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TRANSFORMATION AND EXPRESSION OF GENE IN MICROALGAE: FROM TOOLS TO APPLICATIONS

Sahar Sadaqat, Muhammad Usmaan, Mizna, Sana Shakoor, Rida Hameed, Sara Ajmal, Amina Yaqoob, Momna Muhammad, Mobeen Babar, Arzoo Ishaq, Maham Irfan, Noor ul Ain, Muhammad Awais, Muhammad Saad Bhutta, Abdul Raouf, Mehwish Imtiaz, Naila Shahid, Ayesha Latif, Aneela Yasmeen, Saira Azam, Tahir Rehman Samiullah, Abdul Qayyum Rao* and Ahmad Ali Shahid

Centre of Excellence in Molecular Biology, University of the Punjab, 87-West Canal Bank Road Lahore-53700, Pakistan

*Corresponding author: qayyumabdul77@yahoo.com

ABSTRACT

Microalgae provide an excellent platform for foreign genes expression. Chloroplast and Nucleus based transformation of transgenes in algae is possible after construct designing (including different markers and promoters) and codon optimization. Different and effective promoters and reporter genes are selected for better recombination. Chloroplast mediated transformation is much more effective than nuclear-based transformation for transgene analysis owing to the higher copy number of chloroplast in the unit area. Microalgae-based products are an excellent target for producing a wide variety of bio-products such as vaccines, antibiotics, and drugs against different diseases. These alternative approaches facilitate enhanced biodiesel production through the fast growth of microalgae in a heterotrophic environment. Also, low risks are attributed owing to their nonhuman consumption property. Biosafety and risk assessment should be carried out before releasing genetically modified microalgae into the environment, considering ethical research code. Microalgae provide cheap, efficient, and high-yielding alternatives to plants. The review emphasizes a microalgae-based expression approach with key steps involved in detail.

***Keywords*:** Microalgae, Transformation, Expression, Codon optimization, Biodiesel, Vaccines

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1. INTRODUCTION

Owing to the exponential growth of global population, demand for a number of key industrial products is on the rise. Traditional production systems' costs are on rise due to high mandate and low product yields, which is a strong reason to design solutions for low-cost production (Siddiqui et al. 2020). Microalgae are a varied group of prokaryotic and eukaryotic organisms that play an important ecological role. They are responsible for around half of all global organic carbon fixations (Leon and Fernandez 2007). Photosynthetic microalgae are of vast interest these days as they provide a sustainable approach for the production of large number of products in the field of pharmaceutical, nutrition and biofuels, if genetically engineered. A number of microalgae species have been assigned GRAS (Generally Recognized as Safe) status from US and FDA for human consumption (Torres-Tiji et al. 2020; Sproles et al. 2021). Algal species can be used for producing recombinant proteins by inserting desired gene into the genome of microalgae (Shirakawa et al. 2021). Microalgae give a production stage to the outflow of foreign genes because of their fast development under photoautotrophic conditions and their minimal effort of support, when contrasted with land plants, mammalian cells, yeast and bacteria. Likewise, numerous algal species have created novel metabolic tracks that deliver mixes of commercial esteem (Siddiqui et al. 2020). Microalgae may produce a wide range of high-value bioactive chemicals, including carbohydrates, proteins, lipids, essential fatty acids, pigments, vitamins and antioxidants, among other thing (Udayan et al. 2021). Microalgae are being regarded as prospective bioremediators of urban and agro-industrial effluents, allowing the integration of bio refineries, the utilization of gaseous effluents and the development of added value biomass in this context (dos Santos et al. 2021). Concern about looking young and beautiful has grown in the previous decade, prompting many customers to use anti-aging cosmetics on a daily basis. Cosmetic companies have recently begun to investigate the use of chemicals isolated from microalgae and cyanobacteria in new formulations (Morocho-Jácome et al. 2020). The model diatom

