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Genome Manipulation in Microalgae: A Systematic Map on Improved Traits, Strains, and Their Commercial Applications

Irene Gallego | Michael Meissle | Jörg Romeis

Research Division Agroecology and Environment, Agroscope, Zurich, Switzerland

Correspondence: Irene Gallego (irene.gallego@agroscope.admin.ch)

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ABSTRACT

Microalgae are promising platforms for producing diverse biocompounds relevant to multiple commercial sectors, and the use of genomic technologies in microalgae is rapidly expanding. To examine the research trends, we conducted a bibliographic analysis (systematic map) of this rapidly developing field. Our study presents a descriptive overview of genomic techniques in microalgae for industrial applications, highlighting emerging trends and key knowledge gaps. After the initial identification of more than 23,600 scientific publications, we screened over 5,500 original research studies that were deemed most relevant by AI-based sorting. We then selected 518 relevant studies in which microalgal strains were genetically modified to improve traits of commercial interest. We also compiled information on industrially relevant sectors that potentially benefit from genetically improved microalgae. Overall, more than 60 strains from 10 genera, mainly belonging to green microalgae, cyanobacteria and diatoms, were genetically improved for future commercial applications. Most studies used conventional genetic engineering techniques, with incipient research on gene editing tools. The most frequently improved microalgal traits varied with the type of cell (eukaryotic microalgae vs. cyanobacteria). Genetically improved eukaryotes (mainly green microalgae) produced proteins, lipids and pigments, while cyanobacteria synthetized alcohols, polyols and terpenoids. The main recipient of the improved strains is the human health sector, followed by the bioenergy sector, food and beverage industries, animal farming including aquatic animals, and cosmetics. Manufacturers of bioplastic packaging, chemical industries, environmental remediation companies, wastewater treatment plants, and the agriculture sector may also benefit from the use of genetically improved microalgae and their derived products.

1 | Introduction

Microalgae are microorganisms that produce a wide variety of relevant compounds from atmospheric CO_2 and light, thus contributing significantly to carbon sequestration [1]. They are widely distributed in aquatic habitats and in some terrestrial environments [2], and reproduction is mostly asexual. Regarding the cell type, microalgae can be classified into eukaryotes and prokaryotes. While the former group includes cells with membrane-bound organelles, such as green algae and diatoms (kingdom Chromista, *sensu* [3]); prokaryotes (commonly known as cyanobacteria) lack such membrane compartments in the cell.

Abbreviations: CRISPR, clustered regularly interspaced short palindromic repeats; EU, European Union; GMO, genetically modified organism; NGTs, new genomic techniques; sgRNA, single guide RNA; TALEN, transcription activator-like effector nuclease; ZFN, zinc finger nuclease.

Cultivation of microalgae for commercial use has skyrocketed during the last decades, with applications in food and feed industries, the pharmaceutical sector, agriculture, aquaculture, and promising applications in the bioenergy sector to replace fossil fuels and their derived chemicals [4, 5]. Cultivation systems, such as open ponds and closed photobioreactors or fermenters, have been optimized to increase production and reduce costs. However, microalgal production must still overcome some challenges regarding economic viability and regulatory constraints, particularly within the European Union (EU) [6, 7]. Only a few dozen strains are currently cultivated for commercial use [8]. In the EU, an even more limited number of strains is authorized, since regulation requires a case-by-case assessment that results in high economic costs [7].

The use of genomic tools broadens the biotechnological applications of microalgae as key components in circular bioeconomy models [9]. Genetically modified (GM) microalgae can produce higher biomass yields and accumulate higher concentrations of valuable biocompounds, compared to their wild-type counterparts [10]. Additionally, insertion of genes allows *de novo* synthesis of biocompounds, such as fatty acids, industrially relevant enzymes, or therapeutic bioactive proteins [4]. Currently, a wide range of genomic techniques and tools can be applied, spanning from established techniques of genetic engineering to more recent technologies based on CRISPR-mediated transformation systems [11, 12]. The development of genetic manipulation toolboxes and metabolic engineering models holds promise for further advances in the microalgal sector [9, 13].

According to the Food and Agriculture Organization of the United Nations (FAO), aquaculture encompasses the farming of aquatic organisms, including microalgae [14]. Building on this definition, we review recent advances and highlight key knowledge gaps in the development of genetically improved microalgae for industrial applications. We show temporal trends of the genetic tools used, including both established and novel genomic techniques, and transformation methods utilized over nearly four decades. We then discuss the main recipient strains of such genetic transformations, and their corresponding taxonomic groups, and report which traits have been genetically improved. Finally, to provide an applied perspective, we highlight the industrial sectors that could potentially benefit from such improved traits, i.e., commercially relevant traits.

By applying a systematic map approach, we provide an accurate description of the existing literature on genetically improved microalgae for an envisaged commercial purpose. Our research questions were: (i) which genetic transformation techniques and tools have been used in microalgae to improve a trait of relevance for the industrial sector? (ii) what are the most frequent microalgal strains that have been genetically improved? (iii) what are the most frequently improved traits of interest? and (iv) what are the potential industrial applications of the genetically modified microalgae? We searched for relevant studies in two scientific bibliographic databases (WoS, SCOPUS). We then sorted and screened the studies based on a set of inclusion and exclusion criteria with the help of a machine learning tool. In the final stage, we analyzed and visualized data to report our findings. We thus cannot claim comprehensiveness in this systematic map, but rather a description of the current aquaculture landscape.

2 | Methodology

2.1 | Review Question

We used the PICO framework (P: population, I: intervention, C: comparison/control, O: outcome) to structure our main research question: Which evidence exists on microalgae (P) that have been genetically modified (I) to successfully produce traits with relevance for industrial application (O) compared to non-modified microalgae (C)?

