Trans. Nat. Acad. Sci. & Tech. (Phils.)
1985.7:119-121

THE TECHNOLOGY OF RESEARCH IN THE BLUE-GREEN ALGAE AND RELEVANCE TO PHILIPPINE ECONOMY

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ABSTRACT

An overview of the country's present economy with several examples are mentioned in order to effect solutions discussed by the audience. Among those problems where some thoughts have not been properly discussed are: 1) the application of chemical fertilizers; 2) the inoculation of heterocystous blue-green algae in the ricefields; 3) the inclusion of the science of Genetics; 4) stagnant production of the fishponds along the far-away fertile shorelines; 5) inefficient management and/or unprepared workers might have been the major cause(s) of the present closing of several activities, like factories; 6) Japan, later Hongkong and Taiwan are examples of more productions; and 7) discussion partly of the present sciences – how they are possibly available.

Presentation

In the present condition of the local economy, it appears that all of us must be equally concerned. The rich and the poor alike, indeed all walks of life including some of the politicians who must have taken their time quite individually. It is presently the 84th year of my life when such a condition of the country has been much felt. At this age though I have never thought of the serious effects of the time now felt by many. On the other hand, the Philippines has been a blessed country to live in where the natural resources are practically ready for food. Other countries if not with barren lands have winter and other obstructions.

However, the nutritional well-being of the people in the Orient are generally relevant to the nitrogen of the majority of the lands in agriculture. In our country, the fishes – fresh and salt water are the main sources of cheap proteins. Nature has so adapted itself in the tropical climate so that the pattern of rainfall becomes equally adaptable to the rice production of the people. Later it becomes logical to refer to the blue-green algae systematically as it was done in India and Japan. The people had studied simultaneously that the heterocystous filaments of the blue-green algae are excellent sources of nitrates.

But as a negative example, the application lately of chemical fertilizers had been found to be not only expensive. Besides, chemical fertilizers also pollute the adjacent waters in agriculture where the rivers must be their final drainage. Fishes of these rivers die or weaken slowly as affected by the chemical fertilizers.

An experiment was introduced to inoculate the heterocystous blue-green algae in the ricefields and observed later that the rice plants grew faster and more vigorously. The harvests had been repeated two to three times a year without much decrease of average yields. With such results, the farmers found the inoculation of the blue-green algae to be successful. The experiment should be continued and emulated. But the general Filipino farmers usually continue their old ways. This situation should expect naturally our support, that is, we should extend to them more encouragement. (Refer to the history of the majority of the present farmers).

Some farmers however may include the application of the science of Genetics to improve the grain harvests. Genetics is a necessary tool in many ricefields abroad to improve permanently the grain yield during the rice seasons. A good farmer with his faith in the inclusion of Genetics should expect better rice harvests. Such increase in the rice grains is more permanent than usual where only the weather conditions constitute the natural factors during the seasons.

Another idea is the stagnant production of the fishponds along the far-away fertile shorelines in the country. Observations during a decade show that the Philippines could maintain usually the successful harvest of fish in successive years. And yet, the fishponds along the shorelines had been repeatedly producing the same. To replace the workers in the fishponds became very necessary in order that the fish production may be improved. The former (inexperienced) ordinary workers need their replacement by those who are more trained workers. Eventually, after sufficient recruitment of new efficient workers, the ponds yield fish as expected. In the country, there are many usually lopsided industries which have not reached a more systematized management.

In Japan, and now in Hongkong and Taiwan, the workers in the factories are privileged to give suggestions to improve the efficiency of their factories. Accordingly, the laborers have been promoted more often when they suggest better methods of increasing factory production. I have also known some instances where we can maintain reciprocal suggestions from Japanese foreign scientists. Dr. Hisao Ogawa still maintains friendly relations with the Philippines partly due to our past research activities in Bangkok for many years. As a result, more Japanese algologists have decided to come to the Philippines for reciprocal scientific activities in the middle of November, 1985. Reciprocal activities such as this, can also enhance our technology.