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algae species *Phaeodactylum tricornutum* is an appealing option for synthetic biology applications (Scaife and Smith 2016; Brasil et al. 2017). Because of its inherent proclivity for lipid storage, *P. tricornutum* is an attractive choice for biofuel generation. The nuclear, mitochondrial and plastid genomes of this algae species were sequenced due to industrial and academic interest (Bowler et al. 2008; Yongmanitchai and Ward 1991; Cochrane et al. 2020). The chloroplast of microalgae like *Chlamydomonas reinhardtii* is an appealing chassis for producing novel recombinant proteins and metabolites using light. Over 100 different proteins have been successfully produced using methods for introducing and expressing transgenes in the chloroplast genome of *C. reinhardtii* (Larrea-Alvarez and Purton 2020). In the biotechnologically important alga *Picochlorumceleri*, Cas9-guide RNA ribonucleoprotein (RNP) complexes were employed to efficiently create insertions in both alleles of two targeted genes (nitrate reductase (Fig. 1) and carotenoid isomerase) (Krishnan et al. 2020). Transformation approaches are a critical component of functional genomics research. Microalgae genetic transformation is quickly becoming a valuable biotechnological tool to achieve all the objectives discussed above (Ortiz-Matamoros et al. 2018). The microalgal cell has three membrane-bound organelles: nuclei, chloroplasts, and mitochondria. Despite the fact that most of the preceding instances employ nuclear transformation, nuclear expression has the disadvantage of being susceptible to epigenetic effects and random insertion, which can result in fluctuating transgene expression Chloroplast engineering, on the other hand, has numerous major advantages, including the absence of epigenetic gene silencing, selective transgenic location in the genomes, and relatively high expression levels (Leister 2003; Zhang et al. 2017; Lu et al. 2021).

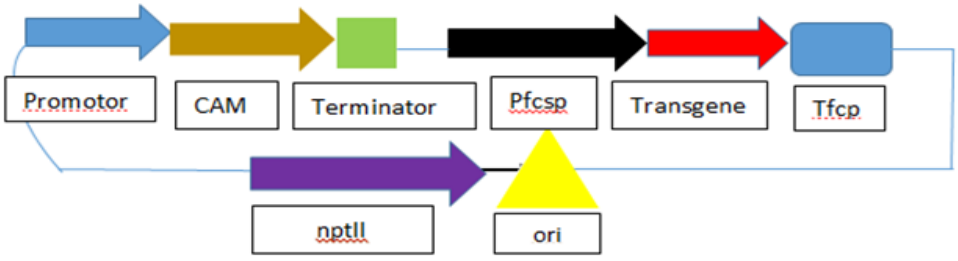

Fig. 1: Systemic view of possible vector construct in microalgae. Annotations of the Plasmid Map: Promoter and Terminator (nitrate reductase) from microalgae, CAM is Reporter gene of chloramphenicol acetyltransferase, PfcpCis Promoter of fcpC gene from microalgae TfcpA: Terminator of fcpA geneoriis pBR322 site for replication. nptIIis neomycin phosphor-transferase gene extracted from Tn5 transposon, it is a reporter gene. AmpR can also be used.

1.1. Nuclear Transformation

For the transformation of microalgae, various transformation procedures have been established. Despite improved capabilities and diverse ways to producing a variety of bioproducts from microalgae, there are technological problems and constraints that must be overcome before algal biotechnology can be commercialized (Gimpel et al. 2015). By various physical/chemical techniques, algal genetic transformation processes create temporal permeabilization of the cell membrane/cell wall, allowing DNA molecules to enter the cell. Thus, with or without external aid, DNA enters the nucleus through the nuclear membrane and integrates into the genome (Hallmann 2007; Rathod et al. 2017). In Chlamydomonas cell wall contains low level of chitin or cellulose so protoplast is generated by incubating with autolysins which are named as hydroxyl-proline-specific protease (Jaenicke et al. 1987; Patil et al. 2020). Biolisticdelivery includes DNA coated gold or tungsten which is delivered with high velocity through particle delivery system by transcending the cell wall which is a physical barrier (Brown et al. 2020).). The protoplast of microalgae is stirred in the presence of glass beads, polyethylene glycol (PEG), and foreign DNA in the Glass Beads technique (Zhang et al. 2020). Electroporation another method for introducing DNA is by applying electric impulses (Table 1). This method is utilized to transform to protoplast and thin wall algal cell. This method was used to transform *C. reinhardtii* (Ng et al. 2020).). *Agrobacterium*-based transformation of microalgae like in land plants Agrobacterium tumefaciens is used to transfer nucleus using pCAMBIA transformation vector. Similarly, it is also used to transfer nucleus in various microalgae like Chlamydomonas (Kathiresan et al. 2009; Dehghani et al. 2017).