2.2 | Searching

Selection of keywords for search strings was based on relevant literature on the topic, as well as expert knowledge. Search strings contained two parts connected with "AND":

Part 1 included search strings referring to genetic modifications *(gene$ OR genom* OR genetic*) NEAR/0 (edit* OR engineer* OR improv* OR integrat* OR manipulat* OR modifi* OR target* OR tool* OR transform* OR silenc* OR knockout* OR overexpress*)* or biotechnology-related terms that might imply genetic modifications *(biotechnolog* OR nanotechnolog* OR bioengineer* OR [...])*. Part 2 included search strings referring to microalgal taxonomic groups (*microalga* OR picoalga* OR cyanobacteri* OR cyanophy* OR diatom* OR dinoflagellate$ OR haptophy* OR chlorophyc* OR chlorophyt* OR *chlorella OR Spirulina OR [...]*). The complete search strings are available in Supporting Information I (SI I), Table SI 1.

Predefined search strings were conducted in two bibliographic databases, i.e., Web of Science (Clarivate) and Scopus (Elsevier). Since Web of Science has multiple collections, we limited our search to the CORE COLLECTION (SCI and ESCI editions) and the Preprint Citation Index. We restricted the search to records available in English, and we refined results by document type. A dataset was created containing original peer-reviewed scientific articles, conference abstracts and early access articles. We excluded review papers and book chapters, as they generally do not contain original data. The search was conducted on 16th December 2023, and an update was performed on 30th January 2025. We saved the retrieved records and removed duplicates using the reference management software EndNote 21 (Clarivate).

2.3 | Screening and Selection of Relevant Items

We used an open-source AI tool (ASReview LAB) to screen the titles and abstracts of the records retrieved from our search, which were systematically labeled as relevant (1) or irrelevant (0) [15]. Because the software did not allow a third label category ("maybe relevant"), our screening phase is closer to a dual screening-selection phase when compared with conventional systematic literature reviews.

ASReview LAB extracts features from the text of selected prior knowledge (i.e., relevant titles and abstracts) and employs active learning techniques to retrain the model based on each newly labeled record. The active learning cycle of ASReview LAB is based on the interaction of the annotator, who decides on the (ir)relevance of unlabeled records. After each retraining of the model, new labels are assigned to the records, the remaining unlabeled references are re-ranked, and the potentially most relevant references are displayed on top.

Although active learning in ASReview LAB starts after a single label, we selected 20 relevant records and 35 irrelevant records as "prior knowledge". Then, we classified the references displayed at the top of the list. A mixed strategy was used to define the stopping criterion, i.e., a combination of a predetermined strategy (at least 30% of the dataset should be screened) and a data-driven strategy (stop after at least 300 consecutive irrelevant documents).

We used the default active learning model setup, i.e., TF-IDF feature extraction technique, Naive Bayes classifier, Maximum query strategy, and a Dynamic resampling (double) balance strategy, as those settings should provide overall fast and excellent performance [16].

During the screening-selection phase, we only included articles that fulfilled the following criteria, structured by the PICO elements of the review question [17, 18]:

1. Population: Any eukaryotic or prokaryotic microalga obtained from culture collections and/or freshwater, brackish, and marine habitats that were cultured at laboratory, pilot, and/or industrial scale under autotrophic, mixotrophic, or heterotrophic conditions.
2. Intervention: Any genetic modification that aims to increase the production of any compound of commercial interest and/or the functioning of the microalga as a cell factory, including cisgenesis, transgenesis, targeted mutagenesis, or any other gene recombination technique, including NGTs such as CRISPR, TALEN, or ZFN. We excluded random mutagenesis with different mutagenic agents (UV, X-rays, chemicals), since microorganisms genetically manipulated using this method are exempted from the EU Regulation on GMOs (Directive 2001/18/EC). We also excluded studies on domestication of strains and adaptive laboratory evolution experiments.
3. Comparison/control group: The study must also include the microalgal strain with no genetic modifications, or prior to the genetic modification described in the study, as a counterpart to the gene-edited strain (referred to as wild-type).
4. Outcome: Improved trait after gene editing compared to the wild type. The relevant variable(s) can be measured either quantitatively (% increase in yield, % increase in concentration) or qualitatively (improvement as a carrier, cell factory, improved yield, improved concentration).

A full summary of the defined PICO elements, with specific inclusion and exclusion criteria and examples, is shown in SI I, Table SI 1.

2.4 | Data Analysis

The selected studies were labeled with the following categories: taxonomic classification of the genetically modified strain(s) (genus, broader taxonomic group, and/or common name), number of genes modified to enhance the trait of interest (excluding markers, reporters, and promoters), genetic tools and transformation methods used, improved trait, and potential commercial application. We merged all the strains belonging to the same genus and grouped them into the following taxonomic groups: green microalgae, diatoms, cyanobacteria, and others. Additionally, we distinguished between the two main categories of cells (eukaryotic and prokaryotic microalgae).

Regarding the use of genetic tools, we classified the studies into three main groups: DNA gene transfer using conventional methods to introduce novel DNA into the host genome, novel gene editing tools such as CRISPR-Cas-mediated systems, and RNA transfer to silence specific genes. We also distinguished two types of transformation methods used to introduce genetic material into the cell: biological methods, which include natural transformation, conjugation, and *Agrobacterium*-mediated transformations, and physical methods, with biolistics (micro- or nanoparticle bombardment), glass beads (agitation), and electroporation as the main transformation methods. Our criteria to classify the different genetic tools and transformation methods are provided in Supporting Information II (SI II).

The improved traits were classified according to their metabolic and molecular origin, when they were related to biocompound production or enhancement. We aggregated such traits into lipids, proteins, pigments, terpenoids and waxes, esters and acids, alcohols and polyols, polymers, hydrocarbons, sugars, element accumulation (heavy metals, nutrients or organic compounds), hydrogen production, and other traits.

We also assigned different categories to the commercial sectors in which GM microalgae can be potentially used, such as agriculture, animal farming, bioenergy, biomaterials, bioremediation, chemistry, cosmetics, food, pharmaceuticals, and undefined sectors. Our criteria for creating the different industrially relevant groups are detailed in SI II.

Data visualization was done in R (version 4.5.2), using the ggplot2 package. Sankey plots were done with the ggalluvial package.

