Lee 9/12/14

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MTSU Clean Energy Initiative Project Funding Request

There are five (5) sections of the request to complete before submitting. See http://www.mtsu.edu/sga/cleanenergy.shtml for funding guidelines. Save completed form and email to cee@mtsu.edu or mail to MTSU Box 57.

1. General Information	
Name of Person Submitting Request **Dr. James Brian Robertson and Dr. Elliot Altman**	
Department/Office **Biology / SCI 2053 and 2067**	Phone # (Office) **898-2066 and 494-8681**
MTSU Box # **60**	Phone # (Cell)
E-mail **james.robertson@mtsu.edu** **elliot.altman@mtsu.edu**	Submittal Date 9/14/14

2. Project Categories (Select One)			
Select the category that best describes the project.			
	Energy Conservation/Efficiency		Sustainable Design
X	Alternative Fuels		Other
	Renewable Energy		

3. Project Information
a. Please provide a brief descriptive title for the project. **b.** The project cost estimate is the expected cost of the project to be considered by the committee for approval, which may differ from the total project cost in the case of matching funding opportunities. **Any funding request is a 'not-to-exceed' amount. Any proposed expenditure above the requested amount will require a resubmission.** c. List the source of project cost estimates. d. Provide a brief explanation in response to question regarding previous funding.
3a. Project Title **Development of a novel microbial consortium approach to produce ethanol biofuel from tree and grass**
3b. Project Cost Estimate $5,000 – supplies for the Robertson laboratory $4,500 – supplies for the Altman laboratory $9,500 – Total

Supplies include oligonucleotide primers, reagents to prepare high quality DNA, restriction and modification enzymes for cloning, chemicals for treating microbes, plastic consumables, sequencing reagents, media and supplements. (An itemized list of supplies and their costs can be provided if requested).
3c. Source of Estimate Based on MTSU prices from Fisher Scientific, Invitrogen, New England Biolabs, Promega, Sigma-Aldrich and VWR Scientific.
3d. If previous funding from this source was awarded, explain how this request differs? Dr. Robertson was funded in 2012 for a proposal titled "Development of a Biosensor to Detect Hydrogen Production in Photosynthetic Microbes", which involved hydrogen fuel cells and thus is completely different from this proposal.

4. Project Description

(Completed in as much detail as possible.)

a. The scope of the work to be accomplished is a detailed description of project activities.
b. The benefit statement describes the advantages of the project as relates to the selected project category.
c. The location of the project includes the name of the building, department, and/or specific location of where the project will be conducted on campus.
d. List any departments you anticipate to be involved. Were any departments consulted in preparation of this request? Who? A listing may be attached to this form when submitted.
e. Provide specific information on anticipated student involvement or benefit.
f. Provide information for anticipated future operating and/or maintenance requirements occurring as a result of the proposed project.
g. Provide any additional comments or information that may be pertinent to approval of the project funding request.

4a. Scope: Work to be accomplished

US economists, legislators and scientists have realized for a long time now that it is crucial for our country to minimize its dependency on foreign crude oil as a source of energy. In 2013 289 billion gallons of oil were consumed by the US. Most of this oil was purchased from foreign countries and the great majority of it was used to produce gasoline fuel. A number of legislative mandates have been issued recently to address this problem, beginning with the widely endorsed Energy Independence and Security Act of 2007 which called for the US to produce 36 billion gallons of renewable fuels per year by 2022 and specified that half of these fuels had to be made from lignocellulosic biomass feedstocks such as trees or grass instead of corn, which has historically been the feedstock of choice to produce ethanol biofuel.

Ethanol has long been realized as a viable replacement for gasoline. In fact the first car sold by the Ford Motor Company, America's first car manufacturer, was designed to run on ethanol, not gasoline. Ethanol is also more cost effective to make than gasoline. Ethanol is produced from sugars, such as glucose, at a yield of 50%, while gasoline is produced from crude oil or petroleum at a yield of 45%. Our countries agricultural infrastructure can only grow enough corn to produce 15 billion gallons of ethanol per year, which is far short of the nation's goal of producing 36 billion gallons of renewable biofuels per year. Our country's agricultural infrastructure can grow more than enough trees and grass to meet this goal. Unfortunately it is far easier to produce ethanol from corn than it is to produce ethanol from trees or grass.

