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Review

Biotechnological Potential of Korean Marine Microalgal Strains and Its Future Prospectives

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Abstract : Marine microalgae have long been used as food additives and feeds for juvenile fish and invertebrates as their nutritional content is beneficial for humans and marine aquaculture species. Recently, they have also been recognized as a promising source for cosmeceutical, nutraceutical, and pharmaceutical products as well as biofuels. Marine microalgae of various species are rich in multiple anti-oxidant phytochemicals and their bioactive components have been employed in cosmetics and dietary supplements. Oil contents in certain groups of marine microalgae are extraordinarily rich and abundant and therefore have been commercialized as omega-3 and omega-6 fatty acid supplements and mass production of microalgae-based biodiesels has been demonstrated by diverse research groups. Numerous natural products from marine microalgae with significant biological activities are reported yearly and this is attributed to their unique adaptive abilities to the great diversity of marine habitats and harsh conditions of marine environments. Previously unknown toxin compounds from red tide-forming dinoflagellates have also been identified which opens up potential applications in the blue biotechnology sector. This review paper provides a brief overview of the biotechnological potentials of Korean marine microalgae. We hope that this review will provide guidance for future marine biotechnology R&D strategies and the various marine microalgae-based industries in Korea.

Key words : bioactive compounds, biotechnology, industrial applications, Korean marine microalgae, Korean waters

1. Introduction

Since the enforcement of the Nagoya Protocol in 2014, each nation has been competing for obtaining beneficial bioresources and building diverse biobanks and Korea is no exception to these developments. Consequently, the Korean government has consistently ramped up its assertion of sovereign rights over bioresources within Korean territory. Many government-funded research efforts have been made focusing on establishing and maintaining bioresources from various environments across the Korean Peninsula. However, mass-culturable or mass-harvestable organisms have recently drawn much more attentions than others due to their potentials for a wide range of profitable bioindustry. In recognition of the current trends, the Ministry of Oceans and Fisheries of Korea (2019) announced the 1st Administrative Master Plan for Marine Bioresources for the next 5 years in accordance with Article 8 (Formulation, Implementation, etc. of Master Plans) of Act on Securing, Management, Use, etc. of Marine and Fisheries Bioresources. Its main objective is to promote the development of exploitation and management strategies for sustainable utilization of maritime bioresources and economic prosperity of the

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nation. In particular, microalgae were appointed as one of the priority target marine bioresources in the Ministry of Oceans and Fisheries of Korea (2019) Master Plan.

Microalgae are important primary producers in the marine ecosystem and they are well known as the key players in the global cycling of carbon, nitrogen, phosphorus, and other trace elements (Moore et al. 2001; Arrigo 2005; Hutchins et al. 2009; Cloern et al. 2014). As they have evolved unique defense mechanisms to cope with highly competitive ocean environments, they can biosynthesize a vast range of commercially interesting compounds via various pathways (Cardozo et al. 2007). Thus, marine microalgae have been widely recognized as promising sources for a variety of biochemical compounds (Venkatesan et al. 2015). Even though marine microalgae have shown great potential in various fields, the microalgae-based industry in Korea is limited to health goods. The most popular and commercially successful microalgae products in Korea are *Chlorella* dietary supplements (Kim 2012b). There also has been an attempt to mass-cultivate *Chlorella* as aquaculture feed (Ministry of Future Creation and Science 2015). In addition, several marine diatom species have been extensively used as a feed source for invertebrate larvae, especially bivalves and abalone. It should be noted that the marine microalgae biochemical industry in Korea is still in its infancy.

There were two previous reports by Intellectual Property On (2015) and the Korea Institute of Ocean Science and Technology (2018) analyzing the feasibility of marine microalgae commercial opportunities. These trends reports provided lists of microalgal species that contain bioactive compounds with application potential in animal feeds, biofuels, bioplastics, biological control, cosmeceuticals, nutraceuticals, pharmaceuticals, and wastewater treatment. Yet, their geographic origins as well as practical applications for commercialization are needed to be confirmed by further literature searches to ensure that the species that have been recorded in Korean waters are only listed and presented. By this means, an applicable list of the prioritized Korean microalgal species is proposed in this review and this aspect should be carefully considered in the future research and development (R&D) plans as precautions against the current and yet-to-happen Access and Benefit Sharing (ABS) issues at international and national levels. In addition, we attempt to provide a comprehensive overview of the current research status and patents in each industrial field. We hope that this review will provide a reference for both researchers and policymakers when making decisions in the future marine microalgae-related R&D programs.

2. Litarature and Patent Searches

A list of Korean marine microalgae species was created based on the above reports. Patents were searched in the KIPRIS (Korea Intellectual Property Rights Information Service) database (http://www.kipris.or.kr/khome/main.jsp) by using “marine microalgae/phytoplankton” and “biotechnology” as search terms. Microalgae without proper identification to the species level in the reports were excluded from the list. In addition, by searching the Korean marine microalgae (Hur et al. 2015) and phytoplankton (the Library of Marine Samples, LIMS, Korea Institute of Ocean Science and Technology (KIOST), http://lims.kiost.ac/portal/ms/hsPlLstPG.do) databases and conducting literature searches in the PubMed (https://www.ncbi.nlm.nih.gov/pubmed/) and National Digital Science Library (NDSL, http://www.ndsl.kr/index.do) using each species name in the list as a keyword, microalgae that had not been recorded or discovered in Korea were also delisted. Currently accepted species names were then checked in both AlgaeBase (Guiry and Guiry 2019) and WoRMS (2019). Further literature and patent searches in the Research Information Sharing Service (RISS, http://www.riss.kr/index.do) and Korea Science (http://www.koreascience.or.kr/MainPage.jsp) databases were performed using the same methods as above to confirm their geographic origins and applications. In total, we retrieved 34 microalgae species with potential applications in eight main fields (Table 1).

3. Biotechnological Potential of Korean Marine Microalgal Strains

Based on our literature and patent searches on the Korean marine microalgae, the most frequently applied fields are animal feed, cosmeceutical, nutraceutical, and pharmaceutical as summarized in Table 1. These results reflect the current mainstream trends of the microalgal biotechnologies in Korea. Considering the present and future potentials of the Korean microalgae markets, marine microalgae may serve as a basis for a leading biotechnological industry in Korea in the near future.

