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Ecological and biochemical aspects in algal infectious diseases

Kamal BOUARAB[1], Bernard KLOAREG[1], Philippe POTIN[1*] and Juan A. CORREA[2]
[1] *UMR 1931 CNRS-Goëmar, Station Biologique CNRS-INSU-Université Paris 6, Place Georges-Teissier, BP 74, F29682 Roscoff Cedex, France.*
** Fax : 33 2 98 29 23 24 - E-mail: potin@sb-roscoff.fr*
[2] *Departamento de Ecología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Casilla 114-D, Santiago, Chile.*
E-mail: jcorrea@genes.bio.puc.cl

Abstract: Infectious diseases in algae are caused by a wide variety of organisms, from virus to other algae. In recent years, important advances in understanding some of these diseases from ecological, cellular and biochemical view points have been done. In an ecological context, epidemiological studies are very limited and restricted to red algal hosts. Those studies show that infections appear to affect large segments of host populations, and the expression of some diseases is clearly aggregated, with consistent patterns of seasonal fluctuation. This, together with host specificity trials and transplant experiments, strongly suggests a genetic base as determinant of host susceptibility and disease expression.

In a biochemical context, the highly specific association established between the sporophytic phase of the red algal host *Chondrus crispus* and its green algal endophytic pathogen *Acrochaete operculata* provides an excellent model to investigate the biochemical basis of recognition and signal transduction which determines host susceptibility, specificity and pathogenicity. Our results emphasize the role of oligosaccharide signals in modulating resistance against pathogens in marine algae. These results are discussed in the light of recent reports of an oligoalginate-induced oxidative burst in the brown algal kelp *Laminaria digitata* and of oligoagarose-induced responses in the association between *Gracilaria conferta* and a pathogenic marine bacterium.

Résumé: Les maladies infectieuses des algues sont causées par une grande variété d'organismes, allant des virus jusqu'à d'autres algues. Récemment, des avancées importantes ont été réalisées dans la compréhension de ces maladies, d'un point de vue écologique, cellulaire et biochimique. Dans un contexte écologique, les études épidémiologiques sont très limitées et concernent les algues rouges hôtes. Ces études montrent que les infections peuvent affecter une proportion importante des populations hôtes, et que l'expression de certaines maladies est clairement agrégée avec des modes de fluctuation saisonnière constants. Ces données ainsi que les tests de spécificité d'hôte et les expériences de transplantation suggèrent fortement une base génétique comme déterminant de la susceptibilité de l'hôte et de l'expression de la maladie.

D'un point de vue biochimique, l'association très spécifique établie entre le sporophyte de l'algue rouge *Chondrus crispus* et son endophyte pathogène, l'algue verte *Acrochaete operculata* fournit un excellent modèle pour étudier les bases biochimiques de la reconnaissance et de la transduction du signal qui détermine la susceptibilité d'hôte. Nos résultats mettent en évidence l'importance de signaux oligosaccharidiques dans la modulation de la résistance contre les pathogènes chez les algues marines. Ces résultats sont discutés en s'appuyant sur des résultats récents obtenus dans la mise en évidence d'un burst oxydatif déclenché par les oligoalginates chez l'algue brune *Laminaria digitata* et par des oligoagaroses dans l'association entre *Gracilaria conferta* et des bactéries marines pathogènes.

Keywords: seaweeds, diseases, ecology, oligosaccharide signalling.

