Italy and Israel bet on GM microalgae to develop edible COVID vaccine

The rush to develop a vaccine for COVID-19 has extended to Italy and Israel, where scientists are using the tools of genetic engineering to develop algae-based edible vaccines against the disease caused by the novel coronavirus.

As I explained in a previous report about work under way in Mexico to develop an edible COVID-19 vaccine delivered through a tomato, plants have numerous advantages — mainly sanitary, storage and transport — over conventional methods for obtaining recombinant vaccines. In the bibliographic search for that report, I observed that microalgae — a type of small, unicellular algae — is another way to obtain recombinant proteins and edible vaccines, with notable results so far.

Under this approach, a series of experimental developments have already been carried out in oral vaccines against pathogens such as hepatitis B, malaria, human papilloma virus (HPV), hand, foot and mouth disease (HFMD), classical swine fever (CSF) and *Staphylococcus aureus*, with some successful pre-clinical trials. Most of these works use *Chlamydomonas reinhardtii*, a small unicellular and eukaryote algae, as a model for research and production of drugs and vaccines due to its practical laboratory management and genetic transformation.

With this background, it wasn't surprising to find that not only are detection kits, nasal sprays and potential drugs for COVID-19 being developed with algae, but two scientific groups are also working to develop algae-based edible vaccines for the same virus. Before delving into the details of both investigations, my initial doubt fell on the technical and management differences that algae could have compared to field or greenhouse crops.

"Their cultivation can be carried out on land infertile or unsuitable for cultivation, because they are capable of using residues as a source of nutrients and transforming them into a large quantity of high-quality molecules," explained biotechnologist Daniel Garza, the Mexican scientist who leads the tomato-vaccine research for COVID, in an email exchange. Garza previously developed an environmental biotechnology project for air decontamination through microalgae in Mexico, so I took advantage of his expertise before continuing to investigate on algae-based vaccines.

"It's also a sustainable process, because during cultivation they are able to use atmospheric carbon (CO2), removing it and transforming it into high-value biomass," Garza added. "They have an important cost advantage derived from their high growth rate and low cultivation cost, which makes them ideal for expressing new vaccines and replacing those that are expensive."

Photobioreactors at the University of Verona

The first work to develop an algae-based vaccine comes from Italy, one of the countries hardest hit by the COVID-19 pandemic. Specifically, the research is being carried out by the Laboratory of Photosynthesis and Bioenergy of the Department of Biotechnology at the University of Verona, directed by professors Roberto Bassi and Luca Dall'Osto.

This laboratory works with a wide range of phototrophic organisms, including unicellular algae, mosses and higher plants, and also has a strong line of genetic engineering in model plants and unicellular algae to express recombinant products and enzymes with industrial and renewable energy applications.

"The ability to perform genetic engineering, especially on the single-celled alga of the model organism *Chlamydomonas reinhardtii*, has provided the basis for contributing to the development of an oral vaccine against the recently emerged SARS-VOC-2 viral strain responsible for the current pandemic threatening the global health," stated Dr. Edoardo Cutolo in a detailed interview (supplemented by valuable bibliography) with the Alliance for Science. This pioneering project involves Cutolo and his colleague, Dr. Max Angstenberger, in addition to the support of Dr. Simone Barera.

italy x

Image not found or type unknown

Chlamydomonas reinhardtii algae under the microscope. Credit: Dr. Cutolo.

The scientific team applied two different approaches to introduce a DNA sequence that encodes an antigen derived from SARS-COV-2 into the microalgae genome. Remember that the antigen, in this case, is a protein or a protein portion that produces a response immune in our body, finally generating antibodies against the virus. The inserted DNA sequence corresponds to a portion of the Receptor Binding Domain (RBD) of the viral spike protein from the famous virus, required to bind to the ACE2 receptor and thus enter and infect host cells.

“We use both conventional nuclear transgenesis and chloroplast transformation. In the second case, we aim at integrating the transgene inside the semi-autonomous polyploid genome of the photosynthetic organelle,” Cutolo said. “In the case of *Chlamydomonas reinhardtii*, the chloroplast represents the largest cell compartment, and since it is made of multiple copies of a circular chromosome, it leads to the accumulation of higher levels of recombinant proteins compared to transgenesis in the nucleus.”

