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Feeding and bioremediation relationships between *Chrysochromulina* species and bacteria

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Abstract
Harmful algal blooms (HABs) consider a global environmental problem. A massive fish death occurred in two coastal Red Sea lagoons: Al-Nawras and Al-Arbaeen (Jeddah, Saudi Arabia), and the main cause was associated to bloom of microalgal *Chrysochromulina* species which can feed on bacteria (phagotrophic). There are bacterial strains that capable of inhibiting or lysing HAB species by producing algicidal substances. On another hand, the resistant of pathogenic bacteria to drugs have become a critical global threat. Thus, finding an effective alternative solution is essential, especially that taken advantage of the phagocytosis in *Chrysochromulina* and applied on pathogenic bacteria has not been studied enough yet. The aim of the article is to understanding the relationship that occur between *Chrysochromulina* and bacteria.

Keywords: Feeding, bioremediation, phagotrophy, *Chrysochromulina*, bacteria

INTRODUCTION

The release of untreated sewage to estuarine waters and the coastal area has been common, mainly comprising street runoff, industrial waste, human waste, and eroded soils [35]. It has been recognized that untreated sewage carries several types of pollutants to the coastal zone, and due to this reason, it is considered the principal source of pollutants in the globe. Therefore, international agencies and coastal nations' ocean waste disposal system regulations have reduced the volume of wastes by applying sewage treatment plants (STPs). Historically, these systems have been focused on the reduction of conventional pollutants, such as sludge or total suspended solids (TSS), pH, fecal coliform bacteria, biological oxygen demand (BOD), oil and grease [97].

However, dumping sewage along the Saudi coasts consider a significant problem. In 2016, Jeddah city witnessed the phenomenon of fish deaths in two coastal lagoons: Al-Nawras and Al-Arbaeen, and sadly, this event has not been adequately studied yet. Both lagoons are famous for receiving sewage which means that bacteria contaminate the water.

The massive death was associated with the bloom of microalgal *Chrysochromulina* sp. and laboratory examinations of the dead fish gills and water samples from Al-Nawras and Al-Arbaeen lagoons have confirmed the presence of the alga. Although the genus *Chrysochromulina* is a photosynthetic organism with the ability to be mixotrophic by consuming particulate matter, bacteria, other small algae and protists (phagocytosis)[27], very little research has studied this type of nutrition in *Chrysochromulina* (especially the feeding on bacteria considered very limited). Most recent studies *Chrysochromulina* were about viruses that infect some algae species and their genome [such as 69, 99].

In general, algae and bacteria have a complex relationship, which may be symbiotic, antagonistic, and competitive [65]. An example of the competitive relationship is when nutrients are limited, such as phosphate, the competition for nutrients between bacteria and algae suppresses [86 cited in 65]. For example, Thingstad et al. [101] pointed out that in phosphorus-poor environments, bacteria tend to be stronger than other competitors for inorganic P. This competition makes bacteria have a high biomass P-content, which allow mixotrophic algae to gain large amounts of P by grazing on bacteria, which may cause blooms [76].

This article aims to understand the relationship between *Chrysochromulina* and bacteria better and take advantage of it as possible.

1. Red sea ecology

The Red Sea is a semi-enclosed warm body of water located between arid and semi-arid regions that are characterized by excessive evaporation rates, which explain the high salinity of surface water mass [4 cited in 108]. However, this site between deserts made the sea unique among the world of the seas because no rivers or permanent streams are flowing into it. Therefore, the Red sea is supplied with terrigenous materials only during heavy rainfall, flash floods and dust storms [85]. As a result, the Red Sea environment is considered one of the most biodiverse globally [82]. The warm tropical water of the sea hosts fauna and flora, particularly coral reefs, dolphins, numerous fish species and numbers of unique marine habitats such as mangroves, coral reefs, algae and seagrass beds [85].

Unfortunately, the sea environment is affected by human activities such as increasing population and the associated social activity and development, maritime transport, recreation and tourism, dumping of unwanted wastes such as plastic, landfilling, oil spills, pollutant discharges and effluent from sewage treatment plants and treated water from desalination plants. All these activities have caused threats to life in the Red Sea [103, 85]

1.1 Jeddah coast:

Along the Red Sea, the coastal areas are incised by lagoons of different shapes and sizes [85]. At the eastern coast of the Red Sea, particularly in the middle, lies the Bride of the Red Sea, Jeddah. The city is the traditional gateway to Makkah, giving it the advantage of connecting to the other parts of the world through a highly developed maritime transport network. Jeddah Islamic Port is considered one of the Red Sea's greatest ports [102].

During the past decades, Jeddah has grown very fast in all aspects of life (the urban area, industry, transportation, trade, ... etc.) [89]. Regrettably, the environmental conditions in the coastal area have changed due to these development activities in last years [1]. Rasiq et al. [84] pointed out that domestic and industrial water supply, treatment and disposal of sewage, water draining from both natural and manufactured sources, commercial and recreational uses for the marine environment, loading/unloading operations in Islamic Harbor, fishing vessels, the desalination plant, all of these are considered the most prominent sources for organic and inorganic pollutants in the coastal area of Jeddah.