Microorganisms are used to convert sugars into ethanol and while almost any sugar can be converted into ethanol, glucose is the most preferred sugar by microorganisms. Corn contains starch that is easily converted to glucose, which can be used to produce ethanol. Trees and grass contain cellulose and hemicellulose, which are also a significant source of sugars, however, it is a lot more difficult to extract the sugars from trees or grass. Lignocellulosic biomass must be treated with high heat and or steam and aggressive depolymerization agents such as concentrated acids or bases to obtain the sugars. Unlike corn grain, which yields the single sugar glucose, trees and grass yield a complex mixture of sugars; arabinose, galactose, glucose, mannose and xylose, which must be independently converted into ethanol. Additionally, the aggressive conditions used to liberate the sugars from lignocellulosic biomass also releases acetate, furfural and hydroxymethylfurfural, which are potent inhibitors of microbial growth.

A number of laboratories have been working to develop microorganisms that can convert the mixed sugar streams generated from lignocellulosic biomass into ethanol. Their strategies, to a fault,

have focused on developing genetically engineered single "do it all" "super bugs" that can remove the inhibitors and convert all of the sugars into ethanol. This approach has yet to yield any practical microorganisms that can be used to effectively and economically produce ethanol in an industrially relevant biorefinery. The problems noted include the inability to remove the inhibitors fast enough for the microorganisms to grow and the inability of the microorganisms to consume the mixed sugars at the same time. Researchers have found that regardless of what genetic manipulations they try, glucose is always consumed first and preferentially, and the other sugars cannot be utilized until the glucose is consumed; a fact that results in very inefficient processes.

The Altman laboratory in collaboration with colleagues at the University of Georgia have developed an alternative strategy that can efficiently detoxify the inhibitors found in lignocellulosic hydrolysates and convert the mixed sugar stream into useful products. Instead of a single microorganism, this approach uses a consortium of microorganism, where each microorganism in the consortium has been genetically engineered to only be able to consume a single sugar at peak efficiency. The approach also utilizes two separate stages. In the first stage a consortium of microorganisms that can consume the inhibitors as carbon sources, but are unable to consume any of the sugars that are to be used to produce ethanol, are used to detoxify the lignocellulosic hydrolysate. In the second stage another consortium of microorganisms are used to convert the mixed sugars into useful products. In this consortium each microorganism can only consume one of the sugars present in the mixed sugar solution. The xylose consuming microorganism, for example, can only consume xylose and cannot consume glucose or the other sugars. To date this approach has been used to engineer bacteria that can produce important green biochemicals, such as succinate and pyruvate, which are utilized in industry to make plastics and other useful products. The next logical step is to use this approach to address the country's energy objectives.

The purpose of this proposal is to utilize the consortium approach to produce fuel ethanol. Because the organism of choice to produce ethanol is yeast, new strains will need to be engineered to employ this technology. The Altman laboratory will focus on the development of the strains necessary to detoxify the inhibitors present in lignocellulosic hydrolysates, while the Robertson laboratory will focus on the development of the strains necessary to convert the mixed sugars into ethanol. This is a natural collaboration as the strengths of both the Altman and Robertson laboratories will be needed to engineer the required strains. The Altman laboratory has significant expertise in utilizing genetic engineering to produce industrially important green chemicals, while the Robertson laboratory has significant expertise in the genetic manipulation of yeast. The two

biggest hurdles remaining to economically generate ethanol from lignocellulosic hydrolysates are effective approaches to 1- remove the inhibitors and 2- efficiently convert the mixed sugars into ethanol. This proposed research solves both of these problems.

