



Edible microalgae: potential candidate for developing edible vaccines

Merin Grace Jiji¹  · Merin Ann Ninan¹ · V. P. Thomas¹ · Binoy T. Thomas¹

Received: 14 February 2023 / Revised: 5 April 2023 / Accepted: 12 April 2023 / Published online: 27 April 2023
© The Author(s) under exclusive licence to Society for Plant Research 2023

Abstract

Infectious diseases are always a threat to all living beings. Today, in this world pathogens have no difficulty reaching anywhere. Every year new and deadly diseases are born and most of them are caused by viruses. Vaccines can provide life-long immunity against infectious diseases, but the production cost of vaccines is unaffordable for a layman and traditional vaccines have certain limitations with storage and delivery. However, edible vaccines have shifted this paradigm and have received acceptance all over the world, especially in developing countries. Microalgae are one of the potential candidates for developing edible vaccines. Modifying microalgae as edible vaccines are gaining worldwide attention, especially in the world of science. Microalgae can augment the immune system as they are a promising source for antigen carriers and many of them are regarded as safe to eat. Moreover, they are a pantry of proteins, vitamins, minerals, and other secondary metabolites like alkaloids, phenols, and terpenes. In addition, being resistant to animal pathogens they are less sophisticated for genetic modification. This review analyses the potential scope of microalgae as an edible vaccine source.

Keywords Chloroplast · Edible vaccine · Immunostimulants · Microalgae

Introduction

In this millennium, it is obvious that biomedical sciences are in an advanced phase through innovative technologies (Gartland et al. 2013). Even though many diseases have been eradicated, novel infections are rapidly emerging in the modern world. Epidemics and pandemics have always had socio-economic impacts on affected populations, but in the present interdependent world, the impacts are truly global (Bloom and Cadarette 2019). Furthermore, infectious diseases force humans to consume high doses of antibiotics which eventually weaken our immunity (Barney 2017). In this scenario, vaccines have a prominent role in reducing the encumbrance of infectious diseases. Moreover, they are considered safer than therapeutic medicines (Andre et al. 2008), but conventional vaccines have safety issues as they are produced from attenuated pathogenic organisms (Bhatia and Dahiya 2015). Here the significance of edible vaccines

comes into play as they are subunit vaccines that can trigger mucosal immune responses, which is the first line of defense against pathogens (Bhatia and Dahiya 2015; Kurup and Thomas 2020). In order to create an edible vaccine, the gene of interest should be inserted into plant cells by genetic engineering techniques (Jan et al. 2016) as shown in the Fig. 1.

DNA of the desired pathogen for antibody production is inserted into the chloroplast genome of *Chlamydomonas reinhardtii* through one of the four methods (plasmid of an agrobacterium, gene gun, electroporation, glass beads). When the microalgae is genetically modified to produce antigenic proteins, it can be orally consumed which will trigger T-helper cells and B-cells to generate antibodies against that particular pathogen (Criscuolo et al. 2019). Besides the chloroplast genome, the nuclear (Geng et al. 2003) and mitochondrial (Larosa and Remacle 2013) genomes are also utilized in foreign gene expression.

The concept of utilizing transgenic plants for edible vaccines began in the late 20th century (Saxena and Rawat 2013). Plant-derived vaccines are economically effective, heat stable, and can be directly consumed (Streatfield et al. 2001). Despite the advantages, plant vaccines have some limitations because they need more time for large scale production, their growth is limited by environmental conditions,

✉ Binoy T. Thomas
bttkripa@gmail.com

¹ Phycotechnology laboratory, Post Graduate and Research Department of Botany, Catholicate college, Pathanamthitta, Kerala 689645, India