2. *Chrysochromulina*

2.1 Taxonomy:

The genus *Chrysochromulina* belongs to *Prymnesiophyceae* (=*Haptophyceae*)[38], mostly marine, photoautotrophic, unicellular flagellated algae with the unique structure called haptonema. The name *Prymnesiophyceae* was based on the genus *Prymnesium* and was given by Hibberd [44] as an alternative class name to *Haptophyceae*. Moreover, *Coccolithophyceae* is another name for *Haptophyceae* and Prymnesiophyceae. However, Edvardsen et al. [18] established that the class can be divided into four orders: *Phaeocystales* (clade A), *Prymnesiales* (clade B) *Isochrysidales* (clade C) and *Coccolithales* (clade D). The previous study, which relied on 18S rRNA analysis, divided the order *Prymnesiales* (which *Chrysochromulina* relate to) into two sub-groups: Clade B1 and Clade B2. Clade B1 contains *Prymnesium* species and some of *Chrysochromulina* species with non-saddle shapes (maybe irregular to spheroid shapes) with haptonema that may equal or shorter than the two flagella. In contrast, clade B2 contains only *Chrysochromulina* spp. with the saddle shape. And because *Chrysochromulina* species fall into both clades, the genus was considered paraphyletic.

More recently, the gene-sequencing study by Edvardsen et al. [19] reconstructed the taxonomy of the *Prymnesiales*. As a result, the emended the family *Prymnesiaceae* and erected the *Chrysochromulinaceae fam. Nov.* (fig.1). The *Prymnesiaceae* emendation was as follows: (1) Placed five species of the genus *Chrysochromulina* (*Chrysochromulina polylepis*, *Chrysochromulina minor*, *Chrysochromulina kappa*, *Chrysochromulina chiton* and *Chrysochromulina palpebralis*) into the genus *Prymnesium* (Table 1). (2) Five other species of the genus *Chrysochromulina* (*Chrysochromulina ericina*, *Chrysochromulina fragaria*, *Chrysochromulina brevifilum*, *Chrysochromulina herdlensis* and *Chrysochromulina hirta*) are moved in a new genus named Haptolina (Table 1). (3) Another new genus is added to *Prymnesiaceae* called *Pseudohaptolina*, and the type species of this new genus is *Pseudohaptolina* arctica which was previously an unnamed species, cited as *Chrysochromulina* sp4 [23].

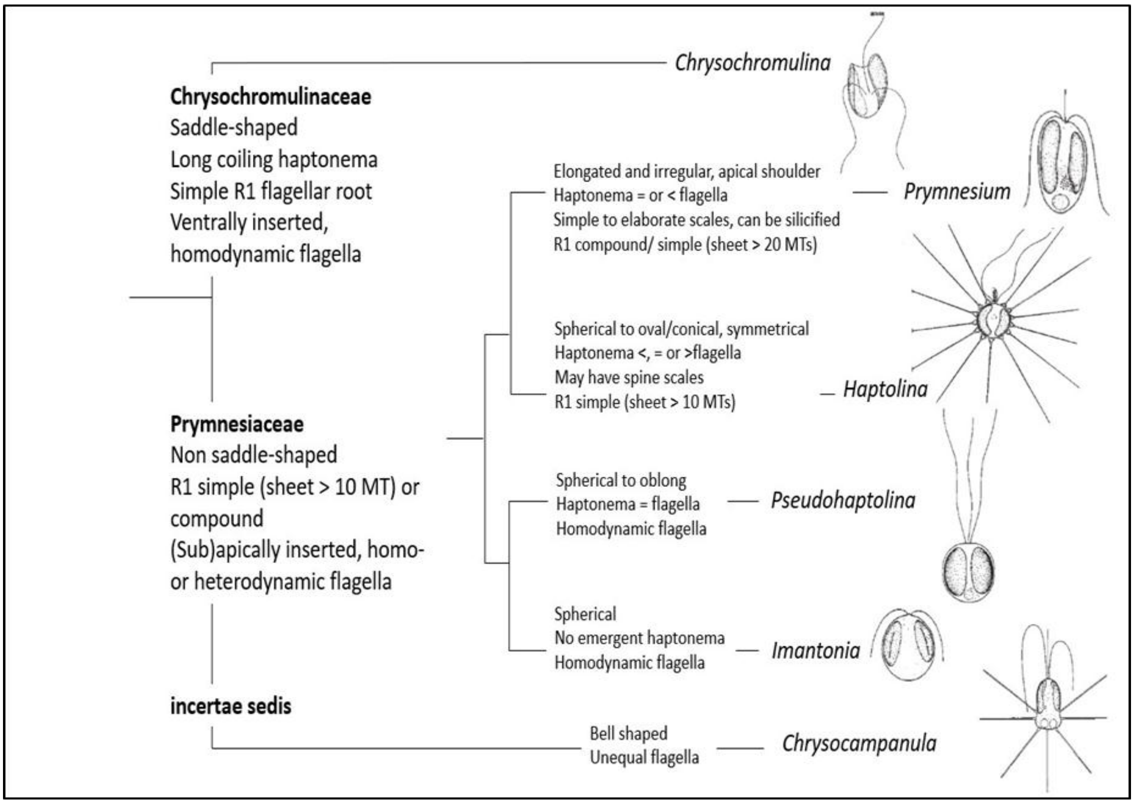

Figure 1: Typical morphological features in the genera of *Prymnesiales* (adapted from [19]).

As for *Chrysochromulinaceae*, it contains members with the saddle shape only (fig.3) (with some exceptions such as *Chrysochromulina leadbeateri* (fig.6) [19] has spherical shape [25]. It is essential to mention that the study was based on 18S rDNA, 28S rDNA and plastid 16S rDNA sequences. Recently, a web application for identifying *Chrysochromulina* species was overviewed by Chrétiennot-Dinet et al. [8].