There are many similar suggestions which I may be able to discuss with you, but time has been limited to 5 minutes. I feel that by closing the reading of the paper it has introduced to us some ideas where all can participate freely in the exchange of opinions.

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Adoracion T. Arañez, Discussant

Increased commercial use of microalgae will probably depend on the successful development of genetically improved strains that grow more rapidly, synthesize valuable products more efficiently and tolerate a wide range of environmental conditions. This could be made possible through some techniques. Hybrid strains of microalgae may be produced by protoplast fusion from different algal species. This requires an enormous amount of basic research on their genetics and physiology. Hybridization and selection cannot be done to improve the blue-green algae because their method of reproduction is asexual.

In higher organisms, in addition to variations as a consequence of the sexual method of reproduction, variations may also be induced by mutagens. At present, some crops have been improved by mutation breeding. However, the different species and even varieties have different sensitivity to mutagens. In green algae, *Scenedesmus* is more susceptible to ethyl methanesulphonate and gamma radiation than *Chlorella* (Tibayan-Arañez, 1984). There are many reports attesting to blue-green algae as resistant to mutagens. Kumar (1964) mentioned blue-green algae as more resistant to ionizing radiation than other algae or higher plants. Kraus (1969) reported that the blue-green algae show a wide range of radiation resistance. *Micrococcus radiodurans*, a blue-green algae, can repair double strands breaks (Dean *et al.*, 1966). An excision and repair mechanism for damaged DNA involving an enzymatic system which is apparently not damaged by high exposure to radiation is observed also in the same species by Lett *et al.* (1967). Werbin and Rupert (1968) reported the presence of a photoreactivating enzyme capable of reversing nucleotide base dimerization lesions resulting from ultra violet irradiation in *Plectonema boryanum*, another blue-green algae. On the other hand, Singh and Tiwari (1969) reported true branching mutant, mutant with heterocysts in chains of sometimes up to eight heterocysts, mutant with more phycocyanin, and mutant with heterocysts that have three polar nodules in *Nostoc linckia* treated with ultraviolet light.

In my present study on *Nostoc linckia* treated with ethyl methanesulfonate, aside from the effect of the treatment on the ability of the algae to multiply, effect on akinete formation and some morphological variations in old cultures, no other changes have been observed. However, the experiment is still going on and further observations are being done.

I am not aware of any work done on the genetic materials of blue-green algae. The cells do not divide by mitosis and meiosis, hence the genetic materials cannot be observed under the light microscope. There are very limited studies of blue-green algae under the electron microscope and these few studies do not include the nature of the genetic material. Electron microscope study of blue-green algae *Anabaena cylindrica, Mastigocladus laminosus* and *Nostoc muscorum* by Chapman and Salton (1962), shows the presence of multi-layered envelopes of sheath (the cell wall and plasma membrane), photosynthetic lamellae and a variety of intracellular granules. Jensen and Bowen (1961) reported that their electron microscope studies of blue-green algae show basic differences between blue-green algae and higher plants and animals rather than similarities. Chapman and Salton (1962) considered the blue-green algae as more closely related to bacteria than other algal groups.

I hope in the future, somebody will work on the genetic materials of some blue-green algae as has been done on bacteria so that modern techniques, as protoplast fusion and genetic engineering, of improving the existing forms could be applied.

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Flordeliz R. Uyenco, Discussant

Friends and colleagues in the Biological Sciences – I wish to thank Dr. Gregorio T. Velasquez for inviting me to discuss his paper this morning. I realize that my role as discussant of the paper of an esteemed professor and scientist, however, is not a simple one.