1.2. Chloroplast Transformation

Chloroplast plays an important role in the reduction of atmospheric carbon dioxide by utilizing it in the production of most abundant protein of the Earth, RuBisCO. In recent decades, genetic modification of chloroplasts

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has been in practice for producing therapeutic proteins and enzymes used in daily life (Ruhlman et al. 2010; Daniell et al. 2021). High levels of desired proteins expressed in chloroplast can be obtained due to high copy number of that organelle. It has a full genetic system with 100–200 genes, the majority of which are connected to photosynthetic light reactions or transcription and translation processes (Green 2011; Bo et al. 2020). Multiple genes can be produced as polycistronic transcription units in the chloroplast concurrently under the control of a single promoter, allowing for the coordinated synthesis of enzymes from certain metabolic pathways or subunits of a protein complex (Kwon et al. 2018). The main stable framework for the chloroplast of *C. reinhardtii* was built up utilizing biolistic delivery. By homologous recombination of the transgenic marker into the goal mutant strain, the outside DNA was supposed to rescue three mutants of the chloroplast atpB quality and restore photosynthetic activity (Boynton et al. 1988; Kwon et al. 2018).

1.3. Markers for the Chloroplast Transformation

Different markers are being used for chloroplast transformation on the basis of their growth, metabolic activity and many other factors listed below.

1.4. Photoautotrophic Growth

Chlamydomonas has the advantage of being able to grow in non-photosynthetic environments while utilizing acetate as a carbon source. Thus, non-photosynthetic mutants were used as recipient strains and retrieval of their photosynthetic action was used as a reporter system to restore expression of the mutated atpB gene, which codes for ATP synthase, and the tscA gene, which codes for a small RNA involved in trans-splicing of the psaA transcript (Goldschmidt-Clermont 1991; Day and Goldschmidt-Clermont 2011). When a foreign gene is added into the plastid genome, native expression cues such as a chloroplast promoter, a 5′UTR with translation initiation signals, and a 3′UTR are required. A variety of chloroplast expression signals have been used in Chlamydomonas, including those from atpA, psaA, psbA, and psbD (Day and Goldschmidt-Clermont 2011; Michelet et al. 2011).

1.5. Metabolic Enzymes

Plastid transformation selectable markers are mainly based on bacteria genes. The expression of bacterial gene ptxD in the chloroplast that encodes an NAD (P)-dependent phosphite oxidoreductase to allow utilization of phosphite as a source of phosphorus (P) (Sandoval-Vargas et al. 2019). The approach aimed that most organisms cannot use phosphite, therefore, growing the transgenic microalga in phosphite provides a selective advantage over competing species (Dyo and Purton 2018).

1.6. Resistance to Antibiotics

Plastid's translation machinery has preserved prokaryotic characteristics, so mutations in ribosomal proteins or rRNA can provide resistance to antibiotics like spectinomycin, streptomycin, and erythromycin. These serve as indicators of chloroplast transformation. Another option is to employ marker genes that code for enzymes that alter and inactivate antibiotics chemically (Day and Goldschmidt-Clermont 2011). Resistivity against the spectinomycin was given by presenting the bacterial-determined aadA quality in the genome of chloroplast, which encodes for aminoglycoside 3′ adenyl- transferase (Tabatabaei et al. 2017). When flanked by suitable chloroplast expression signals (promoter and UTRs), this bacterial gene confers significant levels of antibiotic resistance in Chlamydomonas (Bock 2015).