3 | Results

We identified 23,645 scientific publications that matched our search strings in the selected databases (SI I, Figure SI 1). After deduplication ($n = 7153$) and removal of review articles, book chapters, and editorials ($n = 974$), we generated a dataset with 15,518 records to be screened. More than 30% of these records were manually screened ($n = 5503$ articles) with the AI tool ASReview LAB. We stopped screening the database after we reached 300 consecutive irrelevant papers, retrieving 651 relevant articles. After a more thorough review of the full texts of

items initially classified as relevant, the dataset was reduced to 518 articles. We discarded references that were incorrectly indexed as original papers ($n = 3$) and papers with a focus on method development ($n = 34$), with strains that were not genetically improved in the actual paper but in previous ones ($n = 23$), without clear commercial application ($n = 21$), with a focus on gene functioning rather than commercial application ($n = 21$), on traits not commercially relevant (production system, $n = 19$), with limited access ($n = 6$), on random mutagenesis ($n = 2$), on traits that did not improve after genetic modification ($n = 2$), where the genetically improved strain was not a microalga ($n = 1$), and duplicates ($n = 1$) (SI I, Figure SI 1). The complete list of articles is provided in Supporting Information III (SI III).

DNA gene transfer through genetic engineering has been applied to microalgae to improve commercially relevant traits since 1987. An exponential increase in the number of studies using DNA gene transfer is observed after 2009, plateauing since the late 2010s (Figure 1). RNA gene transfer was first applied to microalgae in 2013, with consistently low frequencies over time (maximum = 3 publications per year). The use of gene-editing tools started in 2014, when the first study using TALEN was published. CRISPR-mediated tools appeared in 2016 and showed the highest values in 2022, with 6 studies out of 36 for that year (Figure 1).

We identified eight different genetic transformation methods, classified into two main groups: biological- and physical-mediated transformations. Physical methods are the most frequently used methods. They include the use of agitation-based glass beads, biolistics (particle bombardment), and electroporation. Electroporation was the most common method (110 studies altogether, Figure 2, SI I Table SI 1). Biological methods include conjugation, natural transformation, and *Agrobacterium*-mediated transformation. The latter was the least frequent method used during the studied period 1987–2024 (Figure 2, SI I Table SI 1). All these methods seemed to develop in parallel over time (Figure 2).

Regarding the origin of the modified gene, we identified that 71.8% of the studies included only heterologous genes (genes of unrelated species), 22.1% of the studies modified only endogenous genes (genes of the same species), and 6% of the studies modified both endogenous and heterologous genes to improve one or more traits of interest ($n = 518$ cases) (Figure 3a). Out of a total of 518 genetic modifications identified, 46.5% of the studies included the modification of a single gene, while 53.5% of the studies improved multiple genes. We observed that both one-gene and multigene studies increased similarly over time (Figure 3b).

Out of the 531 strains that were genetically improved, 54.6% were eukaryotes, belonging to the taxonomic groups of green algae (41.8%), diatoms (7.2%), and other microalgae (5.6%), while the remaining 45.4% were prokaryotes (cyanobacteria) (Figure 4).

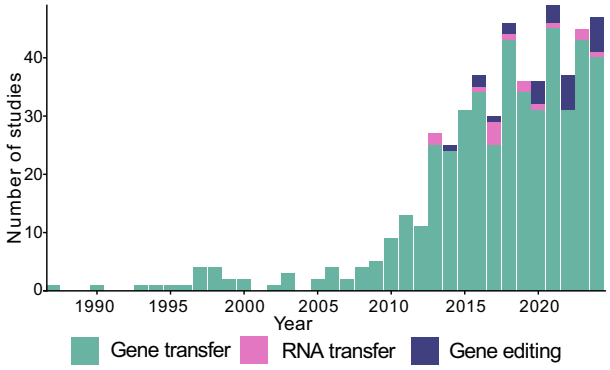

FIGURE 1 | Evolution of genomic techniques used to genetically improve microalgae. Genomic techniques are divided into DNA gene transfer, RNA transfer, and gene editing (CRISPR-mediated transformation and TALEN). No studies using ZFN to genetically improve microalgae were documented. Data plotted are the number of studies per year. $n = 518$ studies.

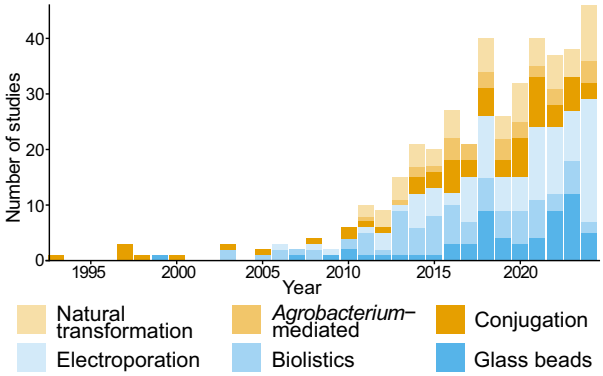

FIGURE 2 | Evolution of genetic delivery transformation methods used on microalgae. Genomic transformation methods are divided into two main groups: Biological (natural transformation, Agrobacterium-mediated transformation, and conjugation), and physical transfer and gene editing (electroporation, biolistics, and glass beads). $n = 417$ cases.