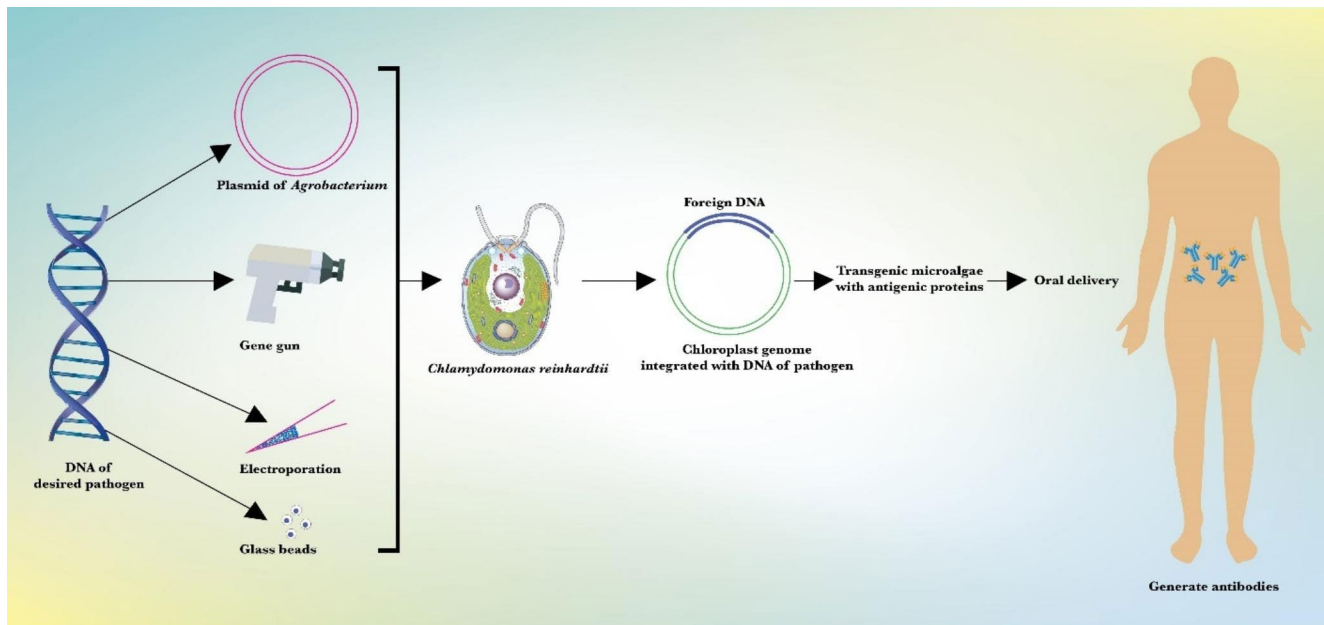


Fig. 1 Graphical concept of edible vaccines from microalgae

and there is a chance of releasing transgenes into soil bacteria or other related plant species (Gangl et al. 2015). Like plant vaccines, transgenic microalgae have also proven to be useful in the production of edible vaccines (Demurtas et al. 2013) and they have enormous industrial benefits over plant vaccines (Charoonnart et al. 2018; Specht and Mayfield 2014).

Microalgae has been farmed at a commercial scale as a health food since 1960 (Mobin and Alam 2017). Today, there is a considerable increase in the utilization of microalgae as a food source (Koyande et al. 2019). Many species of microalgae have desirable characteristics that make them suitable candidates for human consumption (Kusmayadi et al. 2021). Microalgae are an excellent feedstock since they do not compete for land to create different valuable products (Kothari et al. 2017). They can be cultivated in three most prominent growth systems namely open-air ponds, photobioreactors, and heterotrophic bioreactors to generate a large amount of algal biomass (Diaz et al. 2023). According to FAO, global algae production increased 60 times from 1950 to 2019 (FAO, 2021) which emphasizes how far the market trend for algae has grown.

Microalgae represent a promising source for pharmaceuticals, nutraceuticals, cosmetics, and the fuel industry (Borowitzka 2013). Unlike other microorganisms, algae contain chlorophyll and accessory pigments, that make them a potent source for bioactive compounds (Eriksen 2008; Okuzumi et al. 1990). Nowadays, scientific technologies have paved the way for the discovery of biomedicines from microalgae (Costa et al. 2020). Moreover, they are green cell factories and widely used in molecular pharming (Hallmann

2015). Remarkably, owing to a small polyploid genome in their chloroplast which is derived from the cyanobacterial ancestor of this organelle, they are capable to accumulate a large number of proteins (Gutiérrez et al. 2012). One of the green algae *Chlamydomonas reinhardtii* P.A Dangeard 1888 is used as a model organism for expressing recombinant therapeutic proteins in their chloroplast (Mayfield et al. 2003). These therapeutic proteins have the potential to make the microalgae highly desirable for vaccine production (Rasala et al. 2010). Apart from this, microalgae have many other advantages such as easy preservation through lyophilization, rapid biomass accumulation, and they are not restricted by growing seasons. All these attributes ensure their position in the biomedical industry (Specht and Mayfield 2014).