4b. Scope: Benefit Statement

This proposal could lead to new microorganisms that will allow the mixed sugars obtained from trees and grass to be efficiently and economically converted into ethanol fuel and thus finally bring to fruition the lignocellulosic biomass ethanol plants that have been called for in numerous legislative mandates since 2007. This proposal also represents an important economic opportunity for the state of Tennessee, which does not have an ethanol biorefinery. States with ethanol biorefineries are able to hedge against increases in gasoline prices when the price of crude oil increases because they can generate their own gasoline ethanol mixtures and avoid the costs of shipping ethanol from elsewhere in the US. The success of this proposed research would place MTSU in position to be the advocate for the first ethanol biorefinery in Tennessee.

4. Project Description (continued)

4c. Location of Project (Building, etc.)

This research will be conducted in Dr. Robertson's lab in SCI 2050 and DR. Altman's laboratory in SCI 2070. The close proximity of our two laboratories allow for a highly effective collaboration.

4d. Participants and Roles

Dr. Robertson will supervise the construction of the strains necessary to convert the sugars arabinose, galactose, glucose, mannose and xylose into ethanol.

Dr. Altman will supervise the construction of the strains necessary to remove acetate, furfural and hydroxymethylfurfural from the mixed sugar streams that are obtained from trees and grass.

4e. Student participation and/or student benefit

This research project will be completely student-driven. Dr. Robertson and Dr. Altman will lend their expertise and advice to guide the project but the hands-on construction and development will be done by the students. Portions of this research will be completed as modules by the students in BIOL 4550/5550 (Biotechnology) and BIOL 4450/6450 (Molecular Genetics). Both of these courses are integral to the Biology undergraduate core and are offered in both the Fall and Spring semesters. BIOL 4550/5550 accommodates 48 students each semester while BIOL 4450/6450 accommodates 24 students each semester. Thus a large number of undergraduate students will be benefited by this proposal.

4f. Future Operating and/or Maintenance Requirements

This is a one-time investment in research material needed to construct the strains required to implement the consortium approach to produce ethanol from the mixed sugars obtained from trees and grass.

4g. Additional Comments or Information Pertinent to the Proposed Project

This proposal is for technology development with a proposed timeframe of two semesters to construct the strains required to create the microbial consortium. Large scale production of ethanol is beyond the scope of this proposal. However, should this research be successful, the goal of scaling up production may be worthy of another proposal in the future.

5. Project Performance Information

Provide information if applicable.

a. Provide information on estimated annual energy savings stated in units such as kW, kWh, Btu, gallons, etc.
b. Provide information on estimated annual energy cost savings in monetary terms.
c. Provide information on any annual operating or other cost savings in monetary terms. Be specific.
d. Provide information about any matching or supplementary funding opportunities that are available. Identify all sources and explain.

5a. Estimated Annual Energy Savings (Estimated in kW, kWh, Btu, etc.)

See Below

5b. Annual Energy COST Savings ($)

This project by itself does not produce energy cost savings, however it is the beginning of a series of steps that can ultimately lead to reducing our nation's dependence on fossil fuels and the need to import them from foreign countries. Successful completion of this work can provide the impetus for Tennessee to develop regional ethanol biorefineries so that our locally produced grasses, agricultural residue, and tree remnants can provide an avenue for fuel cost savings. Research that benefits development of locally produced renewable fuels reduces transportation costs and provides flexibility to offset the volatility of the fuel market.

5c. Annual Operating or Other Cost Savings. Specify. ($)

5d. Matching or Supplementary Funding (Identify and Explain)

All of the equipment needed for this research has been purchased using other funds. Additionally, student labor for this project will be funded through teaching assistantships.

Linda Hardymon

From: James Robertson
Sent: Friday, September 12, 2014 2:45 PM
To: Center for Energy Efficiency
Subject: Research Proposal
Attachments: CEI Proposal Robertson Altman 2014 final.doc

Dear Clean Energy Initiative Committee,
Here is a research proposal for an alternative fuels project headed by a collaboration between two labs at MTSU (Robertson lab and Altman lab).
Feel free to email me if you have a question about the application.

Thanks,
Brian

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