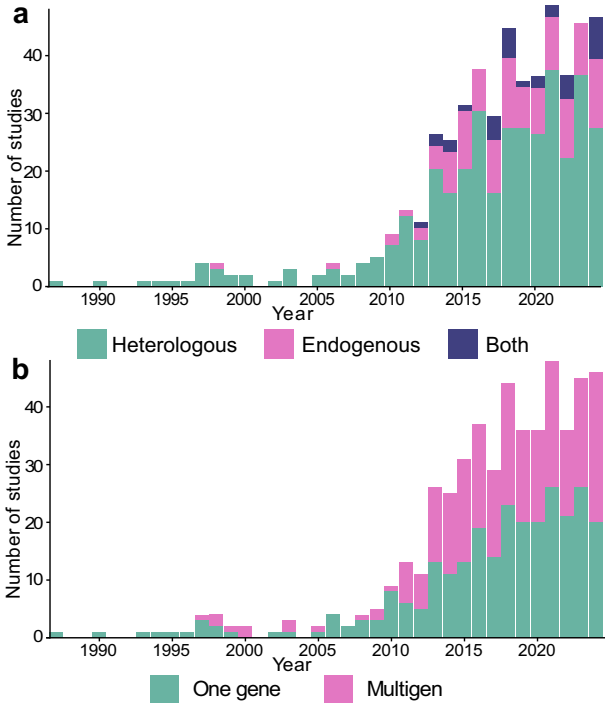

FIGURE 3 | Evolution of genetic transformations based on (a) the origin of the genetic material, (b) the number of genes (one vs. multiple) genetically improved. $n = 518$ cases.

Chlamydomonas (26.6%), *Chlorella* (5.8%), *Phaeodactylum* (5.5%), and *Schizochytrium* (4.1%) were the most frequently transformed eukaryotic genera (Figure 4, Table SI 1, Figure SI 1). Less frequently transformed eukaryotic strains include *Nannochloropsis* (3.4%), *Dunaliella* (2.4%), *Thalassiosira* (1.1%), *Haematococcus, Neochloris, Scenedesmus*, and *Tetraselmis* (all 0.8%) among others. Within the prokaryotes, *Synechocystis* (20.7%), *Synechococcus* (18.5%), and *Anabaena* (4.5%) were the most frequently transformed genera (Figure 4, SI I Table SI 1). Less frequently transformed cyanobacteria include *Arthrospira*

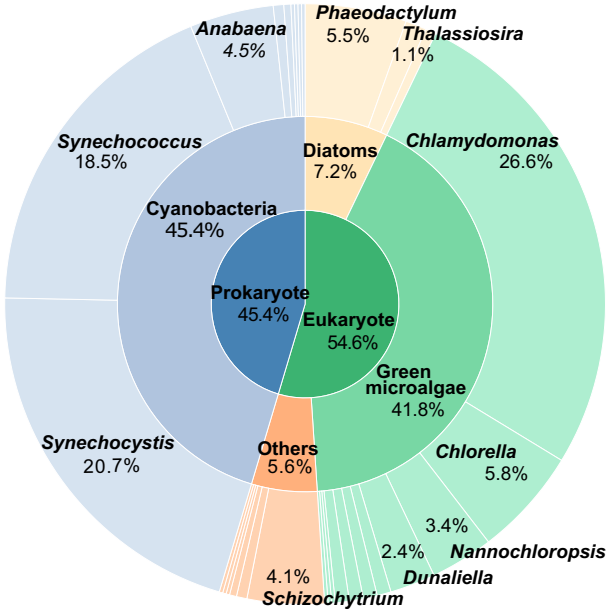

FIGURE 4 | Overview of the relative abundance (%) of genetically improved microalgal strains by cell type (eukaryotic vs. prokaryotic cells, inner ring), taxonomic group (common names, central ring), and genus level (outer ring). Groups with a relative abundance greater than 1% are annotated with their name and percentage. $n = 531$ cases.

(0.6%), Nostoc, Cyanothece, Fremyella, and *Leptolyngbya* (all < 0.4% relative frequency, SI I, Table SI 1). *Chlamydomonas, Synechocystis*, and *Synechococcus* were the genetically improved genera with the highest rates of increase over time (SI I, Figure SI 1).

We identified a total number of 581 cases of traits that were genetically improved in microalgae for potential commercial purposes. The most frequently improved traits were the expression of proteins with 146 cases (25.1%), followed by the enhancement of lipid content, with 111 cases (19.1%). Additional common traits included the production of pigments (10.8%), alcohols (8.8%), terpenoids and waxes (8.4%), esters and acids (6.5%), polymers (4.8%), hydrocarbons (2.6%), hydrogen production and sugars (1.9% in both cases), and the ability to accumulate nutrients, metals and contaminants (1.4%) (Figure 5, SI I Table SI 1). Traits that were more rarely modified (occurrence < 5 cases) include the production of ketones, vitamins, alkaloids, hydrogen peroxide, toxins, glucosides such as dhurrin, gases (oxygen, nitrogen), and other intracellular particles and molecules e.g., electrons, NADPH and nanosized polyphosphate bodies. (SI I, Table SI 1). The production of proteins, pigments, terpenoids and waxes shows a clearly positive trend over time (SI I, Figure SI 1). Lipid enhancement also shows a pronounced increase over time, but this trend seems to drop during the last years (SI I, Figure SI 1). Interestingly, eukaryotic and prokaryotic microalgae showed differentially improved traits. Eukaryotes were predominantly improved to express proteins for pharmaceutical use and animal farming, to produce lipids (for food, animal farming and bioenergy), and pigments (mainly for food and animal farming) (Figure 5a). In contrast, prokaryotic cyanobacteria were mostly genetically improved to produce alcohols and polyols (mainly for bioenergy), to produce terpenoids and waxes with applications in cosmetics, pharmaceuticals, bioenergy, food and chemistry industries, and to produce esters and acids relevant in the biomaterial and pharmaceutical sectors (Figure 5b).

