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SECCIÓN II:

INDUSTRIAL AND ENVIRONMENTAL BIOTECHNOLOGY MINI REVIEW

Una mirada a los estudios de transcriptoma en algas pardas de importancia industrial, en especial del género *Macrocystis*

A peep into the transcriptome studies of the industrially important brown algae with special focus on *Macrocystis* genus

Resumen

Las algas pardas son una fuente comercial de diferentes polisacáridos / hidrocoloides utilizados para producir alimentos, productos farmacéuticos y otros productos biotecnológicos útiles. Por otra parte, la transcriptómica se ha convertido en una herramienta que no solo proporciona información valiosa para la comprensión de los procesos fisiológicos de una especie, sino que también permite la identificación de genes candidatos industrialmente importantes. Este estudio describe el transcriptoma de algas pardas y su aplicación industrial con un enfoque especial en *Macrocystis integrifolia*.

Abstract

Brown algae are a commercial source of different polysaccharides/hydrocolloids that has been used to produce food, pharmaceutical, and several other useful biotechnological stuff. Nevertheless, transcriptomics has become a tool that not only provides valuable information for the understanding of the physiological processes of a species but also allows the identification of industrially important candidate genes. This study describes the transcriptome of brown algae and their industrial application with a special focus on *Macrocystis integrifolia*.

Palabras clave

Macrocystis integrifolia, transcriptómica, polisacáridos de pared.

Keywords:

Macrocystis integrifolia, transcriptomics, wall polysaccharides.

Introduction

Marine macroalgae or seaweeds have been shown to have several complex polysaccharides such as alginates, fucoidans, laminarin, and mannitol that are of great interest to the pharmaceutical and food industry and nevertheless, the recent application of biotechnological tools has maximized the exploitation of those natural resources (Popper et al. 2011, Michel et al. 2010). Due to uncommon structure and beneficial properties, those seaweed polysaccharides not only exert promising therapeutic effects against several human ailments (Fedorov et al. 2013) but also showed a growth-promoting effect in several agricultural and horticultural crops (Battacharyya et al. 2015).

Macrocystis integrifolia, is considered as a potent brown seaweed species for the production of industrially important polysaccharides (Wei et al. 2013). In recent years the global market of macroalgal products is growing rapidly (FAO 2014) and it assumed that by the year 2023 it will reach approximately USD 850 million worldwide. The global algae industry has seen many changes, focused throughout its history, from the exploitation of marine algae as fertilizers and source of potash through the production of iodine and hydrocolloids (Synytsya et al. 2015). Many researchers predict that in near future commercial algal hydrocolloid industry could be a boom due to their extensive use in cosmeceutical (Balboa et al. 2015), nutraceuticals (Humaya & Kim, 2015), and pharmaceutical products (Pan-gestudi & Kim 2015).

On the other hand, seaweeds are often exposed to a wide range of abiotic stresses including heat, UV rays, salinity, desiccation and tidal flow and thus could be a genetic resource to understand the adaptation mechanism for such variable environments and that knowledge could be applied into agricultural research for crop improvement. One of the classic examples is salt tolerant transgenic rice with a gene of a red seaweed *Porphyra yezoensis* developed by Kishimoto et al. (2013). Another aspect could be the characterization of novel genes and the determination of their functions for economically important compound production in brown algae using RNAi technology. Recently, Kumar et al. (2016) defined the importance of metabolomics in marine algae to investigate its unique metabolism where the characterized candidate genes could help to identify the economically important metabolites and their functions; leading to the relationship between regulatory networks and metabolic pathways in marine algae.

Brown algae are widely distributed in temperate and subtropical zones and are an important component of intertidal and subtidal ecosystems (Charrier et al. 2008). Macroalgae can acclimatize to variable CO₂ availability by CO₂ concentration mechanisms (CCMs), which is recently gaining interest due to the increased levels of seawater carbonate in recent decades (Raven & Berardall, 2014). The storage and accumulation of carbohydrates by brown algae is a critical physiological process that mostly obtained by the production of laminarin and

mannitol where mannitol can represent up to 20 – 30% of its dry weight depending on the species of brown algae (Reed et al. 1985). Studies on *Ectocarpus siliculosus* have suggested that mannitol may act as an osmoprotectant in brown algae (Dittami et al. 2011). Likewise, fucoidans participate in the construction of cellular walls of macroalgae and provide rigidity. The presence of sulfated groups in the aforesaid polysaccharides facilitates their binding with different cations including K⁺, Na⁺, Ca²⁺, Mg²⁺ which in turn help macroalgae to adapt in saline as well as heavy metal contaminated water through ion exchange mechanism (Skriptsova 2015). Alginates are naturally occurring polysaccharides present in the cell wall of brown algae to serve a structural role as well as to act as an ionic barrier. Based on the latest available data for 2009, the estimated worldwide annual sale value of alginate is US\$ 318 million (Bixler & Porse, 2011).

Despite an enormous functional diversity, the marine algae are not morphologically or anatomically complex; therefore, the survival strategies adopted by marine algae to withstand the high fluctuation of oceanic conditions, such as the drying and immersion cycle and the activities associated with multiple stressors arising from the periodic rhythms of the tides, and cyclical environmental conditions that do not cross terrestrial plants, but the physiological effects can be analyzed at molecular levels (Kumar et al. 2011). Oertel et al. (2015) recently reported a technique for editing the genome of *Ulva mutabilis*, which consisted in the development of suitable vectors and the transformation of *U. mutabilis*. Nevertheless, since 1990 this research line is gaining strength in terrestrial plants and has now advanced with the development of genome-editing molecular tools such as TALEN and CRISPR / Cas9 (Kumar & Jain 2015) which could also become promising for the algal genome edition.