Both methods have advantages and disadvantages. On the one hand, the chloroplast not only allows greater accumulation of the antigen necessary for a vaccine due to its large size in the microalgae, but also facilitates a more stable integration of the transgene, avoiding the random integration problems that most common when the nucleus is genetically modified. But on the other hand, the cell nucleus has a machinery that allows subsequent modifications, such as glycosylation, of the new protein (or antigen), giving it functionality to generate adequate immunization.

“Of note, in this project we employ selection methods that don’t rely on antibiotic resistance genes,” Cutolo said in reference to a supposed risk widely cited by critics of this technology. “But we exploit the metabolic flexibility of this organism and a novel selectable marker strategy based on the selective metabolism of an essential nutrient to produce algae that comply with both health and environmental related concerns.”

One of the great advantages of algae is that they grow and multiply quite quickly. According to Cutolo, if contamination is prevented, it is possible to accumulate up to 1 mg of the recombinant antigen for each gram of biomass of dried algae. Subsequently, the dehydrated/lyophilized algae can be encapsulated to generate an “oral vaccine.”

italy x

Image not found or type unknown

From left to right: Dr. Simone Barera, Dr. Edoardo Cutolo, Prof. Roberto Bassi and Dr. Max Angstenberger. Credit: Dr. Cutolo.

“The cell wall from the dry algae protects the antigens from the harsh acidic and protease-rich gastric environment, enabling the bioactive molecule to reach the intestinal immune system where it can stimulate cellular and humoral responses, hopefully, leading to effective immunization,” Cutolo explained.

When could they have an oral vaccine ready to test on animals? Very soon, according to Cutolo: “Six weeks is a probable date.”

Israeli Technology: From Animals and Fish to COVID-19

Meanwhile, across the Mediterranean Sea, TransAlgae, a biotech company based in Rehovot, Israel, established itself in just over a decade as a development platform for oral animal vaccines, the aquaculture sector and pest-control in agriculture. A few months ago, the company embarked on applying this technology in a vaccine for COVID-19. To achieve this, they have opened a US$5 million investment round to support the development of an oral vaccine based on genetically modified algae, according to Eyal Ronen, vice president of business development of the company.

When asked about further technical, regulatory and obstacle details for this new oral vaccine, thecompany preferred not to respond or provided only brief answers to questions, citing the need forconfidentiality.

However, in an informative publication by company President Daniel Gressel, a strategy similar to that of Italian scientists can be elucidated. For example, they would also be using the DNA sequence from a portion of the SARS-COV2 spike protein as transgene to insert into algae, and according to various previous company patents for vaccines in animals and fish, it’s very likely that they are using the same algae model *C. reinhardtii* for genetic modification and accumulate large amounts of the antigen — modified algae that would be lyophilized to generate an oral capsule.

transalgae x

Image not found or type unknown

Algae grown in TransAlgae fermenters at Rehovot. Credit: TransAlgae

Ronen claims that the algae are genetically engineered to grow in a fermenter. “This increases production rate 30-fold over wild algae. And we can control all the inputs in an accurate way for consistency,” he added.

With this high speed, they calculate that they will begin animal trials in a few months, and in addition, they would seek collaborations and partnerships with companies in the United States to advance the development of the vaccine.

Advantages and challenges of algae-based edible vaccines

In general, microalgae have all the advantages of producing vaccines in land plants, with some additional benefits.

“Microalgae thrive in very simple cultivation media, don’t require complex infrastructure and their cultivation doesn’t compete with crops for arable lands,” Cutolo said. Another important detail to highlight is that microalgae are much more efficient than higher plants to convert sunlight into biomass.

As in plants, the recombinant antigen obtained from the collected algae biomass doesn’t require purification or extraction, since it can dry out and the algae cell wall protects the antigens with a long useful life of up to 20 months — without loss of efficacy — at room temperature. This is very practical for developing countries, which often have problems with cold chain in storage/transport for conventional vaccines.

Perhaps the main advantage is the microalgae fast multiplication speed, which facilitates the work of the researchers. “From a pure technical perspective, algae are preferable since the development of a new algal strain requires approximately one month, while the establishment of a GM plant can last up to one year,” Cutolo said.