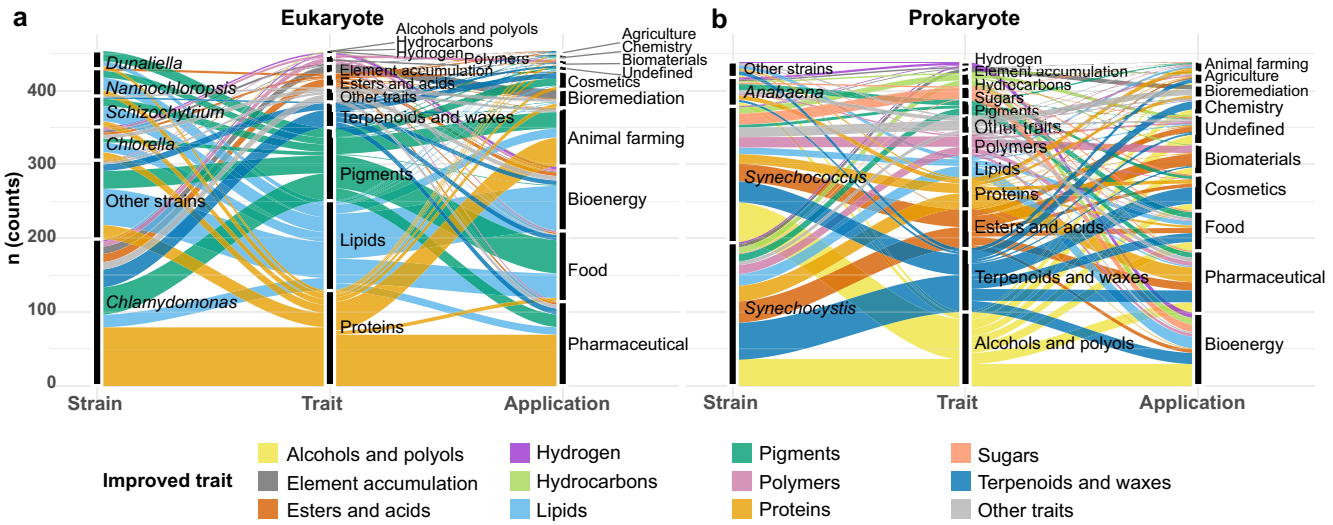

FIGURE 5 | Linkage between strain, enhanced trait and industrial applications in genetically improved microalgae. Panels show data separated by cell type: (a) eukaryotic microalgae and (b) prokaryotic microalgae. Strains were grouped at the genus level. Strains, traits and applications were ranked in order of importance (black columns). Strains and traits with occurrences below 5% of the total number of studies ($n = 519$) were assigned to "Others" categories. Applications that were not clearly targeting one or more industrial sectors in the research study were assigned to "Undefined". The different categories of commercial applications are defined in Supporting Information II.

Overall, the most relevant economic sectors in terms of the highest number of improvements of microalgal traits (total number = 768 cases) were the pharmaceutical sector, with 181 cases (23.6%), followed by the bioenergy sector (156 cases, 20.3%), the food and beverage industries (119 cases, 15.5%), animal farming (82 cases, 10.7%), and cosmetics (64 cases, 8.3%; Figure 5, SI I Table SI 1, Figure SI 1). All these sectors exhibited an upward trend, except for the bioenergy sector, which showed a decline in recent years (SI I, Figure SI 1). When we distinguished between eukaryotic and prokaryotic microalgae, different economic sectors emerged (Figure 5). Eukaryotes are genetically improved for potential applications (in order of importance) in the pharmaceutical sector followed by the food, energy, animal farming, environment (bioremediation), and cosmetic sectors (Figure 5a). Improved prokaryotes were intended for the bioenergy sector, followed by the pharmaceutical, food, cosmetic, biomaterial, and other (undefined) industries (Figure 5b).

Within the animal farming sector, the most common improved traits were proteins, pigments, and lipids, mainly oriented toward the production of feed additives and delivery systems, and to a lesser extent toward biomass production and bioremediation in recirculating aquaculture systems (RAS) (Figure 6). Feed additives were broadly used in both terrestrial and aquatic animal farming without a defined target animal, whereas delivery systems were mainly developed for specific groups such as fish, crustaceans, mollusks, livestock, or poultry (Figure 6).

4 | Discussion

Cultivation of microalgae can contribute to human health, and provide environmental benefits by supporting different ecosystem services [14]. Several policy-driven initiatives and stakeholder platforms worldwide aim to accelerate the development of a sustainable and innovative algae sector, reflecting the growing interest in algae-based solutions [19, 20]. In parallel, genetic modification of microorganisms for industrially relevant applications has rapidly increased over the past decades [21, 22]. In the case of microalgae, we show that efforts to genetically improve strains for commercial use began in the late 1980s, but research has dramatically increased during the last 15 years. We anticipated that the recent developments in genomic techniques would positively impact the volume of research on improved microalgae. However, the application of gene-editing tools accounts for only a small percentage, with nearly all studies relying on CRISPR-mediated systems (and only one using transcription activator-like effector nucleases, TALEN). The preference for CRISPR over other gene editing tools points to its various advantages, namely the simplicity of target design, high editing efficiency, option for multiple gene editing, low cost, and a quick cycle time [23]. In basic research, only a few attempts to use ZFN and TALEN nucleases in microalgae have been reported (see review in [24]). CRISPR/Cas nucleases, however, have been widely used in basic research since 2014, and alternative solutions to mitigate Cas9 toxicity were quickly developed [24, 25]. Other advances to improve the possible off-target effects and the variable efficiency (within and among strains) have been proposed elsewhere [26, 27]. Additionally, toolboxes and protocols tailored to microalgal strains and their industrial applications have been developed [28, 29]. Yet, the adoption of CRISPR tools in microalgae remains limited, possibly due to the absence of libraries for most microalgal strains, unlike those of higher eukaryotes [25]. Recent developments, such as the construction of single-guide RNA (sgRNA) libraries, availability of pan-genomic and epigenomic resources, and mini-genomes,