In general, members of *Prymnesiales* (such as *Prymnesium*, *Chrysochromulina* and *Imantonia*) are characterized with two equal or sub-equal smooth flagella, and the cell body is commonly covered by scales and absence of eyespot [63]. However, although Haptophytes are particularly common in marine plankton, this does not prevent the presence of some species in freshwater [24].

Table 1: Species of the genus *Chrysochromulina* that have been placed

Basionym	Synonym	Reference
Chrysochromulina polylepis	*Prymnesium polylepis*	[19]
Chrysochromulina minor	*Prymnesium minor*	
Chrysochromulina kappa	*Prymnesium kappa*	
Chrysochromulina chiton	*Prymnesium chiton*	
Chrysochromulina palpebralis	*Prymnesium palpebralis*	
Chrysochromulina brevifilum	*Haptolina brevifila*	
Chrysochromulina ericina	*Haptolina ericina*	
Chrysochromulina fragaria	*Haptolina fragaria*	
Chrysochromulina hirta	*Haptolina hirta*	
Chrysochromulina herdlensis	*Haptolina herdlensis*	

In this article, the new definition will be used according to Edvardsen et al. [19] and the names described as *Chrysochromulina* in the literature will be modified.

2.2 Cell structure:

Most Haptophyta species have cells usually smaller than 20 µm (nanoplankton) [38] and, according to Edvardsen et al. [18, 19], the cells of many *Chrysochromulina* species have a typical shape which is saddle form. Anyway, the following are the most critical cellular structures:

2.2.1 Flagella:

Cells in Haptophyta usually have either two equal (isokont) or subequal (anisokont) flagella [38]. However, *Chrysochromulina* species have two smooth flagella and are approximately same in length, but there are two marine species *Chrysochromulina birgeri* and *Chrysochromulina quadrikonta* are quadriflagellate [56, 38, 107]. Both the flagella and haptonema are inserted ventrally (except *C. leadbeateri* [19](fig.6) inserted apically [25]). Eikrem et al. [24] reported that the flagellar action in *Chrysochromulina* is homodynamic and displays an undulating motion. When prymnesiophytes swim. Usually, both flagella are active where the ventral part is directed anteriorly and the tip is bent backward along and behind the cell [38]. The axoneme in flagellum is consists of the usual "9 + 2" arrangement of microtubules [24].

2.2.2 Haptonema:

The haptonema is the most distinctive feature of most members of the Haptophyta. Yoshida et al. [107] describe it as a unique filamentous appendage inserted between two smooth flagella and sometimes the haptonema is much longer than the flagella. The haptonema in the *Prymnesiales*, particularly in *Chrysochromulina* is highly developed [107]. Actually, the genus *Chrysochromulina* was separated from the genus *Prymnesium* depending on the longer and coiling haptonema in *Chrysochromulina* [66 cited in 87].

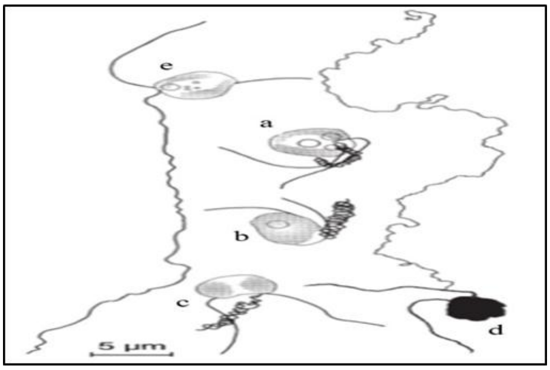

Figure 2: Three cells with coiled haptonema (a, b and c) and two cells with extended haptonema (d and c) [73].

However, haptonema has several roles, such as capturing and gathering food particles (fig.4), adhesion to the substratum and avoiding response [58, 59, 60]. There are two basic movements of haptonema: coiling and bending [63]. Coiling (fig.2) is a sensory response to obstacles [57] occurs when an object hits the alga cell (which swims slowly forward along with the haptonema while the flagella beat slowly backward), so the haptonema coils instantly and the flagella generate a propulsive force resulting in backward swimming [63]. Slow swimming continues when the haptonema extends again (fig.2). It should be noted that rapid swimming occurs with both flagella and haptonema directed backward behind the cell (fig.3) [38]. On the other hand, bending occurs during food capture (fig.4) [60]. Usually, the fine structure of the haptonema is 6 - 7 single microtubules arranged in a circle or across, surrounded by the ER cisterna [24]. Accordingly, the haptonema differs from the flagellum in ultrastructural and behavioral respects.

2.2.3 Chloroplasts:

The genus *Chrysochromulina* contains two well-developed yellow-brown elongate chloroplasts enveloped by four membranes: two inner membranes are the chloroplast envelope and two outer membranes are from the endoplasmic reticulum (fig.3). Each plastid has lamellae composed of three thylakoids [19], but a girdle lamella is generally absent in Haptophyta as Andersen [2] mentioned. A pyrenoid found immersed in *Chrysochromulina* chloroplast [19]. However, *Prymnesiophyceae* chloroplasts contain chlorophylls *a* and c_1/c_2, fucoxanthin, β-carotene, diatoxanthin, diadinoxanthin [109 cited in 63].

