Study Shows Potential for Using Algae to Produce Human Therapeutic Proteins

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Pharmaceutical companies could substantially reduce the expense of costly treatments for cancer and other diseases produced from mammalian or bacterial cells by growing these human therapeutic proteins in algae-rapidly growing aquatic plant cells that have recently gained attention for their ability to produce biofuels.

That's the conclusion of a study, published online this week in *Plant Biotechnology Journal,* which sought to determine whether seven diverse human therapeutic proteins could be produced in *Chlamydomonas reinhardtii,* a green alga used widely in biology laboratories as a genetic model organism, much like the fruit fly *Drosophila* and the bacterium *E. coli.*

"What surprised us was that of the seven genes chosen, four expressed proteins at levels sufficient for commercial production," said Stephen Mayfield, a professor of biology at the University of California, San Diego who headed the study, which involved scientists at The Scripps Research Institute, San Diego biofuel company Sapphire Energy and ProtElix, a protein engineering company in Hayward, CA.

The scientists reported in their paper that all of the algal-produced proteins in their study showed biological activity comparable to the same proteins produced by traditional commercial techniques. And because algae cells can be grown cheaply and quickly, doubling in number every 12 hours, they noted that algae could be superior to current biological systems for the production of many human therapeutic proteins.

"Currently, human therapeutic proteins are primarily produced from either bacteria or mammalian cell culture," they said. "Complex mammalian proteins and monoclonal antibodies are primarily produced by the culture of transgeneic mammalian cells, while simpler proteins are generally produced by *E. coli.* "

"Due to high capital and media costs, and the inherent complexity of mammalian cell culture, proteins produced by mammalian cell culture are very expensive," they added. "Bacterial production is generally more economical in terms of media components, but bacteria are often inefficient at producing properly folded complex proteins, requiring a denaturation and renaturation step that adds significant costs to bacterial protein production."

The scientists said the percentage of human proteins produced in their algal cultures that were properly folded in three dimensions was comparable to the fraction produced by mammalian cell cultures and much better than that produced by bacterial systems. And because algae generate their energy from sunlight and have relatively simple nutrient needs, they said the costs for using them at large scale to commercially produce human proteins should be much lower than for mammalian cell culture, which require expensive fermentation facilities.

To conduct their study, the scientists picked seven proteins that were either currently being used as standard treatments for diseases or are now undergoing human clinical trials. They include human interferon #1, which is used to treat Multiple Sclerosis and costs patients from $1,600 to $2,000 for a one month supply; human erythropoietin or EPO, used to increase red blood production in patients undergoing chemotherapy; and human proinsulin, a hormone with a multi-billion dollar market used to treat Type 1 diabetes. Two other proteins were

human vascular endothelial growth factor or VEGF, used to treat patients suffering from pulmonary emphysema, and high moblility group protein B1 (HMGB1), which activates immune cells and is being investigated for its potential to enhance other cancer therapies. The remaining two proteins were domains 10 and 14 of human fibronectin, which are being investigated for their ability to mimic certain kinds of antibodies.

Mayfield and his colleagues at The Scripps Research Institute demonstrated two years ago that they could produce a mammalian serum amyloid protein from algae and, last year, demonstrated success producing a human antibody. Both of these proteins had biological activities similar to the real proteins from mammalian cells.

"That was the proof of concept," said Mayfield. "It showed us that the system works-that we could produce complex mammalian proteins in algae. What we did in this next study was to say, 'Let's take seven diverse human therapeutic proteins and see if we can express them in algae and report the good and the bad.'"

The scientists found that in algae they were able to produce VEGF, HMGB1 and domain 14 of human fibronectin at levels above one percent of total soluble protein, levels sufficient for easy purification. Domain 10 of human fibronectin could also be produced from algae at these levels when fused to the protein M-SAA, which they had previously used to increase the accumulation of other proteins. Human proinsulin could be produced by algae, but only at lower levels, the study showed, while human interferon #1 and EPO were not produced by algae.

"What our results show is that algae are a robust platform for the production of human therapeutic proteins," said Mayfield. "While not every protein can be produced in algae, a good fraction can, just like in any other system. You can get expression of about 25 percent in bacteria and about 40 to 50 percent in mammalian cells, so we're in the same ball park as these other systems."

What makes algae particularly attractive compared to bacterial and mammalian systems, the scientists say, is its ability to produce proteins cheaply and at very large scale. With algae currently being produced at about $3 per kilogram at commercial scale, the researchers estimate that making recombinant protein would cost about 60 cents per gram prior to purification.

"This is about the same cost estimates for the least expensive protein expression systems presently available, and considerably cheaper than mammalian cell culture," they said in their paper. With expected improvements in the ability to express proteins in algae, "and the continued reduction in algal biomass cost associated with the large scale efforts to use algae for biofuel production, we anticipate at least a ten-fold reduction in the costs over the next few years, which should make algal protein production the least expensive platform available. This reduced cost of goods, coupled with an ability to rapidly scale production in inexpensive bioreactors, suggests that algae may become an economically superior platform for therapeutic protein production in the future."

In a separate, but related effort, Mayfield and his colleagues are using various species of algae to investigate ways of generating renewable forms of transportation fuel from algae that could eventually be competitive with the cost of gasoline.

Other researchers involved in the therapeutic proteins study were Beth Rasala and Michal Jager of UCSD; Machiko Muto of TSRI; Mike Mendez, Philip Lee, Rosa Cardoso and Craig Behnke of Sapphire Energy; and Peter Kirk and Roberto Creo of ProtElix. Grants and other financial support from the National Institutes of Health, Sapphire Energy and the San Diego Foundation supported the study.

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