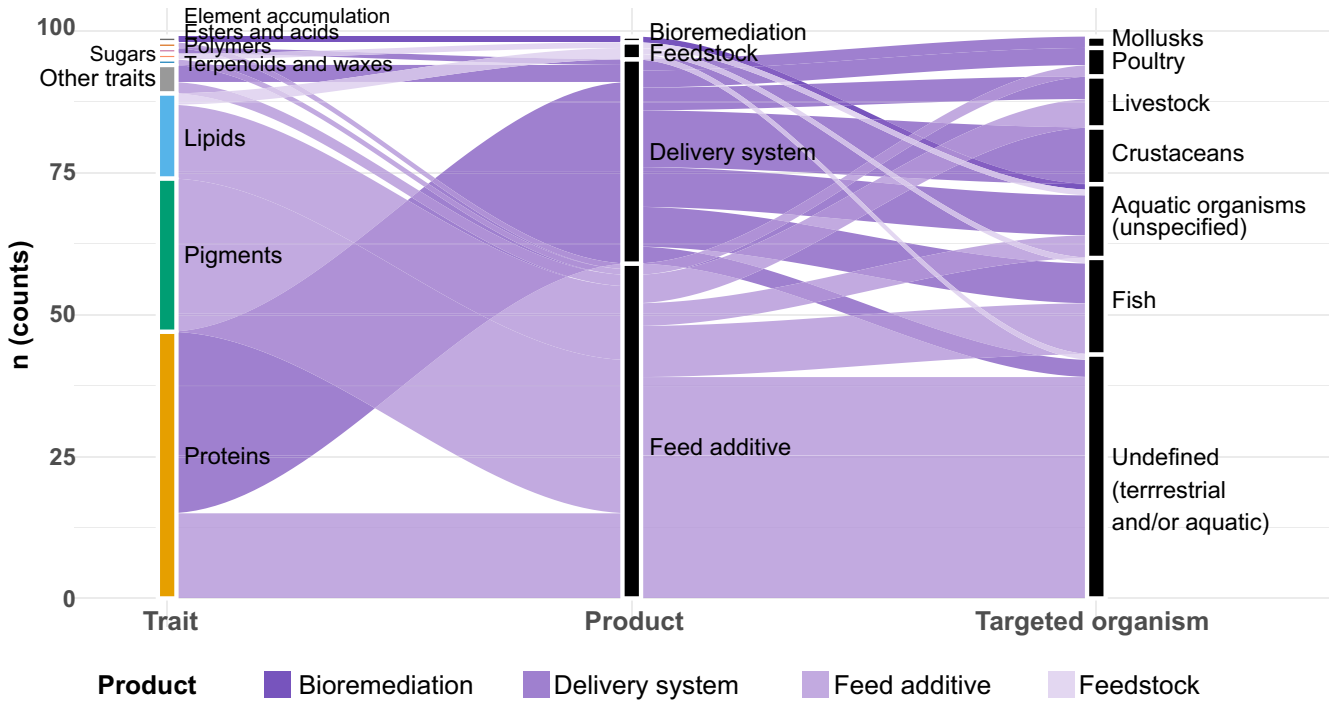

FIGURE 6 | Linkage between genetically improved microalgal traits, derived products, and targeted organisms in the animal farming sector. Traits (left column) follow the same color scheme as in Figure 5. Products are shown in the central column, and targeted organisms in the right column. All categories are ranked in order of importance. When targeted organisms were not specified in the text, they were assigned to the "Undefined" category.

have the potential to further advance microalgal biotechnology [25].

According to our review, physical transformation methods for delivering genetic material into the cell are used more frequently than biological methods. Electroporation and biolistics were the most widely used methods because of their versatility and high transformation efficiency, which is not affected by the presence of rigid cell walls [30]. Both methods, however, require costly equipment. Physical transformation with glass beads, on the contrary, is a cost-effective method per se, but cell wall removal is needed to obtain adequate transformation efficiencies [31]. We observed that for some strains, such as *Chlamydomonas* spp., mutant (cell wall deficient) strains rather than wild types were used when the transformation method was glass beads (data not shown). It appears, however, that in the recent past the use of glass beads has been in decline and electroporation is favored, probably due to the improved efficiencies achieved with the latter, once the required equipment and protocols have been established.

There is evidence for an increasing trend in multiplex gene transfers, particularly after 2010, and a higher number of genetic transformations involving endogenous genes, but heterologous expression in microalgal hosts is the predominant form of gene expression as of today. This might be connected to the still emerging utilization of gene editing tools that we noticed, as CRISPR-mediated systems are excellent tools to simultaneously target multiple genomic sites [32], usually (but not always) endogenous.

Overall, the green eukaryote *Chlamydomonas reinhardtii* and the cyanobacteria *Synechocystis* spp. and *Synechococcus* spp. are the most frequently improved microalgae. All are established model organisms with whole genomes sequenced for decades [33–36], and various genetic and metabolic mechanisms extensively studied [37–39]. *C. reinhardtii* emerges as a solid photosynthetic chassis, with standardized modular toolkits [40] and high-throughput approaches for chloroplast synthetic biology [41]. Genetic modification of the chloroplast genome (plastome) offers advantages over nuclear transformations, and *C. reinhardtii*, with only one chloroplast that encodes part of the light-harvesting and carbon fixation machineries, can be genetically improved to fix carbon and nitrogen, and to produce relevant metabolites [42]. We identified at least 25 studies on chloroplast genetic transformation (data not shown), mostly in *C. reinhardtii*, to express recombinant proteins of therapeutic use [43–45].

Other commercially relevant strains that have been genetically improved include the genera *Chlorella, Nannochloropsis, Dunaliella, Phaeodactylum, Anabaena* (syn. *Nostoc)* and *Schizochytrium*, with varying levels of difficulty to be genetically manipulated. For some genera, such as *Nannochloropsis* and *Phaeodactylum*, molecular toolboxes have been developed [46, 47]. Other genera, however, such as *Chlorella*, are more challenging to transform [48], likely due to the presence of rigid cell walls. Most of these strains, particularly *Chlorella*, are widely cultivated, commercialized, and profitable without genetic transformation [9, 49], suggesting that the demand for genetic tools may not be pressing. The microalgal sector currently benefits from genomic tools to produce various biocompounds and molecules (proteins, lipids, pigments, alcohols and polyols, terpenoids, waxes, esters, acids, polymers) and to accumulate nutrients, metals and potentially toxic compounds, among other traits. The potential applications of these traits extend to multiple industrial sectors, including the biopharmaceutical sector, bioenergy, food and beverage industries, animal farming, and to a lesser extent, cosmetics, packaging, bioremediation, chemical industries and agriculture. The application of genomic tools is expanding to some currently narrow niches within the microalgal sector, i.e., the biopharmaceutical and the bioenergy industries. Currently, non-transformed microalgae are mainly commercialized as food (including supplements), feed, cosmetics, and biostimulants, with only a few commercial producers targeting pharmaceuticals and biofuels yet [9, 49].