TransAlgae’s Gressel also emphasized reproduction speed as a clear advantage in one of the few responses he gave me by email. “Algae double every day… if you start with 1 gram, in 32 days you have 8.8 billion grams, which in the case of coronavirus is enough for the world,” Gressel said.

italy x

Image not found or type unknown

Photobioreactor for algae cultivation in the Italian laboratory. Credit: Dr. Cutolo.

However, difficulties aren’t unrelated to this approach. According to Cutolo, a major drawback in the cultivation of algae resides in the omnipresent risk of culture contamination by outcompeting parasites, which occurs both in closed photobioreactors and open high-rate speed systems.

“This issue makes algal biotechnology cost-inefficient because of high management costs to prevent that,” Cutolo said. “However, we are employing a recently introduced sustainable technology that enables the management of target monoculture of algae in non-sterile conditions, making this production system very attractive.”

Regarding biological containment risks, in Cutolo’s experience, these wouldn’t be a problem. “Most of the naturally occurring (wild type) algal strains used for genetic engineering lack essential genes required for nitrogen assimilation (nitrate reductase), making them strictly dependent on the nitrogen source provided in the cultivation medium, thus making their survival in the wild impossible,” he said.

“There is no containment problem because the algae lack nitrate reductase and therefore die outside the photobioreactor.”

What is coming in the future?

Before entering the market, algae-based oral vaccine would face regulatory obstacles similar to those discussed in my earlier tomato vaccine report. Transalgae had no previous problems when it worked with oral vaccines using GM microalgae for the aquaculture sector, as the final product was a “deactivated” algae powder (dead material). Due to this, the material is considered GMO-derived, not a GMO, a distinction that has allowed the company to successfully operate in markets with strict GMO regulations.

Meanwhile, neither the scientists in Verona nor in Revohot are resting on their laurels. If TransAlgae succeeds with a COVID-19 edible vaccine, its planned next step will be to produce an influenza oral vaccine by rapidly growing antigens against a wide variety of strains of the pathogen. The Italian scientists are working in parallel with gene editing, a tool that would allow them to introduce additional modifications to the algae nuclear genome, which could improve the productivity of its biomass.

“Given the urgency dictated by the current situation, but also due to the very likely possibility that similar pandemics will hit our global society in the future, it’s of paramount importance to develop technologies that afford a rapid production of vaccines and biopharmaceuticals that are safe and easy to deliver, especially in those areas which have limited access to medical infrastructure,” Cutolo concluded.

Undoubtedly, the versatile genetic manipulation, speed of reproduction and minimal resources necessary for the growth of algae make it a viable and sustainable alternative and candidate for urgently needed medical and environmental solutions across the globe.

Recommended references

- Specht, E. A., & Mayfield, S. P. (2014). Algae-based oral recombinant vaccines. Frontiers in microbiology, 5, 60. https://doi.org/10.3389/fmicb.2014.00060
- Benedetti, M., Vecchi, V., Barera, S., & Dall’Osto, L. (2018). Biomass from microalgae: the potential

of domestication towards sustainable biofactories. Microbial Cell Factories, 17(1), 173. doi:10.1186/s12934-018-1019-3
- Yefremova, Y., & Purton, S. (2018). The algal chloroplast as a synthetic biology platform for production of therapeutic proteins. Microbiology, 164. doi:10.1099/mic.0.000599
- Gunasekaran, B., & Gothandam, K.M.. (2020). A review on edible vaccines and their prospects. Brazilian Journal of Medical and Biological Research, 53(2), e8749
- Kurup, V. M., & Thomas, J. (2020). Edible Vaccines: Promises and Challenges. Molecular biotechnology, 62(2), 79–90.

Daniel Emilio Norero González serves as a consultant in scientific communication at agricultural biotechnology and food-tech institutions. He has studied biochemistry at the Catholic University of Chile and has worked as a laboratory assistant in plant sciences. He founded the initiative “Yo Quiero transgénicos” (I Do Want GMOs), a citizen project that aims to demystify and inform about GMOs, with special emphasis on biotech crops in Latin America. Daniel can be found on Twitter @DanielNorero

A version of this article was originally published at the Cornell Alliance for Science website and has been republished here with permission. The Cornell Alliance for Science can be found on Twitter @ScienceAlly