1.7. Resistance to Herbicides

The herbicide named as sulfometuron methyl (SMM) that restrains development of microscopic organisms, yeast, green growth and plants is generally utilized as a choice marker for plants and green growth. The objective of SMM includes quality that encodes aceto-hydroxyacid synthase (AHAS), a compound required in the biosynthesis of expanded amino acids (Grundman et al. 2012). It is observed to be an optimum marker for chloroplast alteration after the genome of chloroplast encodes AHAS in porphyridium sp. (red microalgae) resulted in the change of chloroplast genome (Doron et al. 2016).

1.8. Regulatory Elements: promoters, UTRs, and codon optimization

Transformation efficiency is very high if there is perfect homology of plastid DNA flanking sequences. Identification of promoters, 5′-UTRs, 3′-UTRs and insertion sites play a critical role in the successful transformation. Complete chloroplast genome sequences are required for integration of the transgene at exact site via homologous recombination and to identify endogenous regulatory sequences for perfect transgene expression

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(Grevich and Daniell 2005; Ruhlman et al. 2010). In green algae, diatoms, and cyanobacteria, novel endogenous promoters with both constitutive and inducible activities have recently been discovered. Different promoters like rbcL (ribulosebisphosphate carboxylase/oxygenase), psbA (D1 protein of the photosystem II response focus) and atpA (Y subunit of ATP synthase was used to optimize the activity of the promoters and atpA was resulted in the highest regulatory activity (Barrera et al. 2014).

Table 1: This table shows different pharmaceutically important recombinant proteins in different micro algae along with other important information

Microalgae	Method of transformation	Gene expressed	Promoters	Selective marker gene	Products	Expression location
D. salina	Electroporation	HBsAg	ubiquitin	Chloraamphenicol resistant gene	HBsAg	Nucleus
D. tertiolecta	Particle's bombardment	Xylanase/ alpha-glactosidase	psbD	Erythmycin resistant gene	Xylanase/alpha glactosidase	Chloroplast
Porphyridium sp.	Particle's bombardment	AHAS	Endogenous AHAS promotor	AHAS	Acetohydroxy acid synthase	Chloroplast
S. microadriaticum	Agrobacterium	GUS	CaMV35S	hpt gene	ß-glucoronidase	Nucleus
C. reinhardtii	Particle bombardment	E2	atp A	aad A	Swine fever virus structure protein	Chloroplast

Source: Chew et al. (2017); Yan et al. (2016)

1.9. Inducible Expression in the Chloroplast

For a variety of reasons, an inducible chloroplast gene expression system could be highly valuable. Primarily, it may be used to figure out how important chloroplast genes are for cell development and survival. Second, it may be quite useful for expressing proteins that are harmful to algal cells. Third, it would allow for the progressive depletion of photosynthetic complexes, making it possible to create new photosynthetic complexes (Ramundo et al. 2014). NAC2 is an important component used for the stability of psbD mRNA which binds a distinctive target site in the psbD 5′UTR and regulates the expression of a foreign gene which is under the control of psbD 5′UTR (León et al. 2008).