I would like to present a review of the state-of-the-art on the biotechnology in the blue-green algae. Single-cell algae are potential sources of biological materials and chemicals. Since the blue-green organisms are capable of photosynthesis like higher plants, they can produce a wide variety of products from cheap and readily-available ingredients: nitrogen, carbon dioxide and sunlight. Other applications of blue-green algae include sewage treatment (e.g., in oxidation ponds and activated sludge systems), breakdown of toxic pollutants in the ocean and production of single-cell protein for animal feed and human consumption.

Spirulina, a blue-green trichome, is cultivated as SCP for animal feed and human food because of its thin, weak membrane, it has good digestive characteristics. When one looks at the constituent analysis of *Spirulina*, one cannot but marvel at the intricate, ingenious distribution of the nutritional elements necessary to the maintenance of human life. In addition, *Spirulina* contains large amounts of chlorophyll, phycocyanin and carotenoids. The carotenoids are called pro-vitamins which change into Vitamin A. The abundance of these coloring agents is a characteristic peculiar to *Spirulina*. Its dry protein content is 69.5-71% (soybean has only 39% protein) rich in necessary amino acids. Its synthetic digestion rate (2% pepsin for 48 hrs.) is 95.1%. The rate for *Chlorella*, a green alga, is 73.65%.

Microalgae grow much faster than higher plants and they can be cultivated using marginal land and brackish water unsuitable for conventional agriculture. But microalgal culture is more difficult than bacterial fermentation because the algae must be grown in large open ponds exposed to the natural environment instead of in closed vats.

A handful of U.S. companies are now engaged in research and development producing chemicals with marine microalgae. Microbiology Resources has actually reached the marketing stage and produces beta-carotene, a Vitamin A precursor, by culturing the phosynthetic marine alga, *Dunaliella* in open outdoor tanks at its production facility in California. Cyanatech in Washington is setting up a commercial facility for the production of beta-carotene from *Dunaliella*, as well as developing a strain of N_2-fixing algae as an agricultural fertilizer.

In the Philippines, the work of Pantastico and Martinez at UPLB on *Nostoc* as source of nitrogen in increasing the fertility of agricultural lands has been recognized. They are now trying to adapt specific strains that are faster-growing, hardier and more amenable to large-scale culture. Ocean Genetics of California, works with controlled mass cultures of blue-green algae with the goal of producing specialty chemicals, pharmaceuticals and agrochemicals. These companies have so far relied on classical molecular-genetics for gene cloning and transfer experiments. Genetic engineering techniques might eventually be employed to transfer the gene for a desired chemical into species of microalgae in which the substance could be mass-produced and then extracted osmotically. Other countries are also moving into the microalgal biotechnology. Western Australia is evaluating the potential production of beta-carotene and protein-rich algal meals for animal feed. France is evaluating microalgal production of hydrocarbons and polysaccharides.

In the longer term, microalgae may serve as a renewable source of liquid fuels (such as synthetic diesel fuel and gasoline and petrochemical feedstocks). Many species of marine microalgae manufacture energy-rich lipids. These include hydrocarbons similar to those of petroleum and fatty acids; and triglycerides like those of seed oils. Since 1980 the Solar Energy Research Institute of Colorado has been investigating the possibility of producing lipids from microalgae in outdoor mass cultures. It hopes to cultivate a 1000-acre facility for microalgae for energy production, with carbon dioxide provided from underground deposits or from the exhausts of fossil-fuel power plants.

Although costs of production and conversion of microalgal lipids to synthetic liquid fuel is still prohibitively high, microalgae appear to have the genetic potential for rapid lipid synthesis. When certain species of microalgae are starved for nitrogen, they stop dividing and accumulate lipids constituting as much as 90% of their biomass. Researchers in the U.S. and Israel are studying this "lipid trigger" with the goal of inducing a similar accumulation of lipids in unstressed cells, perhaps with the aid of gene-splicing techniques.

The high potential of the blue-green algae cannot be overemphasized. The appropriate technology for their application and utilization is rapidly evolving and it is time that research and development in science and technology in the Philippines should include the blue-green as one of the priority projects in biotechnology.

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