Animal feed

Due to rapid population growth and food insecurity, global aquaculture has become a larger source for food than wild capture since 2014 (Food and Agriculture

Table 1. Korean marine microalgae with biotechnological potential

Gr Tax	Species	Animal feed	Biofuel	Biochemical compound	Biological control	Cosmeceutical	Nutraceutical	Pharmaceutical	Wastewater treatment
CHL	*Auxenochlorella protothecoides*	O	O				O		O
	Chlamydomonas hedleyi					O		O	
	Chlorella vulgaris	O	O				O		O
	Chloroidium ellipsoideum							O	
	Dunaliella salina		O			O	O		
	Dunaliella tertiolecta	O	O				O		
	Heterochlorella luteoviridis	O	O				O		
	Tetraselmis suecica	O	O				O	O	
CRY	*Teleaulax amphioxeia*	O							
CYA	*Arthrospira maxima*						O		
	Arthrospira platensis					O	O		
DIA	*Achnanthes brevipes*	O		O					
	Achnanthes sancti-pauli	O		O					
	Chaetoceros gracilis	O	O			O	O		O
	Chaetoceros didymus	O							
	Cylindrotheca closterium	O					O		
	Ditylum brightwellii			O					
	Melosira octogona	O		O					
	Navicula salinicola	O					O		
	Phaeodactylum tricornutum	O	O				O		O
	Skeletonema costatum						O		
DIN	*Alexandrium andersonii*				O				
	Amphidinium carterae							O	
	Dinophysis acuminata			O				O	
	Gymnodinium impudicum							O	
	Ostreopsis cf. *ovata*							O	
	Paragymnodinium shiwhaense						O		
	Prorocentrum dentatum	O							
	Prorocentrum lima							O	
	Symbiodinium voratum							O	
EUS	*Nannochloropsis oceanica*	O	O	O		O		O	
	Nannochloropsis oculata		O						O
HAP	*Diacronema lutheri*	O							
	Isochrysis galbana	O	O						

CHL, chlorophyte; CRY, cryptophyte; CYA, cyanophyte; DIA, diatom; DIN, dinoflagellate; EUS, eustigmatophyte; HAP, haptophyte; GrTax, the taxonomic group

Organization of the United Nations 2016) and the aquaculture is expected to provide nearly two thirds of global food fish consumption by 2030 (Food and Agriculture Organization of the United Nations 2018). In Korea, the volume of aquaculture production has already exceeded the capture production since 2006 and it is also expected to grow at the pace of market itself (Hur 2015). Modern intensive aquaculture has been extensively practiced in Korea since the 1960s and thereby the aquafeed industries have already established a sustainable market size. Hence, it would not be very challenging to be profitable in this domain. Consequently, many marine microalgae species have long been used as aquaculture feed since they can serve as excellent sources of proteins, lipids, and pigments, vitamins, and trace minerals. *Chaetoceros* (appendix 4-3. *Chaetoceros gracilis*, appendix 4-4. *Chaetoceros didymus*), *Chlorella* (appendix 1-3. *Chlorella vulgaris*), *Diacronema* (appendix 7-1. *Diacronema* *lutheri*), *Isochrysis* (appendix 7-2. *Isochrysis galbana*), *Nannochloropsis* (appendix 6-1. *Nannochloropsis oceanica*, appendix 6-2. *Nannochloropsis oculata*), *Phaeodactylum* (appendix 4-9. *Phaeodactylum tricornutum*), *Skeletonema* (appendix 4-11. *Skeletonema costatum*), *Tetraselmis* (appendix 1-8. *Tetraselmis suecica*), and *Thalassiosira* are the most commonly used genera in aquaculture (Knothe 2009). Most of the Korean isolates categorized as animal feed in Table 1 belong to these genera.

Rotifers such as *Brachionus plicatilis* are routinely cultivated as a live feed for finfish larvae in aquaculture. *Chlorella* (appendix 1-3. *Chlorella vulgaris*), *Isochrysis* (appendix 7-2. *Isochrysis galbana*), *Nannochloropsis* (appendix 6-1. *Nannochloropsis oceanica*, appendix 6-2. *Nannochloropsis oculata*), and *Tetraselmis* (appendix 1-8. *Tetraselmis suecica*) are the most widely used microalgae for marine rotifer production in Korea (Lee 2001). Since marine *Chlorella* spp. are known to be rich in PUFAs, rotifers can obtain the essential fatty acids from live microalgae on which they feed on. *Tetraselmis* spp. have a relatively larger cell size and they can be easily grown in a large scale due to their eurythermal and euryhaline characteristics. Hence, both genera can be economically cultivated in mass outdoor cultures by supplying commercial fertilizer containing basic nutrients. *I. galbana* (appendix 7-2. *Isochrysis galbana*) is one of the most extensively cultivated live feed species on account of its high contents of protein and fatty acids. However, this species requires some specific culture conditions such as nutrients, temperature, and salinity. Therefore, indoor mass cultivation is mainly practiced which often leads to cost increase in production. There are two types of invertebrates that feed on microalgae in aquaculture (Lee 2001). Invertebrate macroalgivores such as abalone, sea cucumber, and sea urchin consume benthic microalgae during the early stages then shift to seaweeds at juvenile stage. *Caloneis*, *Cocconeis*, *Navicula* (appendix 4-8. *Navicula salinicola*), *Nitzschia*, and *Rhaphoneis* are the most commonly used genera for abalone. *C. calcitrans*, *Navicula* (appendix 4-8. *Navicula salinicola*), *Nitzschia*, and *I.* aff. *galbana* (appendix 7-2. *Isochrysis galbana*) have been used for sea cucumber. Sea squirt and shellfish are microalgivores whose diet consists primarily of planktonic microalgae. These microalgivores filter-feed on microalgae for their entire life span. *C. simplex*, *T. pseudonana*, *I. galbana* (appendix 7-2. *Isochrysis galbana*), and *D. lutheri* (appendix 7-1. *Diacronema lutheri*) are the most widely used shellfish live feed. In addition, *C. calcitrans* and *S. costatum* (appendix 4-11. *Skeletonema costatum*) are also fed to shrimp in the zoea stage and *N. hustedtiana* and *Synechococcus* spp. are used for *Portunus trituberculatus*, the Japanese blue crab, respectively. Powdered microalgae such as dried *Chlorella* are also used in aquaculture, but they are not preferred to be used in aquaculture because the input of powders can deteriorate water quality.