2.2.4 Food vesicle:

In the *Prymnesiophyceae* cytoplasm, there are two types of vesicles. The first vesicle contains lipids, while the second is used for storage products (fig.3). Usually, chrysolaminarin (leucosin) is the stored product in Prymnesiophyta [63]. Many of organisms are phagocytic (Table 3). In addition, *Chrysochromulina* is a mixotrophy organism which means that the alga can get food by photosynthesis or by ingestion particulate organic matter (fig. 5) [76], bacteria and other protists [27].

2.2.5 Scales:

Most members of the *Prymnesiophyceae* have cells that are covered with one or more layers of scales [63]. Golgi apparatus is responsible for scales biosynthesis. Some scales are coccoliths (calcified), while others are organic (non-calcified / plate scale). Hallegraeff et al. [38] mentioned that *Prymnesium*, *Chrysochromulina* and *Phaeocystis* produce only organic scales. This organic scale forms the whole scale or the base plate that the whole system is based on. The edges of the base plate can turn up to form different types of scales, such as very shallow tub-shaped scales with edges that barely turned up and very long spine scales. Several species of *Chrysochromulina* have spiniform scales [107]. However, scales in different types can be found on the same organism.

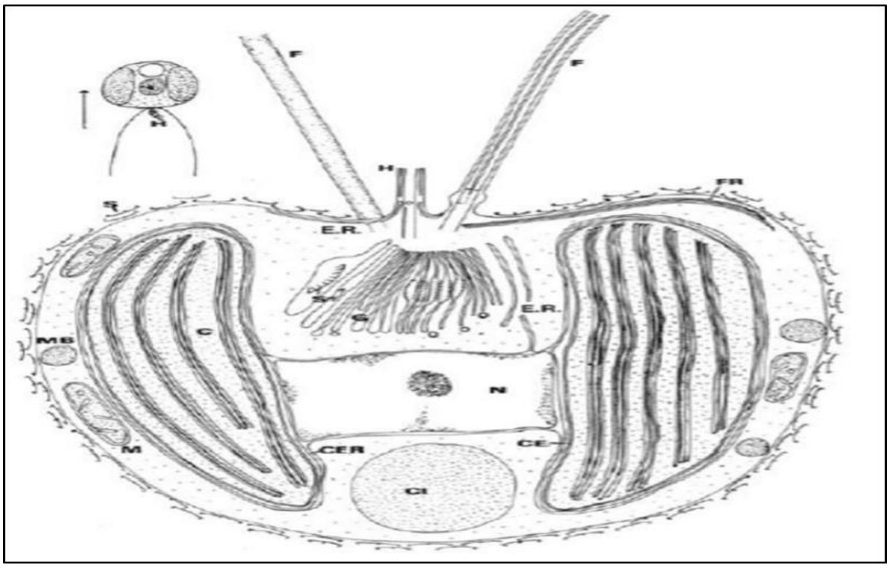

Figure 3: Drawing of a typical *Chrysochromulina* sp. cell (F) flagellum; (H) haptonema; (S) scale; (FR) flagellar root; (MB) muciferous body; (M) mitochondrion; (G) golgi body; (E.R.) endoplasmic reticulum; (C) chloroplast; (CE) chloroplast envelope; (CER) chloroplast endoplasmic reticulum; (N) nucleus; (Cl) chrysolaminarin vesicle. The upper left showsThe upper left shows a rapidly swimming and the arrow hinting the movement direction (Adapted from [44]).

Identification of most haptophytes relies on cell size, length of the flagella and haptonema and scale structure [38]. In fact, at the species level, scale form and ornamentation consider significant taxonomic character [24] and electron microscopical examination of scale morphology is required [38]. Although scales function unknown yet, long spine scales in *H. ericina* may have a role in food capture [59, 38]. That occurs when spines catch prey and are transported to the haptonema, which is delivered at the posterior end of the cell for phagocytosis [59].

Table 2: List some *Chrysochromulina* species, including their habitats, dimensions and shape, flagella and haptonema, toxicity and phagotrophy (redrawn from [70], p. 438 - 441).

Species		*Chrysochromulina acantha*	*Chrysochromulina parva*	*Chrysochromulina cymbium*	*P. polylepis* *	*Chrysochromulina campanulifera*
Habitat		Marine	Freshwater	Marine	Marine	Marine
Shape		Saddle	-	Saddle	Ovoid	Saddle
Length	**Min.**	6	3	-	6	-
	Ave.	8	5	7	9	10
	Max.	10	7	-	12	-
Width	**Min.**	6	3	-	5	-
	Ave.	8	5	7	7	10
	Max.	10	7	-	9	-
Flagella	**Min.**	-	-	-	18	-
	Ave.	20	15	20	23	25
	Max.	-	-	-	27	-
Haptonema	**Min.**	-	-	-	9	40
	Ave.	40	75	60	11	50
	Max.	10	-	-	13	60
Toxic		No	No	-	Yes, No	-
Phagotrophy		Yes	-	Yes	Yes	Yes

* Previously *C. polylepis* [19].

2.3 Nutrition:
2.3.1 Nutrition in algae:

Depending on Lee division [63], the nutrition in algae is either autotrophic or heterotrophic. As it is known, autotroph is defined as any organism that resorts to inorganic compounds as a carbon source. Autotrophic algae can be either photoautotrophic or chemoautotrophic, and the difference between them depends on the energy source. For example, some algae (especially flagellates) are auxotrophic and require an organic compound (usually vitamin) in small amounts, but not energy sources. Furthermore, some photosynthetic algae are mixotrophic and can use organic compounds in the medium. This group of algae is also known as facultatively heterotrophic.