1.10. Promoter Selection and Codon Optimization

Genetic engineering aims to synthesize a plasmid vector comprising components necessary for gene expressional studies. A classic vector creation includes the management of selection marker expression cassettes for the selection of bacterial plasmids or genetic transfer into the host. An extra gene directed expression cassette is present for expression system (Wang et al. 2017). For that purpose, inducible promoter is used to restrict the selection marker expression in transgenic host (Trassaert et al. 2017). So many different promoters which are used for transformation of plants can be used for transformation of microalgae for instance 35S Cauliflower Mosaic Virus (CaMV) promoter and promoter of Agrobacterium p1-2, both were used for reported GUS gene expression in dinoflagellates species, Amphidinium as well as Symbiodinium (Grunennvaldt et al. 2015). Whereas RBCS2 promoter derived from Chlamydomonas reinhardtii (unicellular green algae) displayed high efficiency in contrast to 35S promoter in recombinant Dunaliellasalina (Scranton et al. 2016). Chlorella genetic reformation is relatively slow. Ubiquitin gene promoter performed satisfactory role in expression of mature Neutrophil Peptide-1 (NP-1) of rabbit in Chlorella (Run et al. 2016). While in other research *C. ellipsoidea* was transferred to a vector also having gene for flounder Growth Hormone (fGH) controlled by promoter (35S) as well as phleomycin resistance gene controlled by Chlamydomonas promoter (RBCS2) (Yang et al. 2016).

Effective expression of subject genes in chloroplasts of microalgae is usually best acquired by the usage of regulatory components, taken from genes which express abundantly. For this persistence, promoters involved in the expression of ribulosebisphosphate carboxylase or oxygenase subunits (rbcL), photosystem II reaction centre D1protein (psbA), and ATP synthase Y subunit were utilised. The maximum expression of GUS reporter gene was seen under the control of atpA promoter as well as the 5' UTRs (Doron et al. 2016). Moreover, in another research, atpA in addition to psbD promoters as well as their 5′UTR were observed to manipulate utmost expression of Green fluorescent protein (GFP), a reporter gene, when analyzed it to the promoter of rbcL as well as psbA with 5′UTR. The occurrence of 3′UTRs taken from various genes was required but they had a negligible effect on the gathering

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of transcripts as well as recombinant proteins (Rosenberg et al. 2015). In later studies, the Gold Schmidt Clermont scientists confirmed the fact that the psaA-exon1 along with its promoter as well as 5′UTRs enhances the expression of protein of interest in chloroplast. They also explained that the change in expression rate in different expression systems is also due to the location and number of events of recombination (Michelet et al. 2011). Additional developments amplified the rate of expression in *C. reinhardtii* chloroplast up to 20% of total protein content by concentrating on codon optimization as well as ATP dependent proteases inhibition (Freudenberg et al. 2021). The toxicity levels of transgene could result in adverse effects on gene expression as genotype modification associated with random insertion of gene of interest in nuclear DNA, thus many screenings analysis is needed to separate highest expression level clones. In short, the promoter, UTRs or expression techniques are not the only factors affecting rate of expression. Random integration in nucleus as well as in chloroplast integration site overall affect the expression of transgene (Ahmad et al. 2021).

Even though all of the elements required for effective transcription and translation (promoters, introns, and other regulatory regions) have been included in the chimeric gene design, exogenous gene expression can be minimal or non-existent. Furthermore, expression of the exogenous gene may be reduced if transgenic algal clones are not maintained under selection circumstances (León-Bañares et al. 2004).

1.11. Applications of Microalgae

Microalgae are unicellular photosynthetic organisms that have recently piqued interest due to their potential applications in food, nutraceuticals, medicines, animal feed, cosmetics, and bio fertilizers. Microalgae include a number of high-value bioactive chemicals that have potential health advantages and can be utilized to prevent and treat a variety of diseases. Microalgae have different uses in biotechnology including the biodiesel production, increased lipid content in soybean and pharmaceuticals. They are explained in detail below.

1.12. Pharmaceuticals

Most of the pharmaceutically important products have been made in microalgae especially in *C. reinhardtii* using different genetic engineering techniques. *C. reinhardtii* is an excellent bioreactor for the manufacture of a wide range of pharmaceutically significant proteins, such as vaccines, antibodies, therapeutic proteins, and so on (El-Ayouty et al. 2019).