It is obvious that culturing locally isolated indigenous microalgae species is probably the best option to produce live feed for aquaculture. In this sense, discovering microalgae with desired properties from the seashore areas in Korea is the key strategy for achieving sustainable production of microalgae aquafeed. Therefore, the promotion and development of national service collections are necessary to provide high quality marine microalgal cultures and technical advice to industries as well as academic and research sectors.

Biofuel

With the global energy crisis and the rise in serious environmental issues due to fossil fuel consumption, microalgae have been considered as one of the most promising sources for liquid transportation biofuels. Given their higher photosynthetic efficiency and productivity, microalgae hold more promise than energy crop plants. Many marine microalgae species have ideal fatty acid profiles for biofuels, given that their most common components are palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) acids (Knothe 2009). Many Korean marine microalgae have been demonstrated to be promising biofuel feedstocks (Table 1).

However, the high cultivation and harvesting costs are among the main obstacles to be overcome. The scaling up of the production rates has always been the major problem with microalgae-based biofuel production. It was attempted to cultivate a locally isolated *Tetraselmis* sp. (appendix 1-8. *Tetraselmis suecica*) for cost-effective biodiesel production by using the semi-permeable membrane photobioreactors (PBRs) in the coastal seawater of Youngheung Island in the Yellow Sea, but the productivity was too low to be economically feasible (Kim et al. 2015c, 2016b). However, blend of the *Tetraselmis* sp.-derived biodiesel and petrodiesel (BD3, 3% microalgae biodiesel and 97% petrodiesel) was within the recommended petrodiesel standard specifications and it also showed better engine performance than palm oil-derived biodiesel under cold conditions at -20ºC (Jeon et al. 2017). Until now, there is no industry or a business that commercially produces biodiesel from marine microalgae and makes a profit in

Korea and it is still at the R&D stage like elsewhere in the world. However, the current mandatory biodiesel blending rate in Korea is 3% and the government also considers to increase the rates in order to reduce air pollution since fine particulate matters have become a national concern in recent years. As the majority of biodiesel used in Korea is extracted from imported soy and palm oil and domestic recycled cooking oil, microalgae could be an alternative source of biodiesel. This might be a realistic means for future direction of the industry given the current state that the production of microalgae biodiesel alone is considered neither commercially competitive nor biologically sustainable under the current technologies. Also, the concept of biorefinery should be considered since an integrated system needs to be developed to produce not only biodiesel but also other by-products such as fishmeal and fertilizer by directly using waste water and exhaust gas.

Biochemical compounds

Marine microalgae are under investigation as new raw materials for nanotechnology. Diatoms deposit silicon in their frustules, which can be used in a variety of domains such as biosensors, nanomedicine vehicles, photonic devices, and microfluidics (Mishra et al. 2017). Silica extraction has been attempted from diatoms such as *A. brevipes* (appendix 4-1. *Achnanthes brevipes*), *A. sancti-pauli* (appendix 4-2. *Achnanthes sancti-pauli*), and *M. octogona* (appendix 4-7. *Melosira octogona*) isolated from Jeju Island (Go 2017b). It has been demonstrated that a Korean *N. oceanica* strain *Nannochloropsis* (appendix 6-1. *Nannochloropsis oceanica*) produces nanofibrillar cellulose (Lee et al. 2018). In recognition of the current plastic waste issues in the oceans, *P. tricornutum* (appendix 4-9. *Phaeodactylum tricornutum*) has received great attention since its poly-3-hydroxybutyrate biosynthesis pathway was recently described by Hempel et al. (2011). Poly-3-hydroxybutyrate is a polyester with thermoplastic properties and it can be used in biodegradable plastics. A number of Korean *P. tricornutum* strains have been reported, but no studies on this characteristic in Korean strains have been conducted. Above-mentioned sectors are expected to grow exponentially as regulations change and demands rise.

Biological control

Recently, some phototrophic dinoflagellate species have been revealed to be mixotrophic or heterotrophic being able to feed on diverse prey microorganisms such as bacteria, diatoms, other dinoflagellates (Jeong et al. 2005, 2010; Seong et al. 2006). *A. andersonii* (appendix 5-1. *Alexandrium andersonii*) is able to eliminate *Miamiensis* spp. owing to its mixotrophic nature and thus, is considered as an environmentally friendly control method for scuticociliatosis (Lee et al. 2016b; Jeong et al. 2017; Kim et al. 2017c). It was also demonstrated that *P. shiwhaense* (appendix 5-6. *Paragymnodinium shiwhaense*) preyed on harmful algal bloom (HAB) dinoflagellates, *A. carterae* (appendix 5-2. *Amphidinium carterae*) and *H. akashiwo* (Yoo et al. 2010). Over the past 30 years, the Korean aquaculture industry has experienced economic loss of over 121 million USD due to HABs (Park et al. 2013). To deal with HABs, Korean research groups have attempted to develop a biological means by introducing mass cultured mixotrophic dinoflagellates into red tide patches (Jeong et al. 2008). This method worked well within land-based aquaculture farms, but large scale field application was not as efficient as the mesocosm studies due to its technological and commercial feasibility. Further investigation on biological control is needed to improve the efficiency of red tide control. Regular monitoring of HABs in the Korean waters should be continued and more effective mixotrophic grazers are needed to be identified in the future work.

Cosmeceuticals

Microalgae have developed protective mechanisms to cope with excess light and various reactive oxygen species, and these properties have been widely employed in the cosmetics industry (Ryu et al. 2015). In general, microalgae extracts or active compounds such as anti-oxidants, pigments, and essential fatty acids are incorporated into a variety of cosmetic products (Spolaore et al. 2006). Even though the exact formulation of each product is yet not specified in the current market, microsporines and microsporine-like amino acids (MAAs), tocopherols, phenolic compounds, and terpenoids are the major bioactive compounds that act as anti-oxidants, emollients, and diuretics (Griffiths et al. 2016). *Arthrospira* (appendix 3-1. *Arthrospira maxima*, appendix 3-2. *Arthrospira platensis*), *Auxenochlorella* (appendix 1-1. *Auxenochlorella protothecoides*), *Chlorella* (appendix 1-3. *Chlorella vulgaris*), *Dunaliella* (appendix 1-5. *Dunaliella salina*, 1-6. *Dunaliella tertiolecta*), and *Nannochloropsis* (appendix 6-1. *Nannochloropsis oceanica*, appendix 6-2. *Nannochloropsis oculata*) are widely used in various beauty products, including topical photoprotection, moisturizing care, anti-aging, whitening, and hair loss prevention products (Ryu et al. 2015; Ariede et al. 2017; Guillerme et al. 2017).