We envision the microalgae-based market being more oriented toward the production of recombinant proteins of pharmaceutical and biomedical relevance in the near- and long-term future. Nearly 25% of the reviewed studies targeted the expression of peptides and proteins with antimicrobial activity for disease eradication or control, either in humans or animals (aquatic and terrestrial animal farming) [50–53]. Antigens, oral vaccines and carriers, immunotoxins, antibodies and to a lesser extent, enzymes, accounted for most of the expressed proteins, with a clear application in human health as in [54–57], but also in animal farming against infections [58–61]. The second most frequently improved trait was the production of lipids (19%), with applications in the bioenergy, food and feed industries. The potential use of microalgae as an alternative to fossil fuels is reflected in the literature since the development of genetic engineering tools [62, 63]. However, a lack of economic feasibility and other limitations have hindered the scale-up of microalgal production for biofuel, though research into the topic remains active. We observed that relevant traits for biofuel have diverged from the original trait of interest, i.e., total lipid production, to the production of specific lipidic compounds to reduce costs in the downstream processing (e.g., free fatty acids), other relevant compounds (alcohols, hydrogen, hydrocarbons, etc.), and even feedstock for other microorganisms in fermentation processes (polyols, sugars). Recent studies propose synthetic microbial consortia for sustainable bioenergy production, with microalgae [64, 65]. Another lipid-related trait, the production of polyunsaturated fatty acids (PUFA), seems to be consolidated in the food and animal farming sectors, because of their health benefits and disease prevention [66, 67]. The production of pigments, terpenoids and waxes shows a clearly positive trend, and encompasses a high variety of metabolites, such as astaxanthin [68], canthaxanthin [69], zeaxanthin [70], limonene [71], squalene [72], bisabolene [73], etc., with various applications (colorants in the food and feed industries, fragrances in cleaning products and cosmetics, etc).

Taking a closer look at the animal farming sector, the most relevant products derived from genetically improved traits are feed additives, including colorants, tissue-growth enhancing hormones [74], and health-promoting agents such as enzymes that improve digestibility in monogastric animals [75] (farmed fish, poultry, swine). Feed additives are broadly used across farming without targeting specific animals, as reflected by the fact that nearly half of the studies we identified did not specify a target species. A second major category of products comprises

the use of microalgae as baits or delivery systems for recombinant peptides against diseases. Unlike additives, they tend to be more species-specific, as they target pathogens with narrow host ranges such as Gram-negative bacteria (e.g., *Vibrio* spp. causes lethal infections in fish and shellfish, *Aeromonas* spp. primarily affect fish) and major viral pathogens (White Spot Syndrome Virus in shrimp, Viral Hemorrhagic Septicemia Virus in fish, or avian influenza in poultry). Examples of microalgal delivery systems include oral vaccines developed for shrimp [60], crayfish [76] and fish [77] to mitigate viral infections, as well as the expression of antimicrobial peptides that prevent bacterial infections in fish [50], livestock [51] and poultry [78]. This is particularly relevant amid rising antibiotic resistance, as GM microalgae offer an alternative to conventional antibiotics that currently threaten public health and food safety [51].

The different nature of the biocompounds (and their applications) in eukaryotic versus prokaryotic cells may be explained by the larger body of research on strains of the genera *Chlamydomonas* (eukaryote), *Synechococcus* and *Synechocystis* (both prokaryotes). *Chlamydomonas reinhardtii* served as a host for nearly half of the studies on proteins expressed in microalgae, for human and animal health, and as a chassis for relevant pigments in the food and feed industries. However, *C. reinhardtii* is not generally used to increase lipid yields, since other eukaryotic genera are known to be highly oleaginous, such as *Nannochloropsis, Phaeodactylum or Schizochytrium*. On the other hand, the cyanobacteria *Synechocystis* and *Synechococcus* seem to be excellent hosts for producing alcohols and polyols for various applications, mainly bioenergy, but also pharmaceuticals, food, cosmetics and biomaterials. They are also suitable for producing terpenoids for cosmetic use, and to a lesser extent, in pharma, food, energy and chemistry industries.

To our knowledge, this is the first comprehensive review on genetically improved microalgae from an applied perspective that covers potential applications in all major industrial domains. Recently, Miklau et al. conducted horizon scanning to identify genetically modified organisms (terrestrial animals, fish, algae, and microorganisms), but their search was limited to microalgae potentially released into the environment and to publications between 2012 and 2022. Despite the low number of studies retrieved (36 on eukaryotic microalgae and 5 on cyanobacteria), they also pointed to the bioenergy sector (biofuels) as an important application (44%), followed by biocontrol (16%) and bioremediation (12%) [79]. Other horizon-scanning works on microorganisms and their products obtained with NGTs reported one genetically improved microalgal strain each, namely *Nannochloropsis gaditana* for food and feed [80], and the cyanobacterium *Synechococcus elongatus* [81]. However, the latter was genetically improved as a biocontainment system to prevent possible environmental risks in case of release into the environment.

