DEMI-GREEN

The Evolution of an Algae-Based Nutrition Platform

Abstract

The process begins with the process itself: The discovery, optimization and validation of a largely unknown algal species for a wide range of food and supplementation applications

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1.0 Exploring the Ethical Development of Nutritive Algal Products

ZIVO Bioscience, Inc., a US-based R&D firm, holds intellectual property in the form of a proprietary, optimized strain of filamentous freshwater algae, extracts and bioactive compounds thereof, production methods, post-processing and product formulation concepts for food, supplement and other high-value applications in humans, companion animals and livestock. The intellectual property is supported by *in vitro*, *ex vivo* and *in vivo* studies, as well as nutritional, toxicological and gene activation testing.

The company has engaged in a comprehensive development program beginning with strain discovery through validation, compliance and cultivation to post-processing and productizing, effectively "owning" the entire value chain.

In partnership with the Arizona Center for Algae Technology & Innovation (AzCATI), located at the Polytechnic campus of Arizona State University in Mesa, AZ, ZIVO Bioscience has optimized a largely unknown, wild freshwater algae strain for commercial phototrophic production. Test cultivation has been conducted at AzCATI facilities, as well as the National Center for Marine Algae and Microbiota (NCMA), which is also the repository for the genetic material that supports three issued US patents and several applications pending globally.

Over the last four years, ZIVO Bioscience has also engaged in a wide range of *in vitro, ex vivo and in vivo* studies to validate the beneficial aspects of its optimized strain, safety and nutritional value, cultivation techniques and post-harvest processing to build a novel nutrition platform that can result in potentially dozens of useful applications.

2.0 The Business Case: Affordable. Sustainable. Adaptable.

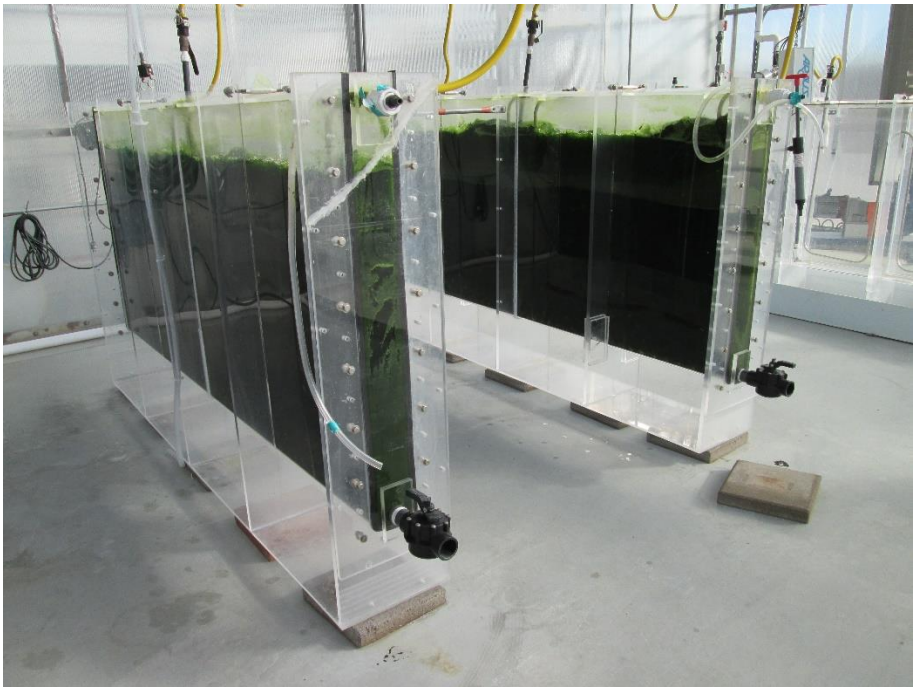
In a departure from R&D typical for the algae industry, ZIVO developed its business model first, and only then selected and optimized an algae strain from its proprietary polyculture that best fit the criteria of low startup cost, sustainability, high yield, continuous harvest, optimal levels of protein, micronutrient and non-starch polysaccharides (NSP's), ease of post-processing and the potential of multiple applications across animal species.

Affordable.