Most of the Korean strains categorized as cosmeceutical in Table 1 belong to these genera. *C. hedleyi* (appendix 1-2. *Chlamydomonas hedleyi*)-derived MAAs have been extensively studied for their unique skin-protecting characteristics (Cho et al. 2014; Jung et al. 2014; Moh et al. 2014; Suh et al. 2014). Small business companies in Korea have launched a couple of marine microalgae-based cosmetic products, but the majority of the microalgae-based cosmetics is dependent on the extracts from freshwater species, *Haematococcus* and *Spirulina*. Currently, the Korean cosmetics industry is worth more than 12.5 billion UDS according to the Euromonitor International and it is ever increasing. Because this industry always seeks new, innovative ingredients and consumer demands for natural products increase, the marine microalgae-based cosmetic industry is expected to grow steadily.

Nutraceuticals

A certain group of microalgae species, such as *Chlamydomonas* (appendix 1-2. *Chlamydomonas hedleyi*), *Chlorella* (appendix 1-3. *Chlorella vulgaris*), and *Dunaliella* (appendix 1-5. *Dunaliella salina*, 1-6. *Dunaliella tertiolecta*) have long been used as nutraceuticals as they can synthesize diverse bioactive compounds, such as carotenoids, essential amino acids, omega-3 and omega-6 fatty acids, and trace minerals (Paniagua-Michel 2015; Jha et al. 2017). All the Korean species in this category discussed in this review (Table 1) have certain properties that benefit health and therefore, they have potential to be used as nutraceutical resources.

The Korean microalgal market has long been dominated by *Chlorella* since 1990 and by *Spirulina* in recent years. In addition to these widely marketed tablet products, there are also food items available such as bread, noodles, and pasta that include these microalgal material. However, commercial *Chlorella* production in Korea is entirely based on freshwater strains grown heterotrophically in fed-batch fermenters. Powdered *Spirulina* cultivated in open pond raceway is mostly imported from abroad, but it is also locally grown in a tubular PBR. There are a number of marine microalgae-based docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) supplements on the market in Korea as they can attract vegetarians and vegans. Since the presence of dioxins, mercury, and polychlorinated biphenyls in large predatory fish species has become a growing public health concern, DHA and EPA extracted from marine microalgae are now a growing opportunity not only in Korea but also throughout the world as alternative to fish oils. The nutraceuticals industry has already established a solid foundation in Korea and the use of microalgae in nutraceuticals is expected to gain more and more popularity because demands for new source of better nutrition with natural ingredients are increasing. Therefore, isolation and characterization of new microalgal species from more diverse maritime environments are needed. Since only small number of microalgal species are routinely cultivated as nutraceuticals, strains with desired active compound production in low cost media and viability under versatile growth conditions are required.

Pharmaceuticals

The Korean species categorized as pharmaceutical in Table 1 show anti-inflammatory, anti-microbial, anti-viral, and/or anti-cancer activities. Medicinal effects of microalgae are also attributed to carbohydrates, pigments, PUFAs, and vitamins, but only marine microalgae-derived toxins are discussed in this section. There are 5 major phycotoxins groups; amnesic shellfish poisoning, ciguatera fish poisoning, diarrhetic shellfish poisoning, neurotoxic shellfish poisoning, and paralytic shellfish poisoning. Among them, dinoflagellate biotoxins with pharmaceutical potentials are gonyautoxins, okadaic acid, pectenotoxins, saxitoxin, yessotoxins, and zooxanthellatoxins (Camacho et al. 2007). In particular, new toxin compounds from Korean dinoflagellate isolates, such as *D. acuminata* (appendix 5-3. *Dinophysis acuminata*), *G. impudicum* (appendix 5-4. *Gymnodinium impudicum*), *O.* cf. *ovata* (appendix 5-5. *Ostreopsis* cf. *ovata*), and *P. lima* (appendix 5-8. *Prorocentrum lima*) have gained attention because they have potential for use as not only standard toxins but also anti-tumor drugs (Camacho et al. 2007; Assunção et al. 2017). Since various types of zooxanthellatoxins have been identified from the cultured *Symbiodinium* spp. (Onodera et al. 2004, 2005; Fukatsu et al. 2007), the Korean *S. voratum* (appendix 5-9. *Symbiodinium voratum*) strains obtained from waters off Jeju Island (Jeong et al. 2012, 2014) should be further investigated for their potentials in novel toxin compound isolation. It is assumed that their unique survival mechanisms make marine dinoflagellates promising as a source of new active substances with high potential uses in medicines. Even though above-mentioned drugs are still in pre-clinical or early clinical stages of development, yet this opens up new possibilities for the microalgae-based pharmaceutical industry in Korea. Hence, further efforts on the establishment of undiscovered dinoflagellate cultures from Korean waters and the identification of novel toxins are warranted.

Wastewater treatment

Microalgal wastewater treatment is an effective means to remove carbon, nitrogen, and phosphorus. Numerous studies have used wastewater as microalgae culture media, since microalgae can efficiently uptake nutrients from contaminated water (Craggs et al. 1997; Jiang et al. 2011; Cho et al. 2013; Whitton et al. 2015; Miranda et al. 2017). Greenhouse gas reduction can also be achieved by the biological transformation of CO_2 from organic carbon and the atmosphere into microalgal biomass. It was calculated that the production of 1.0 kg of dry microalgae biomass fixes 1.83 kg of atmospheric CO_2 (Slade and Bauen 2013). Currently, the carbon trading price in Korea is approximately 25.0 USD per ton of CO_2, according to the Korea Exchange. Because the current supply does not meet the increasing industry demands, the price has gradually increased since the introduction of carbon emission trading in 2015. This can offer new opportunities for the microalgae industry to profit and benefit from carbon trading if microalgae cultivation systems can be integrated with wastewater treatment plants. Even though microalgae-mediated wastewater treatment offers advantages over conventional methods, industrial- or commercial-scale trials have never been conducted in Korea due to the current limitation in the integrated wastewater treatment technologies.