As our literature review exclusively focuses on traits genetically improved with potential practical application in the various industries, we excluded articles on basic research and physiology (including transcriptomics), such as those aiming to understand the functional role of one or more genes, but lacking a clear commercial viewpoint (> 588 papers) and methodological studies (design of vectors, markers, reporters, toolkits, etc., > 411 papers). We also excluded studies on traits with no direct commercial value but rather focused on the optimization of the production system (> 260 papers). For instance, the use of phosphite instead of phosphate [82], the ability to use lignocellulose as a carbon source [83], higher tolerance to photoinhibition [84], the reduction of the antenna to increase photosynthetic efficiency [85], improved tolerance to environmental stress (salt, temperature, etc.) [86, 87], or enhanced tolerance to toxic compounds produced by the host microalga, such as butanol [88]. Research on optimization and upscaling of already developed strains (e.g., [89]), was also discarded (> 180 studies), as we focus exclusively on studies showing the improvement of the trait of commercial relevance, excluding process development steps (optimization, scale-up, etc.). Strains obtained with random mutagenesis methods, such as UV-mutagenesis or chemical-mediated mutagenesis [90] were also excluded as this method does not involve targeted genetic technology (> 50 studies). We tried to sharpen our inclusion criteria, particularly those concerning the commercial application of the improved strain (and trait). However, this process was not always straightforward, and some cases required additional discussion and judgment to determine whether the study should be included or excluded. We also acknowledge that our review is not fully comprehensive, as the search phase focused exclusively on original scientific articles in English retrieved from two databases. Grey literature and other publication types, e.g., book chapters, conference abstracts and review papers were excluded, as well as scientific articles in other languages, such as Spanish or Chinese. We also excluded patents to avoid possible overlap with existing scientific publications. We acknowledge that our findings may have differed under alternative inclusion and exclusion criteria; however, the criteria applied were intentionally aligned with our research questions.

The use of an AI-based tool to screen a relatively large dataset (over 15,000 items) was originally conceived to optimize time resources. Since the software specification leaves the stopping criterion to the reviewer's choice [15], we decided on a very conservative approach and selected a double stopping criterion, i.e., screening at least one third of the total dataset, with no less than 300 irrelevant papers consecutively, to ensure that almost all possibly relevant papers were identified. Recommendations for datasets with more than 5000 references estimate screening 34% of the available references [91], although most authors using similar tools usually target 15% of the dataset, or a maximum of 50 irrelevant papers in a consecutive sequence [92]. As an additional quality control measure to validate the robustness of the AI-generated results, we manually checked 5% of the unscreened papers (526 references out of 10,551), identifying only one relevant study. As a consequence, the impact of missing papers was minimal, and we can expect that it did not alter the overarching findings of our review.

During the screening, we discarded nearly 5000 papers that were out of scope of our review. The main reasons we marked them as "irrelevant" were the absence of genetic modifications (> 1650 papers, data not shown), and hosts other than microalgae such as bacteria, yeast, fungi, macroalgae, or animal cells (> 850 papers, data not shown), that might express microalgal genes instead.

In summary, we identified major trends in the use of genomic tools, improved traits, recipient strains and commercial

applications, with potential implications for future microalgal production. The use of gene editing tools is still incipient in applied research with microalgae, but the extension of genomic tools to a higher number of strains other than the ones currently exploited is foreseeable. We expect this trend to change once standardized toolkits, reliable delivery methods and better off-target control are established for more microalgal species. Most of the commercially relevant traits we summarized in this study may benefit from gene editing tools, alongside production-system traits such as resource utilization (nutrients, light) or stress tolerance. Integration with other synthetic biology tools, such as metabolic engineering platforms or inducible systems, will further expand their applicability. Multi-gene genetic transformation is now a reality for increasing the production of industrially relevant compounds in microalgae and expanding the use of genera such as *Anabaena, Nannochloropsis, Phaeodactylum, Neochloris, Thalassiosira* or *Haematococcus* beyond traditional model organisms and commercially relevant wild-type strains. Nevertheless, model organisms, such as the prokaryotic *Synechococcus* spp. and *Synechocystis* spp. hold promising applications across most identified commercial sectors, whereas eukaryotic *Chlamydomonas reinhardtii* may revolutionize the pharmaceutical sector through the production of recombinant proteins. New approaches such as chloroplast synthetic biology offer promising opportunities for the expression of these recombinant proteins with applications in human and animal health. Aside from the pharmaceutical sector, we envision that the food and feed industries may also greatly benefit from current genomic advances with microalgae, mainly through the production of pigments, lipids, and proteins. The bioenergy sector is also advancing in the development of biocompounds that reduce downstream efforts and target microalgae as a substrate for sustainable bioproduction. The animal farming sector (particularly aquatic organisms) may benefit substantially from these advances, especially for disease control without relying on conventional antibiotics that increase selection pressure and drive the persistence of resistant bacteria. Other economic sectors, such as cosmetics, packaging, bioremediation, chemical industries and agriculture could also benefit from genetically improved microalgae and their products. We anticipate this trend will intensify in the future, supported by advances in biotechnology and innovation-friendly regulation.

Author Contributions

Irene Gallego: conceptualization, methodology, data curation, formal analysis, visualization, writing – original draft, investigation, writing – review and editing. **Michael Meissle:** conceptualization, methodology, writing – review and editing. **Jörg Romeis:** conceptualization, writing – review and editing, funding acquisition.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data supporting the findings of this study are available in supplementary information. All supporting information associated with this publication is provided online.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Supplementary Information I:** This supplementary material includes all tables, figures and methodological details supporting this study. **Table SI 1.** List of search strings (in two different terms linked by the connector "AND") that were used in this review during the searching phase. **Table SI 2.** PICO elements of the study: Population, Intervention (method), Comparison, and Outcome (trait and commercial application), with inclusion and exclusion criteria. Examples are shown in brackets. **Table SI 3.** Frequency table of genetic transformation methods, categorized into biological and physical methods. **Table SI 4.** Frequency table of strains genetically improved, categorized into cell type, taxonomic group and genus level. **Table SI 5.** Frequency table of traits genetically improved, categorized into the main biocompound groups. **Table SI 6.** Frequency table of commercial applications, categorized into pre-defined industrial sectors. **Figure SI 1.** PRISMA Flow Diagram of the review process. **Figure SI 2.** Improved strains over time. **Figure SI 3.** Improved traits over time. **Figure SI 4.** Trends over time by economic sector. **Supplementary Information II.** Definitions of genetic transformation methods, genetic tools, and commercial applications included in this study. **Supplementary Information III.** Dataset and variables included in this study.