Subunit vaccines contain only a few antigens, which reduce immunogenicity and their purification process often leads to the elimination of components that trigger innate immunity (Vetter et al. 2018). Nonetheless, the potential efficacy of the same can be increased by immunostimulatory compounds like adjuvants and immunostimulants (Reed et al. 2013). In this context, microalgae being a source for immunostimulants and most of the microalgae are “Generally Recognized As Safe” (GRAS), attain importance as an ideal delivery vehicle for vaccines (Ramos-Vega et al. 2018).

Lately, an Irish company has been developing an algal vaccine for fish so that they can be promptly immunized against infectious diseases. Instead of vaccines, fish can be fed antibiotics but their consumption can cause biomagnification (Horizon 2020). Recently countries like Italy and

Israel have been working on algae to produce edible vaccines against the novel corona virus (Norero 2020). Furthermore, certain geographic regions are prone to pandemics and they cannot afford high-cost vaccines (Madhav et al. 2018). Therein lies the importance of microalgae as edible vaccines since they are more economical than plant based vaccines (Gunasekaran and Gothandam 2020). This article intends to provide an overview of the edible vaccines produced from microalgae. There is a proverb that says “Your happiness is a reflection of your health.” If microalgae can bring this happiness, then why don’t we have it?

Microalgae as edible vaccines

Plastids are one of the primary requirements for the expression of vaccine antigens (Chebolu and Daniell 2010). The chloroplast of algae plays a pivotal role in expressing therapeutic proteins (Dyo and Purton 2018) and it possesses certain striking features making it an important tool in biotechnology. Maternally inherited chloroplast genome helps the plants to produce stable proteins (Takeyama et al. 2015) and, it is a safe storehouse for proteins due to the lack of endogenous protease (Liang et al. 2020). Moreover, chaperones in the chloroplast help in proper folding and can be utilized effectively in vaccine development (Daniell et al. 2005). In addition, because the algal chloroplast has a rigid cell wall, vaccines produced in them can thrive in harsh conditions. An algal chloroplast-derived vaccine antigen remained stable for more than 1.5 years at room temperature in lyophilized form and was comparably immunogenic when tested in comparison with the antigen stored at 4 °C (Dreesen et al. 2010).

The first algal based edible vaccine was a fusion product of foot and mouth disease virus particle and cholera toxin B (Sun et al. 2003). In their study, the transgenic chloroplast of *C.reinhardtii* was used for vaccine expression. Even though antigen expression levels were low, it marked history in microalgal biotechnology. In another study, E2, a structural protein of the Classical Swine Fever Virus (CFSV) was introduced into the chloroplast of *C.reinhardtii* by biolistic bombardment. Although antigens were expressed in chloroplasts, no immune response was detected after the oral immunization (He et al. 2007).

Certain surface proteins of pathogens are difficult to produce in traditional recombinant systems because of tandem repeats of epidermal growth factor-like domains (Gregory et al. 2012). These surface proteins Pfs 25 and 28 of *Plasmodium falciparum* Welch 1897 cause malaria in humans. The chloroplast of *C.reinhardtii* had been modified to produce those surface proteins and in response, antibodies were generated that hinder the sexual development of parasites.

It was the first demonstration that algae can successfully produce an unmodified and aglycosylated version of these proteins (Gregory et al. 2013). Chloroplast transformation through homologous recombination eliminates the variability in gene expression, gene silencing and enhances the accumulation of the recombinant antigen (Habibi-Pirkoohi and Mohkami 2015). *C.reinhardtii* was also used for the production of vaccine against Human Papillomavirus (HPV), one of the central causes of cervical cancer (Franco et al. 2012). An attenuated form of E7 oncoprotein named E7GGG of this virus was used to express in the chloroplast of *C.reinhardtii* by glass bead method. Although the antigenic proteins produced were insufficient to trigger antibody production, they can be overwhelmed by future studies (Demurtas et al. 2013).

Even though most work has been performed in *C.reinhardtii*, a halophilic green alga named *Dunaliella salina* (Dunal) Teodoresco 1905 was used for expressing Hepatitis B surface Antigen (HBsAg) by electroporation and stable transformants were obtained (Geng et al. 2003). Transformations for expressing antigenic proteins in nucleus are rare (Potvin and Zhang 2010). However, nuclear transformation of the two strains Elow 47 and UVM 11 of *C.reinhardtii* successfully produced the antigenic protein P24, a crucial protein of HIV made by the glass bead method (Barahimipour et al. 2016).