Microalgal biomass can also be utilized as feed and fertilizer as well as biofuel feedstock. However, biosafety should be concerned when utilizing wastewater-grown biomass for human consumption and animal and fish feed since microalgal cell surface is negatively charged due to the presence of various functional groups and therefore microalgae can easily bind with positively charged heavy metal ions (Rawat et al. 2016). On the other hand, this property of microalgae could be applied in rare-earth metals and precious metals recovery. Several studies have demonstrated that microalgae could be used as biosorbent for recovery of rare and therefore valuable metals including gold, lanthanum, and palladium (Ju et al. 2016; Corrêa et al. 2017; Shen et al. 2017; Inoue et al. 2018). Microalgae have higher uptake capacity than other biosorbents such as bacteria, fungi, and yeast due to their cell wall constituents (Monteiro and Carlos 2012). In addition, microalgae also have advantages over the other microorganisms since they do not require oxygen and specific nutrients for growth and they can be cultivated both aerobically and anaerobically (Kumar et al. 2015). A number of marine microalgal species have shown their potential as an efficient biosorbent for a variety of metal species (Matsunaga et al. 1999; Brinza et al. 2007; Wang and Chen 2009). Hence, further investigation on phytoremediation and recovery of heavy metals from the heavily polluted shores is needed. Microalgae-based biosorption metal removal and recovery could be a useful alternative to the conventional systems and this may open up new opportunities for the microalgae-integrated wastewater treatment technologies in Korea.

4. Future Perspectives on the Korean Marine Microalgal Biotechnology

In the early days of microalgae-based industry, microalgae merely provided a source of various bioactive compounds for food supplements and nutraceuticals and a feed for many marine aquaculture species. However, the technology has evolved to include integration of many advanced fields. The growing importance of aquaculture in Korea is going to emphasize the necessity of adaptation to automation and information technologies in animal feed industry. This integration will create a variety of new applications in the near future and this may promote Korea as a world's leading nation in terms of smart aquaculture system. Furthermore, chemical structures of novel compounds isolated from marine dinoflagellates were identified and their biochemical pathway-specific effects to target organisms were investigated (Lee et al. 2015b; Yoon et al. 2017). Also, enhanced biological control of target phytoplankton has been successfully demonstrated by a number of studies using the interaction between toxic dinoflagellates and their predators (Jeong et al. 2003; Park et al. 2006; Kim et al. 2015b, 2017c, 2017e). These are only some examples of the future applications in the microalgae-based technologies of Korea. Hence, selective promotion and encouragement on the cutting-edge R&D programs for the novel Korean marine microalgal strains isolated and established from Korean waters are needed in order to create new trends in the technology and this will be a driving force for the nation to become one of the leading countries in the microalgal biotechnology.

5. Conclusions

To our knowledge, this review is the first report focusing on Korean marine microalgae with high biotechnological potentials and their current status and future trends. We hope that this paper may provide ideas and suggestions for future marine R&D strategies and direction for marine microalgae-based industries in Korea. Future investigation should be focused on discovering new Korean strains belong to the 34 species within 8 categories discussed in this review as priority. It should be stated that the coastal and oceanic waters around the Korean Peninsula are one of the marine microalgae biodiversity hotspot areas. As marine microalgae hold enormous promise in a wide range of fields, more innovative studies are needed to be carried out to render the marine bio-industry more economically competitive.

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[Appendix]

KOREAN MARINE MICROALGAE WITH BIOTECHNOLOGICAL POTENTIAL

1. Chlorophyte

1-1. Auxenochlorella protothecoides

Auxenochlorella protothecoides has long been known as *Chlorella protothecoides* (Darienko and Pröschold 2015) until it was assigned to the genus rank in 1987 (Kalina and Punčochářová 1987). This species has been extensively used in a variety of domains, including foods (Piechocki et al. 2018), animal feeds (Jeon et al. 2012, 2013), biofuels (Choi et al. 2011; Trimbur et al. 2015), and wastewater treatment (Im et al. 2015). A marine *A. protothecoides* strain MM0011, was isolated near Ulleung Island and its biochemical characteristics have been described by Jang et al. (2017). Similar to previously reported strains (Lane et al. 2017; Patel et al. 2018), this strain has potential for application in the fields of nutraceuticals, animal feeds, and biofuels because it is rich in PUFAs and lutein.

1-2. Chlamydomonas hedleyi

Several studies have reported that MAAs derived from *Chlamydomonas hedleyi* strain KMMCC-188, which was isolated from the South Sea of Korea, exhibit skin-protecting properties (Cho et al. 2014; Jung et al. 2014; Moh et al. 2014; Suh et al. 2014). MAAs display UV-protection, anti-inflammation, and anti-aging activities, indicating strong application potential in the cosmetics industry. MAAs have been patented as skin protectants (Moh et al. 2015) and wound-healing agents (Kim et al. 2018a).

1-3. Chlorella vulgaris

Chlorella vulgaris is probably one of the most widely studied and used algae species and it has been commercialized since the 1990s. It can be found in freshwater as well as terrestrial habitats; only few studies have been conducted with marine strains. Lee (2005) reported that strain KMCCC-073 is rich in essential EPA and Ko et al. (2016) showed that strain KMMCC-120 can accumulate neutral lipids and starch. Strain KMMCC-128 has been tested as a biodiesel feedstock by continuous cultivation (Shim 2010). The psychrophilic isolate KCTC 11964BP has been patented for use as a rotifer feed because it can be cultivated in the winter months (Hur and Bae 2013). In addition, *C. vulgaris* strain FC-012 has potential for food-waste leachate treatment as water qualities, such as pH, salinity, and odor, were improved by this strain (Kim et al. 2005).