The cultivation model developed by ZIVO and its partners eschew complex and costly fermentation systems, photo-bioreactors, panels and tubes associated with microalgae production in favor of the most basic and cost-efficient models: a covered, shallow pond constructed of inexpensive, readily-available materials obtainable in many parts of the world. The ideal algal strain would flourish in the simplest of production environments at the lowest possible cost without a necessary dependence on fossil fuels to maintain high yields -- an affordable approach that can be practiced most anywhere.

Covered pond at Arizona Center for Algae Technology & Innovation facility

Even though the strain was optimized in laboratory and clean-room conditions, the objective was to develop a strain that would grow rapidly and continuously with yield targets per liter as good or better than phototropic species currently under cultivation, in conditions that would reduce the likelihood of contamination and competition. Those targets have been largely achieved, and ZIVO is now conducting commercial scale-up efforts and various post-processing experiments, with the intent of feeding the minimally processed biomass, extracts or pelletized product first to monogastric subjects, followed by ruminants.



Culturing ZIVO strain algae in flat-panel photobioreactors at the Arizona Center for Algae Technology & Innovation

Water is critical to any form of agriculture for obvious reasons. However, clean water is quite expensive in many parts of the world, both monetarily and in level of effort to secure an abundant and reliable supply to support farming of any kind. Under ideal conditions, a fast-growing alga such as the ZIVO

strain may produce up to 38 times the amount of protein as soybean using about 33% of the water consumed in growing soybean, according to various industry sources. Evaporation rates can vary according to local climates and cultivation pond size, but the larger point is that water consumption is dramatically lower than soybean cultivation and the water can be recycled 3 to 4 times.

Sustainable.

Optimized phototrophic microalgae such as the ZIVO strain are uniquely suitable for sustainable cultivation as they efficiently capture solar energy. The energy efficiency (food energy output/kg/energy input/kg) is 5 times higher than soy, twice that of corn, and over 100 times higher than grain-fed beef[1].

Yield per acre is equally important. Under ideal conditions, the same amount of land used for growing corn could produce up to 125 times more protein from microalgae like the ZIVO strain,[2] in part due to continuous harvest year-round. The ZIVO strain in particular can be harvested weekly year-round, with no re-start or re-inoculation necessary. It will grow continuously as long as sunlight, water and nutrients are supplied. Test cultures at the AzCATI facility in Arizona remained in continuous culture for more than one year, undergoing partial weekly harvests.

ZIVO optimization goals consist of yield targets, nutrient consumption, water usage/recovery, lowest-cost production methods, low energy requirements, carbon dioxide sequestration, short grow/harvest cycles, continuous harvest, high protein and NSP levels at harvest, among others. Genetic mapping ensured cultivation of the parent ZIVO strain and provide the basis for IP protections.

Adaptable.

Uses for the ZIVO strain are wide-ranging, from non-GMO plant proteins and beneficial micronutrients for human consumption to companion animal supplements and livestock productivity. Initially, the focus is on humans, followed by cattle, both dairy and beef, where incremental use of algae feed ingredients derived from or consisting of algal biomass in general appears to produce beneficial results in early testing.

For human food ingredient and dietary supplement ingredient uses, the company is conducting the necessary studies and compiling data to meet US/EU compliance requirements as dried algal biomass. The powdered form contains approximately 45% pure protein, according to the National Food Laboratory. The powder is almost completely odorless and tasteless, which will favor rapid adoption and consumer acceptance. Initial toxicology tests conducted by Greenwater Laboratories have indicated no neurotoxins, bacterial LPS or chemical contamination from the cultivation/harvest process.

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[1] Habib, M. B., Parvin, M., Huntington, T. C., & Hasan, M. R. (2008*). A Review on Culture, Production and Use of Spirulina as Food for Humans and Feeds for Domestic Animals and Fish*. FAO.org

[2] Amy Westervelt, Forbes, May 4, 2012, "*Forget Fuel, Algae Could Help Feed the World"*

As a poultry feed ingredient, ZIVO follows others into the poultry nutrition market, where early indications are that small amounts of algal biomass are beneficial to animal health, which in turn is very likely to increase productivity. The company is taking the position that speed of growth, low cost of production startup and a unique blend of protein, micronutrients and non-starch polysaccharides may result in a feed ingredient less expensive and more healthful than competitive offerings in poultry applications.

