AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
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TITLE: Renewable biodiesel from crop residues

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SUMMARY: This proposal focuses on a new and valuable use for Oregon wheat straw--the production of biodiesel. The development of renewable energy sources to replace fossil fuels is an urgent national priority and lignocellullosic biomass which includes crop residues is a recognized and attractive starting material. The generation of biodiesel from wheat straw holds particular promise because it allows dual use of cropland—harvest of wheat for food and subsequent harvest of a second "crop" (straw) to process into biofuel. Biodiesel production in the U.S. currently exceeds 1 billion gallons/yr and is expected to increase in response to the national "Renewable Fuel Standard" mandate established in 2008 for continued targeted increases in future use of renewable biofuels. The data obtained in this study will provide key information needed to convert a largely underutilized crop resource into a valuable byproduct.

OBJECTIVES:
1. Evaluate a limited number (2-5) of oleaginous yeast strains for the ability to accumulate fat (lipids) following growth in wheat straw-derived liquors (*yr 1*)
2. Optimize growth conditions for 1-2 of the most promising strains (*yr 2*)

PROCEDURES:

We evaluated a limited number of so-called "oleaginous yeasts" that have the natural ability to accumulate ≥20% fat (lipid) on a dry weight basis following growth on sugar. Yeast cultures were grown aerobically at room temperature for 48 hours in a complex laboratory medium containing either xylose or glucose as carbon source. At the end of the growth phase, cells were washed and incubated in either a xylose-only or glucose-only solution for 36 hours to allow lipid accumulation. Growth was assessed by counting cells or monitoring turbidity (A_{600}). Cells were ruptured by bead beating and lipids were extracted using the Folch procedure. Lipid yields were measured gravimetrically. Individual acyl groups were determined and quantified by fatty acid methyl ester analysis. In order to evaluate possible value of the aqueous extract as a substitute for commercial yeast extract, the aqueous extract was dried, dissolved in a small volume of distilled water to make a 5 or 10X concentrate, sterile-filtered, and stored at 4°C. This yeast extract was then used as an ingredient in formulating the complex laboratory medium used to grow cells to allow comparison with commercial yeast extract.

SIGNIFICANT ACCOMPLISHMENTS:
1. Determined that for *Lipomyces starkeyi* 78-23, high lipid accumulation can occur by simply incubating a fully-grown culture in a sugar solution. Most studies have used a more expensive sugar-rich medium lacking nitrogen.

2. Determined that the aqueous extract from *Lipomyces starkeyi* 78-23 had greater potency than commercial yeast extract. That is, it is a richer source of nutrients needed for yeast growth.

BENEFITS & IMPACT:
Both findings have practical value. Finding #1 suggests a simpler and more economical lipid accumulation phase for *Lipomyces starkeyi*. Finding #2 establishes that two valuable products can be harvested from *L. starkeyi*. One is the original target--the lipid that can be converted into biodiesel. Unexpectedly, the aqueous extract was also found to have value as a source of nutrients for growing *Lipomyces starkeyi*. This finding could potentially reduce reliance on commercial yeast extracts.

ADDITIONAL FUNDING RECEIVED: none; rejected grants were submitted to the DOT/USDA-funded Sun Grant Western Regional program and to the USDA-NIFA Biomass Research and Development Initiative (BRDI).

FUTURE FUNDING: we will consider future RFPs from the above and related national programs that focus on renewable biodiesel from non-standard lipid sources.