In contrast, if the algae are heterotrophic, they will utilize organic compounds as a carbon source for growth. Moreover, heterotrophic algae also can be either photoheterotrophic or chemoheterotrophic. Both photoheterotrophs and photoautotrophs are use light as an energy source, while chemoheterotrophs and chemoautotrophs resort to oxidizing inorganic compounds for energy.

It should be noted that heterotrophic algae may take one of two paths to absorbing their food. One of these paths is phagocytotic, where food particles are absorbed into a food vesicle to digest. The other path is osmotrophic, where nutrients are absorbed in a soluble form through the plasma membrane. And like other organisms, algae can be saprophytic when they live heterotrophically on dead material. And considered as parasites when they live depending on a living host.

2.3.2 Nutrition in *Chrysochromulina*:

As mentioned earlier, *Prymnesiophyceae* are basically photosynthetic eukaryotes with the ability to be mixotrophic. In fact, Fixen et al. [29] mentioned that this feature could be the reason for the dominance of these planktonic on the ecosystem they founded in it.

Chrysochromulina species, in addition to being photosynthetic organisms, are also phagotrophic consuming particulate matter, bacteria, other small algae and protists [27]. Phagotrophy has been observed in *Chrysochromulina* cultures such as *Chrysochromulina alifera*, *Chrysochromulina ephippium* and *Chrysochromulina pringsheimii* [79, 78](Table 2). Furthermore, most *Prymnesiophyceae* species are auxotrophic algae that require thiamine and vitamin B_{12} for growth [81 cited in 27, 105] and *Chrysochromulina* species are also needed selenium (Se) as a trace element for their growth [81, 104].

According to Isaksson et al. [48], phagotrophic in mixotrophy must be considered as a means of obtaining energy and/or nutrients. Thus, it is reasonable to assume that light conditions and nutrients control the phagotrophic behavior of mixotrophic phytoplankton. This is true because phagotrophy is stimulated in many haptophytes when nutrients have been limited [34]. A previous study by Skovgaard et al. [95] shows that under N- or P-limiting or nutrient-replete conditions, the ingestion in Prymnesium parvum preyed on the microalga Rhodomonas baltica was equally high. Nygaard did another study and Tobiesen [76] reported that when phosphate is limited, ingestion bacteria by *P. parvum* and *P. polylepis* have been higher than nutrient-replete conditions. In fact, under P-limited conditions, it is considered an efficient strategy when P-depleted algae grazing on P-rich bacteria (bacterivory) [48], exceptionally that algae characterized by having a lower phosphate /carbon ratio than bacteria [28] and also have less efficient at uptaking P at low concentrations [9, 6, 36]. Moreover, Dahl et al. [10] mentioned that in a low-phosphate condition, the small and high relative motile flagellates such as *Chrysochromulina* may have a competitive advantage over larger flagellates and diatoms in up taking the nutrient because of their small cell size. So, *Chrysochromulina* species may utilize phosphate from dissolved organic nutrients [27] or surrounded particles (picoplankton or bacteria) [76].

On the other hand, in some haptophytes, ingestion can also be stimulated by low light conditions (inversely proportional) [54 cited in 34]. But, Pintner [81] pointed out that although prymnesiophytes are capable of osmotrophy on dissolved organic carbon at high and low light levels, they cannot use these substances or even they cannot use them these substances or even survive and grow in complete darkness. In contrast, P. polylepis ingestion on fluorescent-labeled bacteria (FLB) under high light conditions is higher with adding humic substances. These differences can be explained by large amounts of bacteria that have been found. In fact, this indicates that in abundant bacterial prey, *P. polylepis* can ingest it separately from the light factor. Hansen and Hjorth [42] reported that the importance of phagotrophy in *Chrysochromulina* species for growth and survival under a nutrient limitation is still obscure and would be interesting to study in the future, especially that *Chrysochromulina* sp. can feed on bacteria and small protists.

Finally, it is important to mention that phagocytosis in *Chrysochromulina* begins when food particles adhere to the haptonema (sometimes to body scales [42]) and are moved to a specific site on the haptonema called the particle aggregating center (about 2 µm distal from the base). Once the collected food particles reach a certain size, it is moved to the tip of the haptonema. Then, the haptonema warps (bends) around the cell to deliver the particles to the posterior end of the cell so they can be injected into a food vesicle [60] (fig.4). When the haptonema begins, the flagella stop moving and beat again when the aggregate is detached from the haptonema [59].

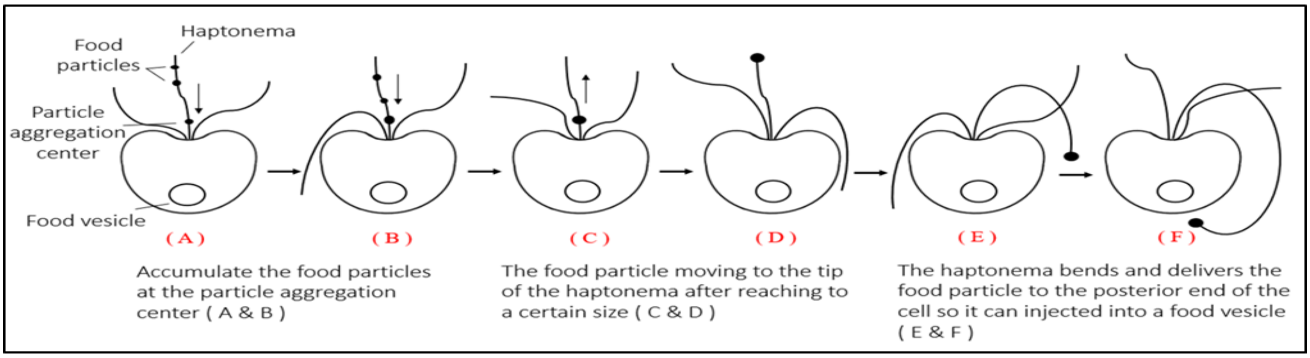

Figure 4: Phagocytosis process in *Chrysochromulina* (redrawn from [63] and [24]).