Initial experimentation into canine models revealed interesting results that require additional studies. Early indications are that potential benefits derived from a healthy nutritional profile are likely, even though the ZIVO biomass would constitute a relatively small part of the diet ingredient mix.

Aquaculture applications have not yet been explored.

3.0 Using the Entire Organism and its Growing Environment

Unlike grain or vegetable crops, all parts of the organism can be consumed. The algal biomass can be harvested and used in its high-moisture form after pasteurization. The cells can be lysed, using the lysate and the cell walls as substrates mixed with other ingredients, or the biomass can be freeze-dried or desiccated whole.

The growing medium, consisting of water, nutrients, vitamins and trace metals, is infused with non-starch polysaccharides, peptides and bioactive compounds secreted by the algae during the grow cycle, creating a valuable supernatant. After pasteurization and salt titration to remove culture nutrients, a low-energy evaporation process can concentrate this supernatant, composed primarily of soluble polysaccharides, which is then mixed back into the biomass, mixed with other feed ingredients, or re-suspended in sterile water.

The concentrated supernatant is remarkably stable, with refrigerated samples retaining important properties and composition after one year. The supernatant, although exhibiting a golden hue and slightly more viscosity than water, is almost completely odorless and tasteless, exhibiting only a slight hint of freshly-mown grass.

As to the algal biomass, several drying methods are under investigation, with an emphasis on low/no energy consumption. Centrifugation and desiccation follow harvesting. The company is working with cultivation experts to evaluate several post-harvest processing techniques to efficiently yield dry or nearly-dry biomass.

Pilot Production

Production capacity was initially limited to the covered ponds and bioreactors at the AzCATI facility in Mesa, Arizona. However, a larger-scale growing installation has been completed in Vero Beach, Florida where biomass output for compliance studies and post-processing tests is now underway. The two raceway ponds, 35,000L and 45,000L respectively, simulate real-world production conditions – in other words, low-cost, low-complexity installations that can be duplicated almost anywhere water and sunlight are available.

Once cGMP protocols are validated summer of 2016, the R&D team intends to scale up production further to large-scale commercial levels in Florida at a demonstration site currently being evaluated. Initial plans are to test 250,000L to 1,000,000L pond sizes, evaluate and adjust, and if objectives are met, commence to contract other growers across the US and worldwide.

Post-processing and productizing

As of spring 2016, experiments are underway to optimize the delivery of dried biomass as a human dietary supplement ingredient and cattle feed additive. The dried biomass resembles a fine, light green powder. The R&D team is looking to maintain integrity and stability of the biomass. Post-processing techniques may include agglomeration and/or encapsulation, followed by some form of low-temp extrusion into caplets, wafers or pellets.

The company's R&D team expects to test several different formulations or product concepts in a rat model, followed by a human study in summer of 2016. It is likely that one or more such formulations will include other approved ingredients, primarily for binding and stability before use, and to also test the breakdown of the encapsulation matrix as the biomass enter the digestive system.

Compliance Strategy

The company's animal feed ingredient and human dietary ingredient compliance pathways were originally designed to work in parallel, each leveraging the findings of the other. Although there is some overlap, the US regulatory environment for novel animal feed ingredients has become more complex in recent years, especially as it related to algae-based products, adding new steps to the process of approval and product registration. Therefore, human product market approval and registration will likely be first.

As a novel feed ingredient with no prior history of use in humans or animals, it is incumbent on the R&D team to provide an abundance of safety data. The R&D team has positioned the algal biomass first as a useful and nutritionally beneficial food ingredient, supplement or feed additive. Extracts and derivatives will be worked through the regulatory process separately at a later date once safety data and some history of use has been gathered for the biomass. These extracts and derivatives may support additional

beneficial claims. Early testing of the raw biomass *in vivo* showed no indications of distress or adverse events.