1.13. Subunit Vaccine

Vaccines have been developed to combat a variety of viral and non-viral diseases. Chlamydomonas chloroplasts can create E2 protein, which contains the antigen for protein against the swine flu virus. This E2 transformed algae when introduced in mice via subcutaneous injections, a significant increase in serum antibodies against the virus was seen (Shamriz and Ofoghi 2016). Microalgae-made vaccine prototypes include four against *Plasmodium falciparum* (malaria) and one against *Staphylococcus aureus*, *Escherichia coli*, *Human papillomavirus*, *Hepatitis B virus*, *Human immunodeficiency virus*, *Ebola virus*, *Influenza virus*, and *Zika virus*. Microalgae-made vaccines against important bacterial (i. e. tuberculosis, anthrax), fungal (i.e. candidiasis), or parasitic (i.e. Chagas) diseases, among others (neglected tropical diseases), are an open field to research and development (R&D) (Hotez 2018). Importantly, preclinical evaluations have been carried out, further clinical studies are the next step to be performed. In those reports, immunizations have been carried out by intraperitoneal, subcutaneous, and intramuscular routes; remarkably, the oral route for immunization has mainly been used (Fig. 2). This fact supports that the first ideal goal of microalgae-vaccine technology is being promoted microalgae used as subunit vaccine production host and delivery vehicle (Ramos-Vega et al. 2021). For the treatment of foot and mouth disease vaccine in *C. reinhardtii*, the VP1 protein was fused with cholera toxin B subunit, which is an important adjuvant and thus the recombinant protein showed the binding affinity towards GM1 gangliosidase (Ramos-Vega et al. 2021). The D. salina genome was effectively converted with the hepatitis B virus surface antigen gene (Soria-Guerra et al. 2014). The viral envelop protein gene VP28 for White Spot Syndrome Virus (WSSV) was successfully transformed into the nuclear genome of *C. reinhardtii* resulting in recombinant *C. reinhardtii* providing a suitable candidate for oral vaccine against this WSSV (Lanh et al. 2021).

1.14. Antibodies Production

Microalgae chloroplasts developed the first monoclonal antibody against herpes simplex virus glycoprotein D. Defensins are the small antibodies like molecules produced against many pathogens. These are the small cationic peptides having same function as antibody. Mature rabbit neutrophil peptide called alpha defensin has a vast range of anti-microbial properties against virus, bacteria, pathogenic fungi etc. have been transformed into a microalgae called *C. ellipsoidea*. Growth hormone can also be expressed in *C. ellipsoidea*. *P. tricornutum* was used for

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expression of human CL4 monoclonal antibody (mAb) and using hepatitis B surface antigens evaluation was done (Hempel et al. 2011). *P. tricorntum* can show expression of recombinant monoclonal antibody against Marburg virus. *C. reinhardtii* has also been used to counter glycoprotein of herpes simplex virus by producing immunoglobulin A (Ig A) monoclonal antibody using microalgae, which lack machinery for N-glycosylation (Mayfield et al. 2003). Genetic or chemical conjugation of eukaryotic toxins and antibody fragments has been used for the production of immunotoxins (Yusibov et al. 2016). These toxins conjugated antibodies reach target cells and release toxins. These toxins disturb translational mechanism of cells and result in apoptosis of these target cells. Single chain variable fragment against CD22 antigen B-cell surface receptor of exotoxin A from *Pseudomonas aeruginosa* has been produced using *C. reinhardtii* chloroplast (Bogner et al. 2010). Erythropoietin from humans had been expressed in *C. reinhardtii* which had the activity of hsp 70 A/rbcS2 (Eichler-Stahlberg et al. 2009). 100 ng HGH from cell extract of modified *C.reinhardtii* when treated to Nb2-11 rat lymphoma cell line showed 8 times increase in cell number after 4 days (Wannathong et al. 2016). Clamydomonas has also been used for expression of Human immuno virus protein. This protein has paved a way for the development of microalgae based oral vaccine for AIDS (Barahimipour et al. 2016).