Microalgae are also utilized in aquaculture biotechnology. Viral diseases are a catastrophe for aquatic life and most of them are endemic (Walker and Winton 2010). *Renibacterium salmoninarum* Sanders and Fryer 1980 is a pathogenic bacterium that causes kidney disease in salmonids. *C.reinhardtii* was genetically modified for expressing antigens of this bacterium and fed to trout and rabbits which later generated antibodies against this antigenic protein (León-Bañares et al. 2004). Chloroplast of the freshwater microalgae *Haematococcus pluvialis* Flotow 1844 was genetically manipulated by particle bombardment and identified that *H.pluvialis* is a good model as an oral vaccine for shrimp and fish (Gutiérrez et al. 2012). White Spot Syndrome Virus (WSSV) causes white spot disease in shrimp. Transgenic *C.reinhardtii* carries VP 28, the viral envelope protein of this virus was orally vaccinated to shrimp and compared with the wild type. Transgenic shrimp shows the highest survival rate than wild type (Kiataramgul et al. 2020).

Even though there are more than 30,000 algal species, only a few microalgae have been genetically transformed (Table 1) to date due to some cell factors. For instance, the cytoplasm of *Arthrospira platensis* Gomont 1892 contains multiple strong endonucleases which makes them inefficient for successful transformation (Shiraishi and Tabuse 2013). However, *Agrobacterium* based transformation of this blue

Table 1 Shows a list of microalgae that has been transformed for vaccine production

Algae	Integration site	Disease	Reference
<i>Chlamydomonas reinhardtii</i>	Chloroplast genome	Foot and mouth disease	Sun et al., 2003
<i>Dunaliella salina</i>	Nuclear genome	Hepatitis B	Geng et al., 2003
<i>Chlamydomonas reinhardtii</i>	Chloroplast genome	Kidney disease in salmonids	Leon-banares et al., 2004
<i>Chlamydomonas reinhardtii</i>	Chloroplast genome	Classical Swine Fever	He et al., 2007
<i>Chlamydomonas reinhardtii</i>	Chloroplast genome	<i>Staphylococcus aureus</i> infection	Dreesen et al., 2010
<i>Chlamydomonas reinhardtii</i>	Chloroplast genome	Malaria	Gregory et al., 2013
<i>Chlamydomonas reinhardtii</i>	Chloroplast genome	Human Papillomavirus infection	Demurtas et al., 2013
<i>Chlamydomonas reinhardtii</i>	Nuclear genome	AIDS	Barahimipour et al., 2016
<i>Chlamydomonas reinhardtii</i>	Chloroplast genome	White spot disease in shrimps	Kiataramgul et al., 2020

green algae was effectively conducted by integrating *gfp:gus* and hygromycin resistance genes into its genome and can be utilized for making edible vaccines near future (Dehghani et al. 2018). Recently, based on a detailed literature survey it has been identified that *Arthrospira platensis* has potential activity against enveloped RNA viruses and can be explored for developing a vaccine against novel Coronavirus disease (COVID-19) (Elaya Perumal and Sundararaj 2020), the deadliest pandemic caused by the virus SARS-CoV-2. In *C.reinhardtii*, the production of the spike protein Receptor Binding Domain (RBD) of this virus was successfully conducted by researchers at the University of California (Robertson 2021), shedding light on the scientific community to develop a low-cost vaccine against coronavirus.

Advantages of algal vaccines

Unlike plants, algal biomass accumulation is extremely rapid, and the whole biomass can be utilized for vaccine production. Moreover, they are resistant to animal pathogens and the expression of heterologous proteins in microalgae is 10- to 100-fold higher than that of plant-based vaccines (Saveria et al. 2022). Notably, *C.reinhardtii* contains only one chloroplast that aids in the stability of specific antigens in their cell line (Sahoo et al. 2020). Since the algal cell wall preserves the recombinant antigen for a lengthy usable life of up to 20 months at room temperature without losing efficacy, it doesn't need to be purified or extracted before use (Rosales-Mendoza 2016). When compared to

plants, microalgae can be produced in a medium that only contains necessary nutrients, negating the need for fertile soil and preventing water contamination from unused fertilizers (Khan et al. 2018). In general, transgenic algae possess several advantages over other recombinant protein production platforms.