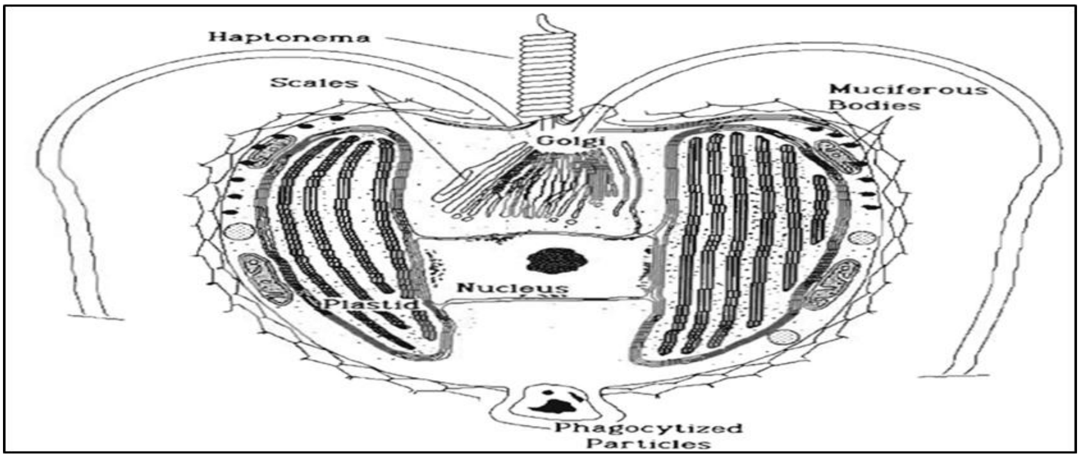

Figure 5: A typical *Chrysochromulina* cell, showing a phagocytized particles (adapted from [27])

Table 3: Phagotrophy in some flagellated microalgae (redrawn from [76], [98] and [7])

Microalgae species	Bacteria that ingested	References
Alexandrium tamarense	Eubacteria (radio-labeled bacteria "RLB") Cyanobacteria (*Synechococcus* sp.)	[76, 49]
C. leadbeateri	Eubacteria (fluorescently-labeled bacteria "FLB")	[53]
Chattonella ovata	Eubacteria	[91]
Cryptomonas sp.	FLB	[76]
Gymnodinium catenatum	Cyanobacteria (*Synechococcus* sp.)	[49]
Heterocapsa triquetra	Cyanobacteria (*Synechococcus* sp.), eubacteria	[49, 91]
Heterosigma akashiwo	RLB	[76]
Karlodinium veneficum	Eubacteria	[76]
Prorocentrum triestinum	Eubacteria	[91]
P. polylepis	Eubacteria (RLB)	[76]

2.4 Bloom:

Algal blooms may be detrimental to the functioning of the ecosystem per se, for example, death of fish and aquatic animals as a consequence of oxygen depletion because of these blooms. As well, the mere physical presence of such densities of phytoplankton may cause damage to other organisms such as fish gills and diatoms [46]. Blooms of toxin-producing species are considered a real threat to the ecosystem because of the damage that causes and can have profound effects on food chain, including human poisonings. Both nuisance and toxic blooms are known as harmful algal blooms (HABs). Microalgae that produce toxins can be found in many algal classes such as: the *Dinophyceae* (dinoflagellates), *Prymnesiophyceae* (prymnesiophytes) and *Raphidophyceae* (raphidophytes) [13]. As regards *Prymnesiophyceae*, the harmful members are found in the genera *Chrysochromulina*, *Prymnesium* and *Phaeocystis* [20].

Chrysochromulina species are ubiquitous in marine waters and makes up a large percentage of the nanoplankton biomass [27, 42], usually in low concentration (10^3 to 10^5 cells·L^{-1}) [22]. Moreover, blooms of *Chrysochromulina* spp. can occur in or around the pycnocline, where light may be a limiting factor [55, 43 cited in 42]. The first recorded *Chrysochromulina* bloom was in 1974 and under the ice in Baltic sea, when a brownish color appeared and the cryoplankton alga *C. birgeri* was the causative [39], but no harmful effects were reported. This genus was not thought to present any kind of menace to the marine environment until the massive 1988 bloom occurred [22].

In 1988, a huge, noxious and monospecific bloom of *P. polylepis* (which was previously known as *C. polylepis*) swept over the Skagerrak and Kattegat straits (an area of about 75,000 km^2) and lasted four weeks. Unfortunately, this bloom caused exceptional mortality of the natural biota in the environment such as fish, zooplankton, phytoplankton, macroalgae, invertebrates and bacteria (which were absent in the water layer that containing the toxic *P. polylepis* cells) [70]. Maximum population densities were very high, ranging from 50 to 100 million cells·L^{-1} [22]. This toxic species *P. polylepis* appears to have a world-wide distribution, blooms of this species were observed in 1988, 1989, 1994 and 1995, all in the Skagerrak or Kattegat [20]. The reasons for the great intensity and toxicity of this bloom are still not understood [64].