In recent years both genomics and genetic engineering are undoubtedly playing a role in the improvement of the algae culture. Currently, techniques such as transcriptome analysis or RNAseq have found to be extremely useful for the interpretation and understanding of the functional perspective of the eukaryotic genome (Wang et al. 2009). The main objectives of comparative transcriptomics are to catalog all the differentially expressed RNAs (transcripts) during a period of development or specific physiological condition (Dos Reis Falcão et al. 2008, Wang et al. 2009). The recent integration of transcriptomic studies in macroalgae not only helps to elucidate the gene expression analysis in response to environmental stress but also facilitates to characterize several algal candidate genes that are involved in different biochemical and metabolic pathways (Nyvall et al. 2003). The whole genome of brown algae *Ectocarpus siliculosus*, which constitutes a biological model for studying physiological and ontogenetic processes (Charrier et al. 2008), has already been sequenced (Cock et al. 2010) and the genomic information obtained has allowed identifying the molecular basis of different metabolic pathways specific to this organism (Graisillier et al. 2014). RNAseq on Illumina sequencing platform has been done with several species of Phaeophyta (brown algae) such as

Saccharina japonica (Deng et al. 2012), *Sargassum thunbergii* (Liu et al. 2014), *Sargassum vulgare* (Kumar et al. 2017), *Ectocarpus siliculosus* (Roeder et al. 2005, Dittami et al. 2009, Martins et al. 2013, Saint-Marcoux et al. 2015), *Macrocystis pyrifera* (Konotchick et al. 2012) and *Macrocystis integrifolia* (Salavarría et al. 2018), as well as Rhodophytes such as *Pyropia haitanensis* (Wang et al. 2015). Dittami et al. (2009) studied the global transcriptome expression pattern of the brown alga *Ectocarpus siliculosus* during a short thermal acclimation at three different abiotic stress conditions; demonstrating that at least one of these stressors regulate almost 70% of the gene expression. On the other hand, Martins et al. (2013) conducted a study on gene expression biased by sex in the brown alga *Fucus vesiculosus* and estimated that gene expression was higher for males with 14% orthologous genes compared to females with 9%.

Although several morphological studies have already been performed in brown algae *M. pyrifera* the molecular studies are still scarce. Taxonomists have reported two ecotypes of the genus *Macrocystis*: *M. pyrifera* and *M. integrifolia*, however, no molecular proof has been reported yet. A recent study conducted along the southeastern Pacific coast concluded that *Macrocystis* has low genetic variability. However, this research demonstrated a different genetic pattern in Peru, including unique haplotypes in two sites of the Central-South coast (Salavarría et al. 2019). A recent transcriptomic study regarding *Macrocystis pyrifera* metabolic function has been carried out by Konotchick et al. (2012) considering several variables such as the gradient of depth, light, temperature, and nutrients, revealed novel gene families (clusters). In *M. integrifolia*, the first de novo global transcriptome analysis using RNAseq technology (Illumina NextSeq 500) was performed by Salavarría et al. 2018, and abiotic stress-induced differential gene expression in intertidal and subtidal conditions has been reported. Results revealed that due to the desiccation and other abiotic stresses in intertidal condition several antioxidant defense genes including molecular chaperon proteins/HSP70, vanadium-dependent bromoperoxidase, serine/threonine-protein kinase, aspartate aminotransferase, and glutathione S-transferase have been overexpressed in *M. integrifolia* (Salavarría et al. 2018).

Conclusions

The importance of the macroalgae is evident from every point of view, from the ecology to be constituted in the habitat of hundreds of marine invertebrates to the economic aspect, for its bioeconomic valuation due to the impulse of the blue bioeconomy. Moreover, the use of molecular techniques promoted the discovery of new attributes with potential industrial applications. The development of transcriptomics and its application in the field of macroalgae is fundamental to understand the biological processes from the molecular point of view and to interpret their physiological and metabolic responses at different adverse environmental conditions which give them the power of adaptability. The expansion of functional genomics in macroalgae will make

it possible to identify new alternatives in favor of food security, biomedical treatments and in the bioeconomic field of the countries. To determine the regulation of the genes involved in important metabolic pathways for secondary metabolites of medical and industrial application will allow generating advances in transcriptomics. On the other hand, the use of current technologies for the editing of genomes, which has had a wide application in vegetables, is also being used in macroalgae.

The challenges ahead will be focused on the improvement of these techniques to be used more frequently in macroalgal functional genomic research, moreover, to open new competitive markets for the use and development of products of macroalgal origin. Brown algae offer not only a viable source of biofertilizers due to the different phytohormones they possess but also phytochemicals that can be used for cancer treatment. On the industrial ground, the genus *Macrocystis* is very important due to the production of several wall polysaccharides, constituting an important competitive alternative in international markets. It remains to face the challenges of the industrialized processing of these resources, which generates remarkable economic income offers opportunities to apply new and innovative molecular techniques. Joint efforts from education, through research, industry and government; it constitutes the props for the construction of new development opportunities, based on a blue bioeconomy, and in this way cope with the environmental impacts that affect us.

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