1-4. Chloroidium ellipsoideum

Chloroidium ellipsoideum was formerly listed as *Chlorella ellipsoidea*, but this ellipsoidal *Chlorella*-like species was reassigned to a new genus by Darienko et al. (2010). Extract of commercially available Korean marine strains of *C. ellipsoideum* has anti-oxidant (Lee et al. 2009b) and anti-inflammatory (Choi et al. 2010) activities. Novel peptides derived from a marine *C. ellipsoideum* showed anti-hypertensive (Ko et al. 2012a) and anti-oxidant (Ko et al. 2012b) effects, and were patented for these purposes (Jeon et al. 2014a, 2014b).

1-5. Dunaliella salina

Dunaliella salina is a halotolerant eukaryotic photosynthetic organism and it is known as the most important species for β-carotene production in the marine bioindustry. Polle et al. (2008) identified a Korean *D. salina* strain, KCTC 10654BP, and this strain was found to have high potential as a β-carotene feedstock (Polle et al. 2008; Ryu 2011). Enhanced biomass production for biodiesel and high PUFA accumulation by using two-wavelength phased culture (465–520 nm and 660–520 nm) with the aid of alight emission diode (LED) was demonstrated with a commercially available Korean marine *D. salina* strain (Kang 2015a). Kim et al. (2017a) developed sodium bicarbonate-based culture of *D. salina strain* JDS 001 isolated from the West Sea of Korea.

1-6. Dunaliella tertiolecta

Dunaliella tertiolecta is known to tolerate a wide range of salt concentrations and has potential for a variety of industrial applications. *D. tertiolecta* strain C-9 has been tested for its potential as biofuel feedstock (Shim 2010) and copepod feed (Min et al. 2006). Enhanced biomass production and PUFA accumulation by using two-wavelength phased culture of a commercially available Korean marine *D. tertiolecta* strain has been investigated by Kang (2015a) and Ra et al. (2015).

1-7. Heterochlorella luteoviridis

Heterochlorella luteoviridis has been regarded as *Chlorella luteoviridis*, but the genus *Heterochlorella* was raised by Neustupa et al. (2009). *H. luteoviridis* MM0014 was isolated from the Jeongja port in East Sea and it had high omega-3 and -6 PUFA and protein contents (Kim et al. 2017d). This species has long been consumed as a food in European countries prior to 1997, along with *C. vulgaris* and *C. pyrenoidosa*, and it is not subjected to Novel Food Regulation (EC) No. 258/97 (Champenois et al. 2015). Therefore, pre-market authorization is not needed and this will be advantageous for future commercial applications in European countries.

1-8. Tetraselmis suecica

Anti-oxidant activities of *Tetraselmis suecica* extract have been reported by using a commercially available Korean marine strain (Lee et al. 2009a). Increased C_{16}–C_{18} fatty acid content of a commercial Korean strain was achieved by using two-stage culture (Go et al. 2012). Swine manure has been used as culture medium instead of fertilizer with a commercial Korean *T. suecica* strain, which can reduce cultivation cost and waste organic substances (Kang 2016). Optimal growth conditions of *T. suecica* strain KMMCC-111, isolated from Deukryang Bay, were determined using different LED wavelengths (Kang 2015b, Kang et al. 2017). *T. suecica* strain P-9, another isolate from Deukryang Bay, has been investigated as an aquafeed for the cyclopoid copepod *Paracyclopina nana* (Min et al. 2006). The use of a commercial compound fertilizer in the cultivation of this strain has been attempted to reduce the production cost (Choo 2014). Anti-bacterial activity of strain P-9 was demonstrated by Kim et al. (2017f). A commercial strain has been tested for biodiesel production (Lee 2011).

2. Cryptophyte

2-1. Teleaulax amphioxeia

Teleaulax amphioxeia is a ubiquitous unicellular cryptophyte marine alga and a number of *T. amphioxeia* cultures from various Korean coastal waters have been established and tested as prey for the *B. plicatilis* and larvae of *Crassostrea gigas* and *Ruditapes philippinarum* (Park 2012; Park et al. 2016). Among them, strain CR-MAL 08-2 showed the highest density and fecundity rates of the rotifer and also excellent survival rate for bivalves and this strain was patented as a live-feed rich in PUFAs and essential amino acids (Kim et al. 2015a).

3. Cyanophyte

3-1. Arthrospira maxima

Arthrospira maxima, more commonly known as *Spirulina maxima*, is a filamentous cyanobacteria species that has been extensively used as a nutritional supplement. As this species thrives under extreme alkaline conditions (Belay et al. 1993), outdoor mass cultivation is common practice worldwide. Many Korean groups have conducted studies on the industrial potentials of this species; however, these studies generally used non-Korean strains. One reported *A. maxima* strain, KORDI-3, was isolated from the estuary of the Dongjin River. Enhanced biomass and amino acid production of strain KORDI-3 was demonstrated by using optimized media (Kang et al. 2013a).

3-2. Arthrospira platensis

Arthrospira platensis, also known as *Spirulina platensis*, is another cyanobacteria species widely used in a variety of fields. *A. platensis* KCTC AG20590 was isolated near Jeju Island (Choi et al. 2007) and this strain produces γ-linolenic acid (Kim et al. 2012) and high-purity phycocyanin (Lee et al. 2016c, 2016d). c-Phycocyanin from a commercial Korean strain has been applied in nanoemulsion formulations to enhance topical delivery (Jung 2016).

4. Diatom

4-1. Achnanthes brevipes

This adhesive diatom was isolated from lava seawater near Jeju Island and it was patented by JNC Fishing Corporation Inc. as a red sea cucumber feed (Go 2017a) and a general sea animal additive (Go 2018) because of its benthic nature and nutritional profile. This species has been reported to occur in various locations, including the East Sea and the West Sea (Lee et al. 2013). In addition, silica was extracted from the biomass of this species and this process was patented for extracting natural silica (Go 2017b). Diatom-based silica has commercial application potential as biosensors, drug and gene delivery systems, and nanoparticles (Dolatabadi and de la Guardia 2011; Mishra et al. 2017).

4-2. Achnanthes sancti-pauli

Achnanthes sancti-pauli was isolated from Jeju lava seawater by the same company that isolated *A. brevipes* and was patented for the same purposes as *A. brevipes* (Go 2017a, 2017b, 2018).