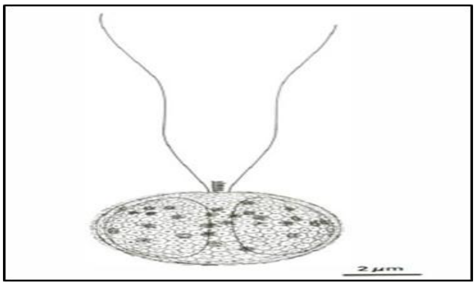

Figure 6: Schematic drawing of *C. leadbeateri* based on light and electron microscopic observations of the Vestfjorden isolate (adapted from [25]).

Once again, in 1991, another massive bloom occurred in the Lofoten archipelago in northern Norway and lasted five weeks. This time *C. leadbeateri* (fig.6) was behind the phenomenon of killing 600 tons of cultured pen-raised Atlantic salmon. In fact, although the other natural biota was unaffected by the bloom, but there was localized high mortality of sea-urchins [50]. The bloom concentration reached 3×10^6 cells·L^{-1} [22]. Stoecker et al. [98] mentioned that the factors that regulated and prolonged this bloom and its toxicity are unknown. However, there are other species of *Chrysochromulina* that have also formed blooms. Table 4 shows some recorded blooms that occurred worldwide and *Chrysochromulina* was the causative of them and associated results. It should be noted that the concentrations of all records blooms were $>10^6$ cells·L^{-1} [22].

Table 4: Recorded events of *Chrysochromulina* blooms

Year	The bloom site	Species	Effect	Reference
1974	Under ice in the Baltic Sea	*C. birgeri*	A brown color in the ice and the water beneath the ice in the Baltic Sea.	[39]
1978 – 1980	5 lakes in Ontario and New Hampshire lakes, North America	*Chrysochromulina breviturrita*	Obnoxious odors	[74]
1988	Along the Danish-Swedish-Norwegian coast	*P. polylepis*	Kills varies organisms and farmed fish	[11, 33]
1991	Vestfjorden sea in Lofoten archipelago, Northern Norway	*C. leadbeateri*	Kills about 600 tons of pen-raised Atlantic salmon	[3]
1991	Small lake in Denmark	*C. parva*	Fish kills	[41]
1991 – 1992	In Swedish coastal lake	*C. parva* and *P. parvum*	Fish kills	[45, 1993, 22]
1996	Nova Scotia in Canada	*C. birgeri*	Fish kills	[22]
2016	Al-Nawras lagoon in Jeddah	*Chrysochromulina* sp.	Thousands of sardine fish were dead	-
2016	Al-Arbaeen lagoon in Jeddah	*Chrysochromulina* sp.	Kills varies organisms	-

2.5 Toxin:

In general, there are two basic ways for algae to be harmful: (1) by producing huge populations in the water body, (2) by producing toxins [38]. The production of large growths of some algae (such as *Chrysochromulina*) can clog fish' gills, leading to their killing [63].

Members of Haptophyta have been associated with allelopathic activities or other toxic or fish kills [22, 20]. A previous study done by Schmidt and Hansen [90] shows that releasing toxins into the water may explain the monospecific *P. polylepis* bloom in 1988 that occurred in Scandinavian waters at high cell concentrations. In fact, *P. polylepis* toxin were examined by Yasumoto et al. [106] and found two ichthyotoxic and hemolytic compounds, but have not been fully elucidated. However, there are common factors that increase cell toxin in *C. leadbeateri* and *P. polylepis* which are: salinity, P-limitation, N or P-limitation, high pH [32, 51].

According to Johansson and Granéli [51], the availability of inorganic nutrients (N and P) to several phytoplankton species that may be toxic is an important factor for the regulation of their growth and toxicity. The N:P ratios in many coastal areas (where most toxic blooms occur) have changed considerably due to human activities that cause a high, erratic input of N and P. Anyway, the potential role of *Chrysochromulina* toxins in phagotrophy is unknown [98].

It should be noted that the toxicity of *Chrysochromulina* varies between species and within the same species (Table 2) [10]. Actually, several *Chrysochromulina* species and strains were tested under P-limiting conditions cultures and found that they are not toxic [17]. For example, when *C. leadbeateri* bloomed in 1991 and caused the death of tons of caged fish deaths, the alga was shown to be quite toxic in the field. But the cultures of *C. leadbeateri* proved nontoxic to the brine shrimp *Artemia salina* [38]. However, previous studies [e.g., 21, 94, 52] have used the brine shrimp Artemia assay to investigate *Chrysochromulina* toxicity. In fact, the test is used extensively in applied aquatic toxicology (for more information see "*Artemia* in aquatic toxicology : a review " by Persoone and Wells [80]).

2.5.1 Allelopathy:

According to Dakshini [12], algal allelopathy defined as an ecological/physiological manifold phenomenon. Chemicals secreted by the alga can affect its own growth (i.e., autotoxicity), other algae or higher plants in its vicinity, microbes associated with it.