Challenges in making algal vaccines

Advances in the genetic engineering of algal genomes have accelerated the synthesis of therapeutic proteins. But genetic instability and low expression were observed in some transformed strains (Rosales-Mendoza et al. 2020). Even though many algae have been successfully transformed, stable *Chlorella* transformants have long been scarce. *Chlorella* is also a promising green alga for recombinant protein expression (Bai et al. 2013). However, they are small and have a resilient cell wall thus effective utilization of them for recombinant protein expression is hindered by a lack of genetic tools (Yan et al. 2016). Another daunting challenge is that recombinant protein yields in algal systems are sometimes low compared to microbial platforms, but this can be overcome by the use of efficient promoters, a better understanding of gene regulation and new selectable markers (Rosales-Mendoza et al. 2020; Taunt et al. 2018). Furthermore, the fact that microalgae as an edible vaccine can only be used after achieving GRAS status remains a challenge. Another significant drawback to growing algae is the constant threat of contamination by parasites that compete for resources. This threat exists in both closed photobioreactors and open high speed systems. To date, only a few microalgae are commercially exploited for vaccine production. Significant research and further study are thus required for making edible algal vaccines.

Conclusion

Algae are well-designed platforms for producing antigens. Most of the studies focused on the green unicellular microalga *Chlamydomonas reinhardtii* owing to some peculiar features. Although many countries have developed a vaccine against the coronavirus, they can develop a variety of side effects, including neurological disorders such as Guillain-Barre syndrome (Waheed et al. 2021). Studies are being conducted on microalgae for preparing a potential vaccine against the novel coronavirus. If the studies succeed in inventing a vaccine against coronavirus, that will be a ray of hope for humankind. The future of edible algal vaccine techniques is very bright as they can be mass produced in a limited amount of time and are cost-effective. Large-scale

acceptance and progress of transgenic algae is still a challenge in many developing countries, and if accepted by society, it will be feasible to produce edible vaccines against various diseases globally. With edible vaccines, many infectious diseases can be wiped out and wide applications in microalgae have infinite possibilities for future study.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