4-3. Chaetoceros gracilis

Chaetoceros gracilis was patented as an aquafeed additive for abalone (Kim et al. 2014), fish (Lee and Lim 2018a), and sea cucumber (Lee and Lim 2018b). This marine diatom has been patented for a wide range of applications, including biofuels (Trimbur et al. 2015), soap and cosmetics (Day et al. 2016), waste consumption (Choi et al. 2011), fucoxanthin production (Kim et al. 2018b, 2018c, 2018d, 2018e), lipid-rich flour production (Piechocki et al. 2018), and food additives (Brooks et al. 2017). Strain KMCC B-52 isolated from Gyunggi Bay reportedly can be used as an ingredient for hair loss prevention and as anti-oxidant source (Jung et al. 2018).

4-4. Chaetoceros didymus

Chaetoceros didymus is a neritic species and it is commonly found in temperate to warm water regions. Strain KMMCC-1199 isolated from surface water in Namhae and it was tested as a feed for blue mussel *Mytilus galloprovincialis* (Lee and Shin 2015).

4-5. Cylindrotheca closterium

Cylindrotheca closterium, also known as *Nitzschia closterium*, is a marine benthic diatom commonly found in coastal waters. Using *C. closterium* strain B-72, high lipid production was achieved in continuous batch culture (Shim 2010). Strain KMMCC-62 has been patented as an animal feed for marine invertebrate larvae of abalone, horned turban, echinoid, and sea cucumber (Hur et al. 2018). This marine diatom has been patented for its potential in fucoxanthin production (Kim et al. 2018b, 2018c, 2018d, 2018e). Strain KMCC B-9, isolated from the estuary of the Nakdong River, has been evaluated as abalone feed (Han and Hur 2000).

4-6. Ditylum brightwellii

A series of studies have investigated the potential of *Ditylum brightwellii* as a bioindicator of water quality (Lee 2014; Lee et al. 2014a; Ebenezer and Ki 2016). Expression of the heat shock protein 20 gene from *D. brightwellii* strain B-326 is responsive to metals and endocrine-disrupting chemicals (Lee et al. 2014a).

4-7. Melosira octogona

Melosira octogona was isolated from Jeju lava seawater and this benthic diatom has been patented for the same purposes as *A. brevipes* and *A. sancti-pauli* (Go

2017a, 2017b, 2018).

4-8. Navicula salinicola

Navicula salinicola was previously known as *Navicula incerta*. An *N. salinicola* strain was isolated from the abalone hatchery of the National Institute of Fisheries Science (NIFS) on Jeju Island, and anti-oxidant activities have been investigated in axenic culture and it was shown that the reactive oxygen species scavenging rates and metal chelating activities of *N. salinicola* extract were higher than commercial anti-oxidant (Affan et al. 2007).

4-9. Phaeodactylum tricornutum

Phaeodactylum tricornutum is also one of the most widely studied species and is utilized in various fields. *P. tricornutum* strain MBEyh07L, isolated near Youngheung Island (Lee et al. 2014b; Lee et al. 2016a), and strain B-128 from Busan (Shim 2010) showed potential as a biodiesel feedstock. Lee et al. (2011) investigated potential oil production by a commercially available *P. tricornutum* strain. Enhanced growth was achieved under blue light (450 nm) with *P. tricornutum* strain KMMCC-309 isolated from the East Sea (Oh et al. 2015; Jeon 2016). In contrast, maximum growth of *P. tricornutum* strain KMMCC-541, originating from coastal region in Buan, Jeonbuk, was attained under red LED light (660 nm). Strain KMMCC-309 is able to remove heavy metals, such as copper (Cu) and zinc (Zn), from contaminated sediment (Lim 2002). Strain KMMCC-255 isolated from Yokji Island is able to biosynthesize EPA and DHA (Lee 2005). Strain B-128 has been used as feed for edible clam species, *Ruditapes philippinarum* (Lim 2002; Cho 2004) and *Meretrix petechialis* (Lim et al. 2008). Fucoxanthin extraction from a commercially available strain has been investigated (Kim 2014a). Cultivation of a commercial Korean *P. tricornutum* strain using livestock wastewater as a medium was demonstrated by Kang (2016). Another Korean strain was isolated from off Seongsan, Jeju, and its optimal growth conditions have been investigated (Lee and Kim 2002).

4-10. Skeletonema costatum

Skeletonema costatum is a non-toxic coastal diatom species that is distributed worldwide and has been used as bioindicator of water quality (International Organization for Standardization 1995). Korean origin has been confirmed for only one strain, B-396, which produces EPA and DHA (Lee 2005).

5. Dinoflagellate

5-1. Alexandrium andersonii

The marine planktonic dinoflagellate *Alexandrium andersonii* strain AAJH201505 was obtained from the port of Jinhae (Lee et al. 2016b). This species is generally known to cause red tides or harmful algal blooms in subtropical and tropical regions. However, unlike other species within the genus *Alexandrium*, this dinoflagellate has mixotrophic ability (Lee et al. 2016b). Furthermore, this species is capable of killing *Miamiensis* spp. at concentrations of 1,000 to 2,500 cells mL^{-1} (Jeong et al. 2017; Kim et al. 2017c). Therefore, *A. andersonii* has potential as an environmentally friendly anti-parasitic against scuticociliatosis caused the main disease of flounder aquaculture in Korea.

5-2. Amphidinium carterae

The benthic dinoflagellate *Amphidinium carterae* strain JHWAC isolated from the coastal area of Hwasun, Jeju Island shows anti-oxidant and anti-inflammatory effects (Shah et al. 2014) and its feasibility for mass cultivation has been reported (Shah et al. 2016). Satake et al. (2017) identified two new amphidinols from a Korean *A. carterae* strain collected from Goseong. Amphidinols are natural anti-fungal agents commonly present in this marine dinoflagellate and have been extracted from strains KMMCC-550 and -551 (Kim 2014b). *A. carterae*-derived amphidinol has been patented for its capability of eliminating and preventing mycoplasma contamination of cell cultures (Han 2016). Furthermore, the anti-microbial, anti-oxidant, and potential anti-cancer activities of *A. carterae* extract have been analyzed and in particular *A. carterae* ethanol extract showed anti-cancer properties against HepG2 and HT-29 human cancer cells (Kim et al. 2017b). Also, DPPH (2,2-Diphenyl-1-picrylhydrazyl) and ABTS (2,2-azinobis-3-ethylenebenzothiozoline-6-sulfonic acid) radical-scavenging activities and anti-bacterial properties against 16 Gram positive and negative bacterial strains were confirmed by the same authors.