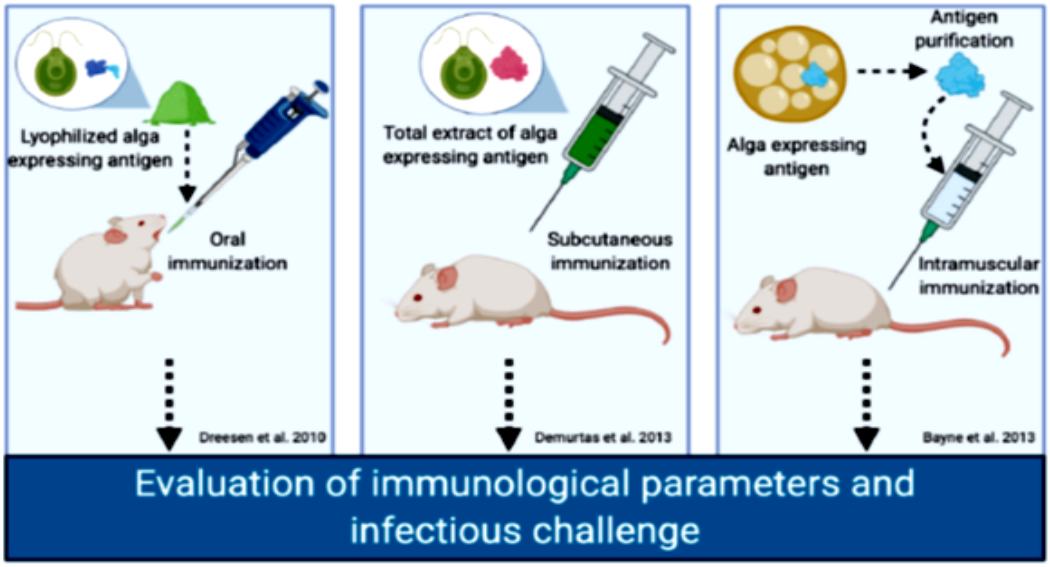

Fig. 2: Examples of evaluations of microalgae-made vaccines under different route schemes (Ramos-Vega et al. 2021).

1.15. Biodiesel

Microalgae being photosynthetic bodies are excessively rich in oil and biodiesel can be obtained by using suitable technology. Microalgae lipids possess two times more energy than other lipids. Microalgae require less space and energy in producing biomass than other crops hence are of great interest for replacing bioethanol. If oil palm can be grown 50% of transport fuel needs can be met while using only 24%of total cropland (Metzger and Largeau 2005). But oil crops cannot completely replace petroleum needs. If microalgae are for biodiesel production only 1 and 3% of cropland would be used to meet 50% transport fuel needs. Microalgae have certain advantages as well. Microalgae double their biomass within 24 hours (3.5h during exponential growth). Microalgae are also rich in oil which can exceed 80% by weight of dry biomass. Oil levels of 20–50% are quite common (Spolaore et al. 2006).

Oil production in microalgae depends on oil content of biomass and their growth rate. More oil producing algal species are the need of time. Different species produce different types of complex oil and hydrocarbons. Among all the oil production from different species of microalgae is not good fit for bio-diesel production but suitable oils are more common. Microalgae potentially a good source of natural organic carbon source such as sugar, can be used to make biodiesel (Ratledge and Wynn 2002; Guschina and Harwood 2006).

1.16. Biosafety Considerations

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In the European Union, all the genetically modified organisms and their products prior to their release in market should get an approval. There is a guidance protocol prepared by Europe Food Safety Authority (EFSA) in 2006 (Wijffels 2015). Theses containers are mostly in closed conditions in which genetically modified algae can be grown, stored, transported, destroyed, and then disposed of in the environment. The contained growth of microalgae can take place in tubular reactors, closed reactors and polyethylene. These advancements are intended to permit proficient development and the reaping of vast amounts of micro algal biomass (van der Vlugt 2020).

1.17. Risk Assessment

Risk assessment of genetically modified microalgae can be checked by testing their effect on animals, plants, humans, and environment. Risk assessments predict the toxicity and associated allergenicity and potential hazards of horizontal gene transfer (HGT) (Gressel et al. 2014). Though, to discourse this potential risk, even on the off chance that it is extremely occasional, the utilization of choice methods based on endogenous metabolic markers that supplement a missing movement in an altered beneficiary strain would be invaluable over the utilization of qualities that present resistance to herbicides or antibiotics. Evacuation of the selection markers is additionally suggested (Monier et al. 2009; Beacham et al. 2017).