Introduction

Marine macroalgae can be infected by a variety of pathogens, including viruses, bacteria, fungi and other algae (Andrews, 1976; Goff & Glasgow, 1980; Correa, 1997). In general terms, the effect of pathogens on their hosts may produce deformations, or necrosis and tissue destruction. In the first case, changes to the normal morphology of the frond, stipe, or both, are induced by the vegetative growth of the invasive organism, as in the case on many fungal (see Kohlmeyer & Kohlmeyer, 1979; Apt, 1988a), bacterial (Tsekos, 1982; Apt & Gibor, 1989) and algal (Apt, 1988b) infections. Deformations may also be the result of cell hypertrophy and hyperplasia of the host tissues located in the vicinity of the pathogen, symptoms which have been reported in algae infected by heterotrophic bacteria and fungi (reviewed by Apt & Gibor, 1989) and cyanobacteria (Correa et al., 1993). In the second case, destruction of the host cells and tissues is usually triggered directly by lytic enzymes released by the pathogens. This situation is well known from several bacterial diseases, including the white rot of *Nereocystis luetkeana* caused by *Acinetobacter* sp. (Andrews, 1977), the summer rot of *Laminaria* sporophytes caused by *Pseudomonas* sp. (Chen et al., 1979; Wu et al., 1983) and the green spot of *Porphyra* associated with the presence of *Pseudomonas* sp. and *Vibrio* sp. (Nakao et al., 1972), among others (see Correa 1997). Degradative diseases with massive destruction of host tissues are also known in fungal infections, such as the red rot of farmed and wild species of *Porphyra* caused by several species of *Pythium* (reviewed by Fujita, 1990). One of the most important fungal diseases resulting in tissue degradation was reported to affect the farming operation of *Chondrus crispus* in southern Nova Scotia, Canada (Molina, 1986; Craigie & Shacklock, 1989). Even though the effect of the pathogen *Petersenia pollagaster* is localized at the tip of the fronds, destruction of these areas affects the meristems and therefore, becomes a major obstacle to achieve adequate growth of the cultivated fronds.

The issue of infectious diseases in marine macroalgae has been reviewed several times since the first and broad paper written by Andrews (1976). The early general reviews (Andrews, 1976; Goff, 1983; Apt & Gibor, 1989) coincided with the fact that most of the information available at the time of the revisions dealt mainly with the description of pathogens and their effects on the cells and tissues of their hosts. Subsequently, Correa (1997) summarized once again the topic, although this time the recent advances resulting from the studies of the rhodophycean host *Chondrus crispus* and its green algal endophytes were available. This information revealed the complexity of the process of host invasion and subsequent colonization of healthy tissues. It also strongly suggested, for the first time in an algal pathosystem, that some host cell wall components, likely polysaccharides or their oligomeric derivatives, were actively involved in determining the degree of host specificity and the outcome of the infection (Correa & McLachlan, 1991). Thus, the need of much more research became apparent, to fully unveil the intimacy of the cellular and biochemical mechanisms regulating the interactions between infecting organisms and their algal hosts. This need was considered critical to predict the outcome of infectious diseases which were emerging, as the world-wide interest in seaweed derivatives and farming was increasing steadily.

The economic importance of understanding the mechanisms of pathogenicity, to improve or prevent the potential effects of pathogens on farmed stocks, is undisputed. In fact, as it will be discussed below, our current knowledge of the role of oligosaccharides as signals involved in the host-pathogen biochemical communication goes beyond the field of phycology, with potential applications in other plant pathosystems. In his review, Correa (1997) also individualized the ecology of infectious diseases as an even wider area where understanding of the host-pathogen interactions was exceedingly poor. In this context, aspects such as epidemiology and knowledge of the factors which determine the expression of a disease in wild stocks of algal hosts were suggested as prime subjects for research. The main gain for approaching the issue of infectious diseases from an ecological perspective is double. It would allow to assess the role of pathogens as direct modulators of a host's population dynamics, and, therefore, indirectly determine the potential capacity of diseases as yet another factor with an influence in shaping the community structure in marine natural systems.

Based on the above, the present review will concentrate on the areas outlined by Correa (1997). Therefore, it will cover the current efforts to understand the role of pathogens in wild algal populations as well as the elaborated sequence of signalling required for a pathogen to effectively colonize an algal host.

The ecology of infectious diseases

As indicated above, lack of information on most of the diseases that affect wild stands of algae still prevents from making broad generalizations. However, a theoretical view based on the understanding of plant-pathogen interactions in terrestrial pathosystems can be used as a starting point to visualize the possible role of pathogens in shaping populations and communities. This approach, included in the latest review on algal diseases (Correa, 1997), highlights the fact that for the few well-studied cases, wild populations of algae appear to tolerate higher rates of infection (i.e. Correa et al., 1987; 1993, 1994; Correa & Sánchez, 1996;

Littler & Littler, 1995) than plants in terrestrial systems (Bourdon & Leather, 1990). In his review, Correa (1997) concluded that it seems likely that these high infection rates without an apparent effect on the host populations may result from the occurrence of different regulatory mechanisms to those found in terrestrial plants.