- Andre F, Booy R, Bock H et al (2008) Vaccination greatly reduces disease, disability, death and inequity worldwide. *Bull World Health Organ* 86:81–160
- Bai LL, Yin WB, Chen YH et al (2013) A New Strategy to produce a defensin: stable production of mutated NP-1 in Nitrate reductase-deficient *Chlorella ellipsoidea*. *PLoS ONE* 8:1–9. <https://doi.org/10.1371/journal.pone.0054966>
- Barahimipour R, Neupert J, Bock R (2016) Efficient expression of nuclear transgenes in the green alga *Chlamydomonas*: synthesis of an HIV antigen and development of a new selectable marker. *Plant Mol Biol* 90:403–418. <https://doi.org/10.1007/s11103-015-0425-8>
- Barney J (2017) Antibiotics found to weaken immune response to disease. <https://news.virginia.edu/content/antibiotics-found-weaken-immune-response-disease>. Accessed 7 Feb 2021
- Bhatia S, Dahiya R (2015) Edible vaccines. *Modern applications of Plant Biotechnology in Pharmaceutical Sciences*. Elsevier Inc., pp 333–343
- Bloom DE, Cadarette D (2019) Infectious disease threats in the twenty-first century: strengthening the global response. *Front Immunol* 10. <https://doi.org/10.3389/fimmu.2019.00549>
- Borowitzka MA (2013) High-value products from microalgae-their development and commercialisation. *J Appl Phycol* 25:743–756. <https://doi.org/10.1007/s10811-013-9983-9>
- Charoonnart P, Purton S, Saksmerprome V (2018) Applications of microalgal biotechnology for disease control in aquaculture. *Biology (Basel)* 7:1–14. <https://doi.org/10.3390/biology7020024>
- Chebolu S, Daniell H (2010) Chloroplast-derived vaccine antigens and biopharmaceuticals: expression, folding, assembly and functionality. *Curr Top Microbiol Immunol* 332:33–54. https://doi.org/10.1007/978-3-540-70868-1_3
- Costa JAV, Moreira JB, Fanka LS et al (2020) Microalgal biotechnology applied in biomedicine. In: Ozcan K (ed) *Handbook of Algal Science, Technology and Medicine*. Mathew Deans, pp 429–439.
- Crisuolo E, Caputo V, Diotti RA et al (2019) Alternative methods of vaccine delivery: an overview of edible and intradermal vaccines. *J Immunol Res* 2019:1–13. <https://doi.org/10.1155/2019/8303648>
- Daniell H, Chebolu S, Kumar S et al (2005) Chloroplast-derived vaccine antigens and other therapeutic proteins. *Vaccine* 23:1779–1783. <https://doi.org/10.1016/j.vaccine.2004.11.004>
- Dehghani J, Adibkia K, Movafeghi A et al (2018) Stable transformation of *Spirulina (Arthrospira) platensis*: a promising microalga for production of edible vaccines. *Appl Microbiol Biotechnol* 102:9267–9278. <https://doi.org/10.1007/s00253-018-9296-7>
- Demurtas OC, Massa S, Ferrante P et al (2013) A *Chlamydomonas*-Derived Human Papillomavirus 16 E7 vaccine induces specific Tumor Protection. *PLoS ONE* 8:e61473. <https://doi.org/10.1371/journal.pone.0061473>
- Diaz CJ, Douglas KJ, Kang K et al (2023) Developing algae as a sustainable food source. *Front Nutr* 9:1–21. <https://doi.org/10.3389/fnut.2022.1029841>
- Dreesen IAJ, Hamri GC, El, Fussenegger M (2010) Heat-stable oral alga-based vaccine protects mice from *Staphylococcus aureus* infection. *J Biotechnol* 145:273–280. <https://doi.org/10.1016/j.jbiotec.2009.12.006>
- Dyo YM, Purton S (2018) The algal chloroplast as a synthetic biology platform for production of therapeutic proteins. *Microbiology* 164:113–121. <https://doi.org/10.1099/mic.0.000599>
- Elaya Perumal U, Sundararaj R (2020) Algae: a potential source to prevent and cure the novel coronavirus – a review. *Int J Emerg Technol* 11:479–483.
- Eriksen NT (2008) Production of phycocyanin - A pigment with applications in biology, biotechnology, foods and medicine. *Appl Microbiol Biotechnol* 80:1–14. <https://doi.org/10.1007/s00253-008-1542-y>
- Food and Agricultural Organization of the United Nations (2021) *Global seaweeds and microalgae production, 1950–2019*
- Franco EL, de Sanjosé S, Broker TR et al (2012) Human papillomavirus and cancer prevention: gaps in knowledge and prospects for research, policy, and advocacy. *Vaccine* 30:F175–F182. <https://doi.org/10.1016/j.vaccine.2012.06.092>
- Gangl D, Zedler JAZ, Rajakumar PD et al (2015) Biotechnological exploitation of microalgae. *J Exp Bot* 66:6975–6990. <https://doi.org/10.1093/jxb/erv426>
- Gartland KMA, Bruschi F, Dunder M et al (2013) Progress towards the “Golden Age” of biotechnology. *Curr Opin Biotechnol* 24:S6–S13. <https://doi.org/10.1016/j.copbio.2013.05.011>
- Geng D, Wang Y, Wang P et al (2003) Stable expression of hepatitis B surface antigen gene in *Dunaliella salina* (Chlorophyta). *J Appl Phycol* 15:451–456. <https://doi.org/10.1023/B:JAPH.0000004298.89183.e5>
- Gregory JA, Li F, Tomosada LM et al (2012) Algae-produced pfs25 elicits antibodies that inhibit malaria transmission. *PLoS ONE* 7:1–10. <https://doi.org/10.1371/journal.pone.0037179>
- Gregory JA, Topol AB, Doerner DZ, Mayfield S (2013) Alga-produced cholera toxin-Pfs25 fusion proteins as oral vaccines. *Appl Environ Microbiol* 79:3917–3925. <https://doi.org/10.1128/AEM.00714-13>
- Gunasekaran B, Gothandam KM (2020) A review on edible vaccines and their prospects. *Brazilian J Med Biol Res* 53:1–10. <https://doi.org/10.1590/1414-431x20198749>
- Gutiérrez CL, Gimpel J, Escobar C et al (2012) Chloroplast genetic tool for the green microalgae *Haematococcus pluvialis* (chlorophyceae, volvocales). *J Phycol* 48:976–983. <https://doi.org/10.1111/j.1529-8817.2012.01178.x>
- Habibi-Pirkoohi M, Mohkami A (2015) Recombinant vaccine production in green plants: state of art. *J Cell Mol Res* 7:59–67. <https://doi.org/10.22067/jcmr.v7i1.45074>
- Hallmann A (2015) Algae Biotechnology – Green cell-factories on the rise. *Curr Biotechnol* 4:389–415. <https://doi.org/10.2174/2211550105666151107001338>
- He DM, Qian KX, Shen GF et al (2007) Recombination and expression of classical swine fever virus (CSFV) structural protein E2 gene in *Chlamydomonas reinhardtii* chloroplasts. *Colloids Surf B Biointerfaces* 55:26–30. <https://doi.org/10.1016/j.colsurfb.2006.10.042>
- Horizon (2020) Algae for fish vaccine. <https://www.enabling-project.com/news-1/2020/2/11/algae-for-fish-vaccine>. Accessed 5 Feb 2021
- Jan N, Shafi F, Hameed O, bin et al (2016) An overview on edible vaccines and immunization. *Austin J Nutr Food Sci* 4:1078.

