. CRIS Project 5325-21410-020-00D

Genetic improvement of Lesquerella, an oilseed producing crop, could provide a biomass source of hydroxy fatty acids, high value biobased raw materials. Scientists at Albany, CA demonstrated genetic modification of Lesquerella using a seed-specific promoter to focus only on plant tissues of interest, those where oil is stored. Next, transgenic lines were successfully modified to incorporate a key gene from castor responsible for hydroxy fatty acid (HFA) synthesis. Fifteen transgenic lines have been made, including 4 lines with increased HFA production. Transgenic Lf lines with enhanced HFA and oil content could be developed for commercial production. Seed oil of Lesquerella fendleri (Lf) contains hydroxyl fatty acids which have valuable industrial uses, for example in manufacture of lubricants, plastics, and cosmetics. Genetic transformation, a key technology for crop improvement, is only successful if all plant cells are modified in a permanent manner. ARS researchers in Albany, CA, developed protocols to overcome the hurdles of formation of mixtures of transformed/untransformed cells in (Lf) transformation, and were the first to generate stably transformed Lf lines. The transformation system will further commercialization efforts by allowing the engineering of varieties with herbicide and disease resistance, and production of oils that have not be obtained through traditional breeding (Chen and Lin, 2010; Chen et al., 2011a, 2011b; Chen, 2011; Chen and Lin, 2012). CRIS Project 5325-21410-020-00D

To engineer seed oils or fatty acid contents in Lesquerella, a promising industrial oilseed for the arid west, it is preferable to utilize seed specific promoters, to limit the changes of oils and fatty acids to the seed, and to avoid the changes in membrane lipids in other parts of the plant. ARS scientists in Albany, CA, have cloned and sequenced the upstream regulatory region of *napA* gene from Rapeseed (*Brassica napus)* and tested the tissue specific expression pattern of this promoter in *Lesquerella*. Five independent transgenic lines were grown to maturity and generated transgenic seeds. The activity of *napA* promoter was examined in various organs and tissues, including leaf, stem, root, flower and developing seeds. For ll transgenic lines, the *napA* promoter showed activity only in developing seeds, not in any other organs and tissues. The *napA* promoter can be used to express a target gene and to facilitate future research on genetic engineering of seed oil in *Lesquerella* (Lin and Chen, 2013a, 2013b, 2014). CRIS Project 5325-21410-020-00D

The toxin ricin continues to be a concern as a byproduct of castor oil production, both as a threat agent and as a biohazard. Researchers at WRRC in Albany, CA have addressed this concern through several approaches. They have identified an approach that improves processing of the castor seed, treating it with an enzyme that eliminates the ricin protein from the seed cake, making it safe to handle. A recent concern raised in some states is the presence of volunteer castor plants in fields resulting in contamination of the field crop with castor seeds if castor becomes a widely cultivated crop. We have demonstrated low level herbicide (glyphosate) spraying, may be effective in eliminating castor volunteers from most fields of major crops. In cooperative research with another research unit at WRRC and a collaborator in Spain, we have identified castor cultivars with reduced levels of ricin for inclusion in breeding programs to eliminate ricin from castor. Each of these approaches can help to eliminate ricin as a problem and a perceived concern, thus fostering domestic production of castor oil, a key chemical feedstock with yields of up to 2500 lbs. of oil/acre, equivalent to replacing 8 barrels of petroleum with a renewable product that can be converted to uses currently supplied by petroleum (Severino et al., 2012; McKeon et al., 2012). CRIS Project 5325-21410-020-00D

The economic competitiveness of biorefineries is improved by development of optimal use of fibers and biorefinery coproducts. ARS Scientists from Albany, CA developed an array of fiber composites and nanocomposites that improved the commercial application of biopolymers in specific commercial markets. The corn-based biopolymer, polylactic acid (PLA), which is produced at a significant scale by Cargill at one of their larger biorefineries, isa stiff polymer with a very low softening point; material properties that have prevented wide-scale use for objects in contact with hot foods and drinks. The research team from Albany, in collaboration with several industrial partners, developed polymer additives that (1) makes the PLA plastic more flexible and (2) raises its softening temperatures. Patents were filed on these additives which were then commercialized as (1) card stock (for book covers and credit cards), (2) as flexible drink bottles, and (3) as single-use food utensils. In collaboration with another commercial partner, fibers from rice shells were ground and added to PLA to create biodegradable plastic forks and knives, with commercialization being carried out in Northern California. USDA's CRADA partner is marketing these single-use compostable utensils at large cafeterias throughout the West (including Google's employee café) and at Whole Foods markets. In similar research with a large Home Products company, wheat proteins were chemically modified to make natural polymer-based superabsorbent polymers. The wheat proteins were heat-treated with phosphoric and/or citric acid resulting to create a modified polymer that now absorbs up to eighty-eight times its weight in water. ARS then incorporated antimicrobial agents into these fibrils and tested them in home studies for application as "green absorbents" in diapers. This team continues to find the best end-use value for rice straw, wheat straw, tree prunings, and other agricultural residues (Teixeira et al., 2014; Robertson et al., 2014, 2011; Medeiros et al., 2014, 2012; Bilbao-Sainz et al., 2014, 2013; Moreira et al, 2013; Imam et al, 2013; Chiou et al., 2013a, 2013b, 2010; Wood et al., 2013; Glenn et al., 2013; Stevens et al., 2010; Rosa et al., 2010). CRIS Project 5325-41000-056-00

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