Recent information, however, indicates that the latter conclusion should be accepted with reservations, based on the observations that endophytic infections of the rhodophycean host *Mazzaella laminarioides* significantly reduced germination of carpospores (Faugeron et al., 2000). This effect on one of the fitness components in the host is similar to what has been reported in other pathosystems (reviewed by Clay & Kover, 1996) and current ongoing research in wild stands of *Mazzaella* will likely find additional similarities between diseases affecting terrestrial and aquatic plants. One of these potential similarities relates to the factors determining the expression of a disease.

Environmental factors are certainly important in either enhancing or diminishing the effect of the pathogen on a host, and this seems to be the case in *Chondrus crispus* which is much more affected by its endophytes in protected bays than in wave-exposed beaches (Correa et al., 1987). Similar is the case of *Mazzaella laminarioides*, where the micro-environmental conditions, in at least two of the studied populations, appear to determine the degree at which the deformative disease affects the host (Buschmann et al., 1996). However, the work by our group (Buschmann et al., 1996) strongly suggested that a patchy spatial distribution of the deformative disease could reflect a genetically structured host population. In this situation, the expression of the disease could be due to the spatially patchy distribution of susceptible hosts, a phenomenon reported for some terrestrial pathosystems (Burdon et al., 1990; Mulvey et al., 1991; Michalakis et al., 1993; Sorci et al., 1997). This hypothesis is currently being tested in the laboratory and in the field.

Although the effect of the infectious diseases can be subtle, there are conditions where they can produce profound impacts on their hosts in a very short period of time. From this perspective, perhaps one of the most impressive examples of major effects of an infectious disease on algal assemblages is the case of the large scale mortality of coralline algae in the South Pacific reefs caused by the Coralline Lethal Orange Disease (CLOD) (Littler & Littler, 1995). The high mortalities were found associated with the presence of a bacterium, although causality has not been demonstrated so far. The disease expanded throughout a geographic range of 6000 Km in a year, indicating that the pathogen was in an early stage of virulence and dispersal. Unfortunately this first and only epidemic reported to affect an alga in the wild, has not been followed up.

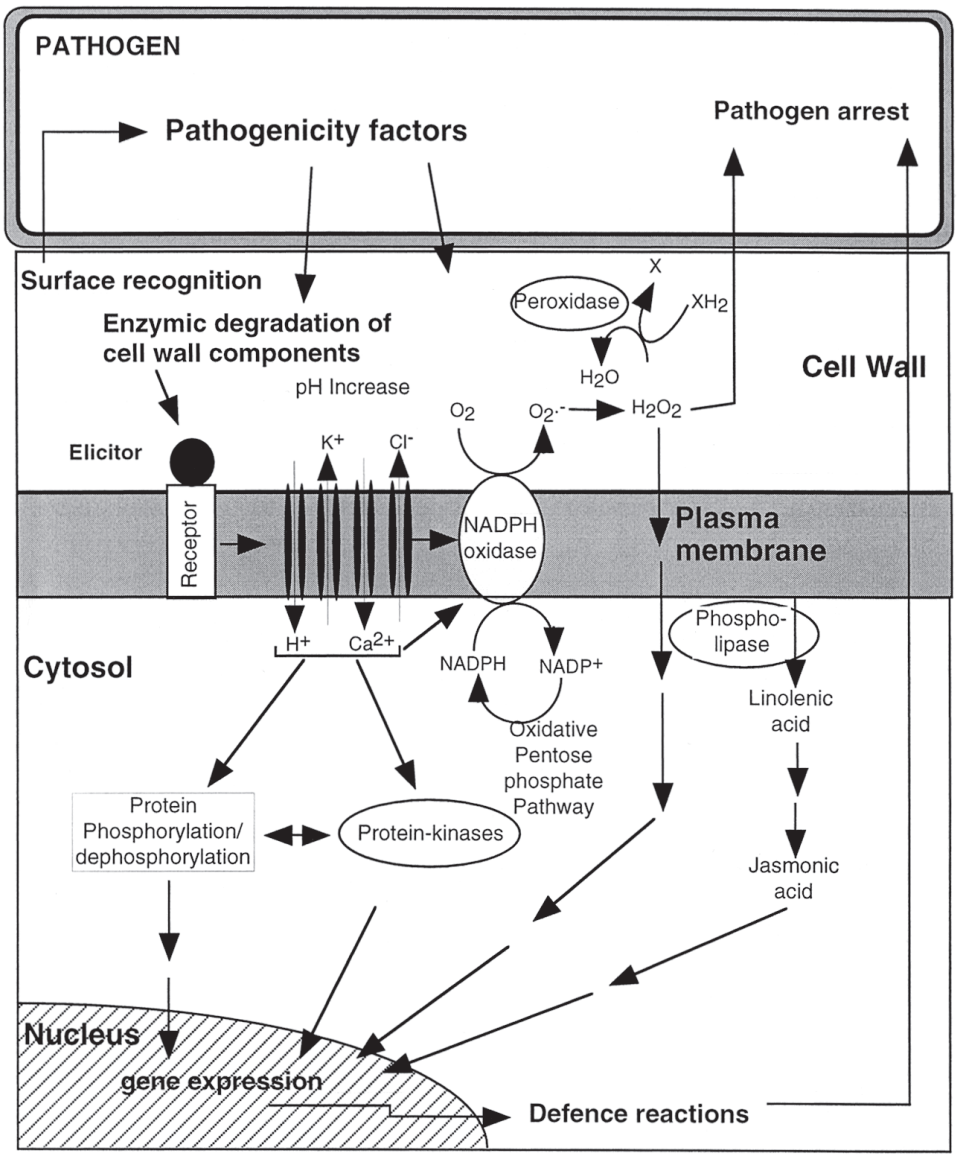
Biochemical aspects of signalling in infectious diseases