- Khan MI, Shin JH, Kim JD (2018) The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. *Microb Cell Fact* 17:1–21. <https://doi.org/10.1186/s12934-018-0879-x>
- Kiataramgul A, Maneenin S, Purton S et al (2020) An oral delivery system for controlling white spot syndrome virus infection in shrimp using transgenic microalgae. *Aquaculture* 521:735022. <https://doi.org/10.1016/j.aquaculture.2020.735022>
- Kothari R, Pandey A, Ahmad S et al (2017) Microalgal cultivation for value-added products: a critical enviro-economical assessment. *3 Biotech* 7. <https://doi.org/10.1007/s13205-017-0812-8>
- Koyande AK, Chew KW, Rambabu K et al (2019) Microalgae: a potential alternative to health supplementation for humans. *Food Sci Hum Wellness* 8:16–24. <https://doi.org/10.1016/j.fshw.2019.03.001>
- Kurup VM, Thomas J (2020) Edible vaccines: promises and challenges. *Mol Biotechnol* 62:79–90. <https://doi.org/10.1007/s12033-019-00222-1>
- Kusmayadi A, Leong YK, Yen HW et al (2021) Microalgae as sustainable food and feed sources for animals and humans – biotechnological and environmental aspects. *Chemosphere* 271:129800. <https://doi.org/10.1016/j.chemosphere.2021.129800>
- Larosa V, Remacle C (2013) Transformation of the mitochondrial genome. *Int J Dev Biol* 57:659–665. <https://doi.org/10.1387/ijdb.130230cr>
- León-Bañares R, González-Ballester D, Galván A, Fernández E (2004) Transgenic microalgae as green cell-factories. *Trends Biotechnol* 22:45–52. <https://doi.org/10.1016/j.tibtech.2003.11.003>
- Liang ZC, Liang MH, Jiang JG (2020) Transgenic microalgae as bioreactors. *Crit Rev Food Sci Nutr* 60:3195–3213. <https://doi.org/10.1080/10408398.2019.1680525>
- Madhav N, Oppenheim B, Gallivan M et al (2018) pandemics:Risks,Impact and Mitigation. In: Jamison DT, Gelband H, Horton S. (eds) *Disease Control Priorities*, 3rd edition: improving health and reducing poverty, 3rd edn. pp 315–345.
- Mayfield SP, Franklin SE, Lerner RA (2003) Expression and assembly of a fully active antibody in algae. *Proc Natl Acad Sci U S A* 100:438–442. <https://doi.org/10.1073/pnas.0237108100>
- Mobin S, Alam F (2017) Some promising Microalgal species for commercial applications: a review. *Energy Procedia* 110:510–517. <https://doi.org/10.1016/j.egypro.2017.03.177>
- Norero D (2020) Italy and Israel bet on GM microalgae to develop edible COVID vaccine. <https://allianceforscience.cornell.edu/blog/2020/06/italy-and-israel-bet-on-gm-microalgae-to-develop-edible-covid-vaccine/>. Accessed 5 Feb 2021
- Okuzumi J, Nishino H, Murakoshi M et al (1990) Inhibitory effects of fucoxanthin, a natural carotenoid, on N-myc expression and cell cycle progression in human malignant tumor cells. *Cancer Lett* 55:75–81. [https://doi.org/10.1016/0304-3835\(90\)90068-9](https://doi.org/10.1016/0304-3835(90)90068-9)
- Potvin G, Zhang Z (2010) Strategies for high-level recombinant protein expression in transgenic microalgae: a review. *Biotechnol Adv* 28:910–918. <https://doi.org/10.1016/j.biotechadv.2010.08.006>
- Ramos-Vega A, Rosales-Mendoza S, Bañuelos-Hernández B, Angulo C (2018) Prospects on the use of *Schizochytrium* sp. to develop oral vaccines. *Front Microbiol* 9. <https://doi.org/10.3389/fmicb.2018.02506>
- Rasala BA, Muto M, Lee PA et al (2010) Production of therapeutic proteins in algae, analysis of expression of seven human proteins in the chloroplast of *Chlamydomonas reinhardtii*. *Plant Biotechnol J* 8:719–733. <https://doi.org/10.1111/j.1467-7652.2010.00503.x>