1.18. Exposure Assessment

On commercial scale, the development of micro algae may cause their spillage to natural ecosystem so an exposure assessment is needed to analyze the route by which these genetically modified algae can interact with natural ecosystem. For exposure assessment digital PCR technique can be used to detect the presence of GM micro algae strains in environment (Kumar 2015).

Conclusion and Future Perspectives

Algae form the base of plant evolutionary tree. It can grow in normal as well as under harsh conditions. Algae can provide biomolecules and chemicals to biotechnology industries. They also provide food to the aquatic animals. They are the powerful source of bio medical molecules such as drugs, vaccines and antibodies (Hallegraeff 2003). Recently a complete carotenoid pathway was developed for *Xanthophyllomyces dendrorhous*, which depends on efficient expression of transgenes in microalgae (Gressel et al. 2014). Foreign gene can be expressed by integrating in chloroplast or nuclear genome. These foreign genes are loaded with desired signals that cause the synthesis of desired proteins. The expression of the genes of interest in microalgae is somehow difficult due to epigenetic mechanisms but recently these problems are being overcome. For the use of microalgae in basic and applied research, better protocols are being developed for knock-in and knock-out of gene by RNAi and CRISPR/Cas system. In conclusion, the change of frameworks for inducible expression is likewise of extraordinary significance, since it will enable us to control the transient expression of toxic particles. At long last, quick headways in genome sequencing, now regularly performed for all life forms, will demonstrate instrumental in propelling these objectives in the near future.

ORCID

Sahar Sadaqat https://orcid.org/0000-0003-1067-8838
Muhammad Usmaan https://orcid.org/0000-0003-0590-7579
Mizna https://orcid.org/0000-0001-7267-4133
Sana Shakoor https://orcid.org/0000-0002-4414-5119
Rida Hameed https://orcid.org/0000-0002-8588-5236
Sara Ajmal https://orcid.org/0000-0003-4804-5462
Amina Yaqoob https://orcid.org/0000-0003-0685-7550
Momna Muhammad https://orcid.org/0000-0001-5441-9171
Mobeen Babar https://orcid.org/0000-0002-7880-2551
Arzoo Ishaq https://orcid.org/0000-0003-4873-6108
Maham Irfan https://orcid.org/0000-0002-3638-9113
Noor ul Ain https://orcid.org/0000-0003-1195-8719
Muhammad Awais https://orcid.org/0000-0002-5722-5935
Muhammad Saad Bhutta https://orcid.org/0000-0002-1721-6807
Abdul Raouf https://orcid.org/0000-0002-5368-2186
Mehwish Imtiaz https://orcid.org/0000-0002-9090-0015
Naila Shahid https://orcid.org/0000-0002-6583-0406

Sadaqat S, Usmaan M, Mizna, Shakoor S, Hameed R, Ajmal S, Yaqoob A, Muhammad M, Babar M, Ishaq A, Irfan M, Ain NU, Awais M, Bhutta MS, Raouf A, Imtiaz M, Shahid N, Latif A, Yasmeen A, Azam S, Samiullah TR, Rao AQ and Shahid AA, 2022. Transformation and expression of gene in microalgae: From tools to applications. Agrobiological Records, 7: 29-39. https://doi.org/10.47278/journal.abr/2021.012

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Ayesha Latif https://orcid.org/0000-0002-7950-4898
Aneela Yasmeen https://orcid.org/0000-0002-5478-0263
Saira Azam https://orcid.org/0000-0002-2064-7093
Tahir Rehman Samiullah https://orcid.org/0000-0003-2004-3801
Abdul Qayyum Rao https://orcid.org/0000-0001-9269-3307
Ahmad Ali Shahid https://orcid.org/0000-0003-4667-8880

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