Algal biomass, wet or dry, will likely never be consumed in its pre-processed, harvested state. As a dry powder or a slurry, it will always be mixed with other ingredients or excipients to create a finished product. As part of the productizing process, the R&D team would mix the biomass at benchtop sample sizes with additives and ingredients commonly found in finished supplements and assess any interactions, degradation or changes in the biomass. Some or most may be tested further, the findings of which would be included in the regulatory dossier. Any applicable learnings would find their way into the livestock regulatory dossier, as well.

4.0 Nutrition as Personal Health Driver

The field of nutrigenomics posits that an optimal nutritional profile results in optimized expression of genetic potential. Cost modeling and initial testing have indicated that premium nutrition ingredients, including minimally-processed algal biomass or extracts, have the potential to improve health. However, the price/value relationship favors the lowest possible cost of these premium ingredients, which in turn favors the modest startup and production costs of cultivating the ZIVO strain. However, adoption is not necessarily driven by business modeling alone.

Non-GMO

Although not common or abundant in its natural setting, the native form of the ZIVO strain is not an exotic and is thought to be widely distributed. However, a commercially viable microalga specie needs to meet performance and economic criteria that make it suitable for widespread cultivation. Wild type strains are usually just the starting point. This requires optimization, whether by artificial or natural means. Regulatory reactions to genetically modified foods, plants or animals are a reflection of consumer concerns, media coverage and/or political motivation. The net effect is that a non-GMO classification becomes a marketing and branding advantage, hastening adoption and expanding market share.

The ZIVO optimization process, utilizing natural selection over the course of many generations, was conducted under laboratory conditions using various growing and competition techniques, but ultimately was tested in pilot-production scenarios simulating real-world cultivation methods and compared against existing algae cultivation methods and yield/econometric models. As stated previously, the nutritional profile offered by the ZIVO strain was the net result of meeting performance criteria and commercial targets.

Inoculation and optimization work at the Arizona Center for Algae Technology & Innovation

Antibiotic-Free Animal Husbandry

Also making headlines and setting industry prerogatives is the move toward antibiotic-free animal husbandry, to include aquaculture. ZIVO scientists believe that a healthy immune response, resistance to infection and disease, better growth and productivity are the result of peak health achieved through optimized nutrition and ideal environmental conditions. A number of studies and scientific sentiment seems to favor the notion that optimized nutrition may be a viable substitute for pre-emptive use of antibiotics for growth or productivity enhancement.

Toward that end, the R&D moved forward with testing the proprietary ZIVO strain as a feed additive that offers a nutritional profile to support a healthy immune response, which is expressed differently in each animal model tested.

Livestock Applications

As a feed ingredient for dairy cows, the Company has conducted *in vitro* and *in vivo* studies to substantiate the usefulness of its processed biomass as a feed additive to fortify generic feed mixes with high-availability non-starch polysaccharides, vitamin A, amino acids and quality protein, which in combination support milk productivity on a herd-wide basis, as well as a healthy immune system.

Among other studies, an *in vitro* study utilizing primary mammary gland epithelial cells was conducted at the University of Wisconsin - Madison Department of Dairy Science, followed by an *in vivo* pilot study of dairy cows conducted at the University of California – Davis agricultural tech facility in Tulare County, CA

with both studies showing promising results. Preliminary nutritional and toxicology studies were conducted at National Food Lab and GreenWater Labs with good results. Final results will be published upon conclusion of a primary study underway. The algal biomass in a commercial feed additive form consists of dried, powdered algae which is agglomerated, encapsulated and pelletized to blend well in mechanized mills and mixers.

In spring of 2016, ZIVO is commencing *in vivo* toxicology/safety studies in the US/Canada for FDA review as a human supplement ingredient and as a cattle feed additive, while simultaneously looking to collaborate with a global food ingredient or supplement marketer to test-market various product formulations.

Summary

ZIVO Bioscience is looking to partner with a global food or supplement company to collaborate on its human supplement initiative, followed by a livestock or companion animal initiative. Research continues, as the ZIVO algae strain appears to be an ideal plant-based source of protein without starchy carbs and is almost completely tasteless and odorless. As a clean, sustainable, non-GMO source of protein, micronutrients and support for a healthy immune response, the ZIVO strain is patent-protected and proprietary. Other nutrition claims may be added as studies continue.

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