In the monospecific bloom of *P. polylepis* in 1988, the abundance of zooplankton was very low [75]. As mentioned earlier, *P. polylepis* inhibited the activity of copepods, planktonic bacteria and heterotrophic protists [70]. First indications of allelopathy in *P. polylepis* provided by Myklestad et al. [71] (fig.7). Adding an abundant amount of *P. polylepis* suspensions to cultures contains the diatom *Skeletonema costatum* that inhibited this diatom. A similar effect was achieved using filtrates from abundant *P. polylepis* cultures, show that toxic substances that the algae released to the medium was the cause. However, a previous study done by Schmidt and Hansen [90] demonstrated that the toxicity of *P. polylepis* on other algae was affected by factors such as pH and maybe CO_2 limitation.

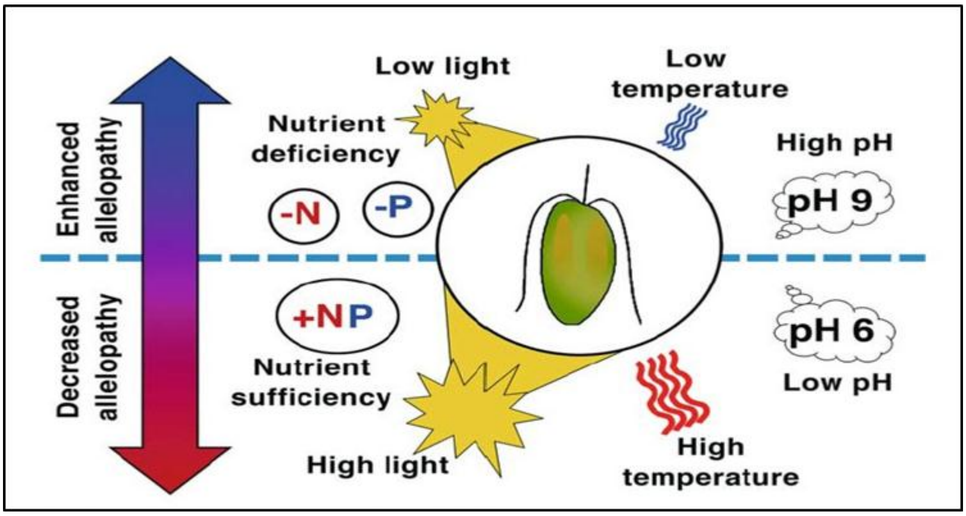

Figure 7: Abiotic factors that affect allelopathy in harmful algae (adapted from [34]).

3. Bioremediation

Concerning 1988 bloom, Simon et al. [93] pointed out that to explain the formation of monospecific blooms, hypotheses involving extraordinary climatic conditions, competition considerations and harmful effects on predators have been proposed. In opposed, Fogg [30] excludes the increase in nitrogen, phosphorus and trace element supplies or unusual climatic conditions to be likely causes of the bloom in Scandinavian waters in 1988. Instead, Fogg [30] stated that "it seems that the organism must have taken advantage of a lull in grazing". Moreover, the algal bloom may be ended or slowed down by the action of algicidal viruses or bacteria.

Nowadays, pathogenic bacteria's resistance to drugs has become a critical global threat [26]. Moreover, the cost of the drugs is high and also they may cause an adverse effect on the host [47 cited in 77]. Thus, finding an effective alternative solution is essential. The antibacterial activities related to marine algae against several pathogens have received special attention [92 cited in 77]. In fact, there have been several reports of bacteria being inhibited by algae [e.g., 14, 37, 16, all cited in 15]. Moreover, in aquaculture systems and for controlling pathogenic bacteria like *Vibrio harveyi*, several algal species were used. The algae have disrupted the quorum sensing communication between these pathogenic bacteria [72 cited in 83].

Back to the allelopathic activity of algae, Rice [88], Mautner et al. [67] found that the red alga *Rhodomela larix* produce brominated phenols, which cause allelopathic interference to several gram-positive and negative bacteria. At the same time, the chrysophyte *Ochromonas malhamensis* inhibited the growth of *Bacillus megaterium*, *Bacillus subtilis* and *Staphylococcus aureus* [40].

A previous study done by Keller et al. [61], show that *Ochromonas* sp. appear to discriminate between and preferentially ingest different types of bacteria. The alga preferred the rod form of bacteria over the coccoid form and vibrio-type. However, it should be noted that there are no studies that have taken advantage of the phagocytosis in *Chrysochromulina* and applied on pathogenic bacteria.

On the other hand, harmful algal blooms (HABs) have increasingly become a public and global environmental problem [31] and seriously undermine the sustainable development of coastal areas. Therefore, it is important to find solutions for HAB [83]. There are bacterial strains capable of inhibiting or lysing HAB species by producing algicidal substances [5]. Some bacteria genera such as *Bacillus*, *Flavobacterium*, *Alteromonas*, *Vibrio*, *Planomicrobium*, *Pseudoalteromonas*, *Pseudomonas* and *Zobellia* isolated from marine environments have algicidal effect [68 cited in 5]. The affection of these algicidal bacteria on algal bloom dynamics may sometimes occur when bacteria increase in abundance concurrently with the decreasing algal blooms [68]. Kouzuma and Watanabe [62] recorded that different taxa of bacteria produce algicidal metabolites. In the previous study, Tan et al. [100] showed that algicidal bacteria have strong activity against *Phaeocystis globosa* (Haptophyta), which causes HAB.

CONCLUSIONS

Since *Chrysochromulina* species can feed on bacteria (phagotrophic), taking advantage of this feature is essential, especially since its application to pathogenic bacteria has not been adequately studied.

VALUE CONFLICTS

Conflicts of interest do not occur.

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