5-3. Dinophysis acuminata

Dinophysis acuminata has received public attention because it is responsible for the diarrhetic shellfish poisoning in tropical, temperate, coastal and oceanic waters. *D. acuminata* HAPCC DA-MAL01, KNU, isolated from Masan Bay, was successfully mass-cultivated for the first time by Park et al. (2006). Follow-up studies to identify bioactive compounds from *D. acuminata* uncovered acuminolide A, an actomyosin ATPase that can aid in the prevention of muscle-weakening diseases (Hwang et al. 2014; Hwang 2015) and has been patented for this purpose (Rho et al. 2016). Pectenotoxin-2, with application potential in anti-cancer research and as a standard toxin, has also been extracted from *D. acuminata* (Yih et al. 2011).

5-4. *Gymnodinium impudicum*

Gymnodinium impudicum, formerly called *Gyrodinium impudicum*, is responsible for bloom formation in Korean coastal waters in association with *Cochlodinium polykrikoides* (Park and Park 1999). Since axenic culture of *G. impudicum* strain KG03G isolated in the Ganggu coastal waters was established by Yim and Lee (2004), and a number of follow-up studies have been conducted to reveal the pharmaceutical potential of exopolysaccharide (EPS) from *G. impudicum* (Yim 2002). Optimal culture conditions for EPS production have been determined (Yim et al. 2003), and the anti-cancer (Bae et al. 2006), anti-viral (Yim et al. 2004; Lee et al. 2005, 2009a; Kim et al. 2013), and immunostimulatory activities (Yim et al. 2005; Lee et al. 2006) of sulfated EPS have been reported and/or patented in these follow-up studies.

5-5. *Ostreopsis* cf. *ovata*

Ostreopsis is a genus formed the toxic algal blooms in the Mediterranean Sea and distributed in temperate and tropical marine areas. New toxin compounds such as ostreol A and B in *Ostreopsis* cf. *ovata* isolated from Jeju coastal waters have been isolated and identified (Hwang et al. 2013, 2018; Kang et al. 2013b; Hwang 2015). These toxins have potential pharmaceutical applications as anti-cancer drugs.

5-6. *Paragymnodinium shiwhaense*

Paragymnodinium shiwhaense was isolated from Shiwha Bay and was described by Kang et al. (2010). In a follow-up study by the same research group, this species was found to be rich in EPA and DHA and to harbor omega-3 biosynthesis-related genes (Jang et al. 2017). This mixotrophic species was also reported that it was able to feed on red tide forming dinoflagellates including *A. carterae* and *Heterosigma akashiwo* (Yoo et al. 2010).

5-7. *Prorocentrum dentatum*

Although *Prorocentrum dentatum* is a common harmful algal bloom species in China, Korea, and Japan (Tang et al. 2006), a Korean strain KMMCC-1101 which was isolated from Haeundae, Busan showed its potential as a good feed for *M. galloprovincialis* (Lee and Shin 2015).

5-8. *Prorocentrum lima*

Prorocentrum lima is a benthic dinoflagellate species that is distributed worldwide. A Korean *P. lima* strain, KNUPL-121117, isolated from *Sargassum fulvellum* at the coast of Geomundo Island, is able to produce a dinophysistoxin and limaol, which shows cytotoxicity against cancer (Jo 2016; Yang 2017; Yang et al. 2017). Another *P. lima* strain, PLKNUAL-23, from a seaweed collected off the Jeju coast, is capable of biosynthesizing a new diol ester that has potential anti-hepatic fibrosis and anti-cancer activities (Lee et al. 2015b; Lee 2016).

5-9. *Symbiodinium voratum*

The genus *Symbiodinium* is one of the dominant dinoflagellates in marine benthic habitats and known as the symbiont of corals (Jeong et al. 2014). Lee (2016) demonstrated that a mixture of glycolipid and peridinin extracted from laboratory-cultivated *S. voratum* strain SvFL 1, isolated from Jeju coastal waters, inhibited hepatic fibrosis.

6. Eustigmatophyte

6-1. *Nannochloropsis oceanica*

Nannochloropsis oceanica is known as a promising source for biofuel and valuable pigment production. A commercially available Korean *N. oceanica* strain has been used in a wide range of applications, including abalone feed (Cho et al. 2018), biofuel (Park et al. 2014; Kang 2015a; Lee et al. 2015a; Ra et al. 2015, 2016), skin-protective violaxanthin production (Kim 2017), and anti-inflammatory, anti-oxidant, and anti-amyloidogenic extracts (Choi et al. 2017). *N. oceanica* strain KMMCC-154 has been also tested for biofuel production (Kim et al. 2016a). Nanofibrillar cellulose production by a commercial Korean strain has been reported (Lee et al. 2018).

6-2. *Nannochloropsis oculata*

Nannochloropsis oculata also is a promising source for biofuel production. Biodiesel production with a commercial strain has been tested (Lee 2011; Kim 2012a, 2013; Kang 2015a; Ra et al. 2015, 2016), and swine manure has been used as culture medium for a commercial Korean *N. oculata* strain, which not only reduces cultivation cost but also recycles waste organic matters (Kang 2016).

7. Haptophyte

7-1. Diacronema lutheri

Diacronema lutheri, previously called *Pavlova lutheri*, is widely used in aquaculture as live feed for a wide range of marine invertebrates (Cancela et al. 2016). Numerous studies have investigated the industrial potential of this species, but these were conducted with non-Korean strains. One Korean strain was isolated from the North Jeju Marine Hatchery at the NIFS and optimal growth conditions have been determined (Lee and Kim 2002).

7-2. Isochrysis galbana

Isochrysis galbana is an unicellular phytoflagellate and is known to be rich in PUFAs. Various studies have shown its high potential in the aquafeed industry, but these were not conducted with Korean strains. A Korean strain was isolated from the North Jeju Marine Hatchery at the NIFS (Lee and Kim 2002), and optimal growth conditions, including temperature, salinity, and N:P ratio, have been determined. Biofuel production has been demonstrated with a commercially available Korean *I. galbana* strain (Lee et al. 2011; Ra et al. 2015, 2016).