- Reed SG, Orr MT, Fox CB (2013) Key roles of adjuvants in modern vaccines. *Nat Med* 19:1597–1608. <https://doi.org/10.1038/nm.3409>
- Robertson S (2021) New platform using green algae tests for SARS-CoV2 virus. <https://www.news-medical.net/news/20210201/New-platform-using-green-algae-tests-for-SARS-CoV-2-virus.aspx>. Accessed 8 Feb 2021
- Rosales-Mendoza S (2016) Algae-based biopharmaceuticals. *Algae-Based Biopharm* 1–166. <https://doi.org/10.1007/978-3-319-32232-2>
- Rosales-Mendoza S, García-Silva I, González-Ortega O et al (2020) The potential of Algal Biotechnology to produce antiviral compounds and biopharmaceuticals. *Molecules* 25:1–25. <https://doi.org/10.3390/molecules25184049>
- Sahoo A, Mandal AK, Dwivedi K, Kumar V (2020) A cross talk between the immunization and edible vaccine: current challenges and future prospects. *Life Sci* 261:118843. <https://doi.org/10.1016/j.lfs.2020.118343>
- Saveria T, Parthiban C, Seilie AM et al (2022) Needle-free, spirulina-produced *Plasmodium falciparum* circumsporozoite vaccination provides sterile protection against pre-erythrocytic malaria in mice. *npj Vaccines* 7:1–11. <https://doi.org/10.1038/s41541-022-00534-5>
- Saxena J, Rawat S (2013) Edible vaccines. In: Ravi I, Baunthial M, Saxena J (eds) *Advances in Biotechnology*. Springer New Delhi, pp 207–226
- Shiraishi H, Tabuse Y (2013) The ApII restriction-modification system in an edible cyanobacterium, *arthrospira (spirulina) platensis* NIES-39, recognizes the nucleotide sequence 5'-CTGCAG-3'. *Biosci Biotechnol Biochem* 77:782–788. <https://doi.org/10.1271/bbb.120919>
- Specht EA, Mayfield SP (2014) Algae-based oral recombinant vaccines. *Front Microbiol* 5:1–7. <https://doi.org/10.3389/fmicb.2014.00060>
- Streatfield SJ, Jilka JM, Hood EE et al (2001) Plant-based vaccines: unique advantages. *Vaccine* 19:2742–2748. [https://doi.org/10.1016/S0264-410X\(00\)00512-0](https://doi.org/10.1016/S0264-410X(00)00512-0)
- Sun M, Qian K, Su N et al (2003) Foot-and-mouth disease virus VP1 protein fused with cholera toxin B subunit expressed in *Chlamydomonas reinhardtii* chloroplast. *Biotechnol Lett* 25:1087–1092. <https://doi.org/10.1023/A:1024140114505>
- Takeyama N, Kiyono H, Yuki Y (2015) Plant-based vaccines for animals and humans: recent advances in technology and clinical trials. *Ther Adv Vaccines* 3:139–154. <https://doi.org/10.1177/2051013615613272>
- Taunt HN, Stoffels L, Purton S (2018) Green biologics: the algal chloroplast as a platform for making biopharmaceuticals. *Bioengineered* 9:48–54. <https://doi.org/10.1080/21655979.2017.1377867>
- Vetter V, Denizer G, Friedland LR et al (2018) Understanding modern-day vaccines: what you need to know. *Ann Med* 50:110–120. <https://doi.org/10.1080/07853890.2017.1407035>
- Waheed S, Bayas A, Hindi F et al (2021) Neurological complications of COVID-19: Guillain-Barre Syndrome following Pfizer COVID-19 vaccine. *Cureus* 13:2–5. <https://doi.org/10.7759/cureus.13426>
- Walker PJ, Winton JR (2010) Emerging viral diseases of fish and shrimp. *Vet Res* 41. <https://doi.org/10.1051/vetres/2010022>
- Yan N, Fan C, Chen Y, Hu Z (2016) The potential for microalgae as bioreactors to produce pharmaceuticals. *Int J Mol Sci* 17:1–24. <https://doi.org/10.3390/ijms17060962>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.