As mentioned above, outbreaks of infectious diseases have been rarely observed in natural seaweed populations (e.g. Littler & Littler, 1995; 1997; Küpper & Müller, 1999), indicating that marine plants have developed effective mechanisms to resist attacks by their natural pests, whether bacteria, fungi or other algae, as effective as the defence strategies elaborated by terrestrial plants. However, in striking contrast to our knowledge on the pathologies and defence mechanisms of many species of Spermatophytes, the biochemical basis of microbial diseases and defence mechanisms have been hardly investigated in marine algae.

Can we extend the current concepts of host-pathogen interactions in terrestrial environment, to marine macroalgae?

Plant pathologists distinguish two types of resistance responses to potential pathogens: the non-host resistance is frequent and the race/cultivar specific host resistance is comparatively rare. Race/cultivar specific resistance is the base in breeding for disease resistance in crops. This interaction is genetically defined by the direct or indirect interaction between the product of a dominant plant resistance gene (*R*) and the complementary product of the corresponding dominant pathogen avirulence (*avr*) gene (Flor, 1971). This process is dependent on the genotypes of the interacting partners. In contrast, basic incompatibility is exhibited by all plant species that respond to potential pathogen without apparent *R*/*avr* gene combinations. Apart from this distinction, however, the biochemical processes occurring in host and non-host resistance are very similar. As an example, localized 'hypersensitive' cell death may occur in both processes (Somssich & Hahlbrock, 1998).

Land plants have developed specific mechanisms to resist the attacks by pathogens (Fig. 1). Long-term, gene-regulated defence responses include the synthesis of pathogenesis-related proteins, production of protease or pectinase inhibitors, stimulation of secondary-metabolite pathways, cell wall cross-linking and lignification, and the hypersensitivity response, i.e. limited necrosis at the site of infection (Fritig et al., 1998; Somssich & Hahlbrock, 1998; Scheel, 1998). Such defence reactions are initiated upon recognition by the host of extracellular signals known as elicitors (Ebel & Mithöfer, 1998). Many elicitors of non-host resistance in plants consist of oligosaccharins, e.g. cell wall polysaccharide fragments with signalling activities (Côté et al., 1998). A number of components of the signalling pathways that network recognition of elicitors to the host defence responses are now identified in land plants (Somssich & Hahlbrock, 1998; Scheel, 1998). One typical



rapid response is the production of active oxygen species (AOS), such as superoxide ions, hydrogen peroxide, or hydroxyl radicals. This so-called oxidative burst is triggered whithin minutes after pathogen recognition, via the activation of plasma membrane-associated NAD(P)H oxidases and/or apoplastic-localized peroxidases. It involves a series of signal transduction events such as calcium efflux and other ion fluxes, plasma membrane depolarization and protein phosphorylation. The oxidative burst is also linked with the activation of phospholipases, leading to the production of lipid hydroperoxides also known as oxylipins or lipoxins. Reactive oxygen species are intrinsically toxic, serve as second messengers and, as peroxidation substrates, they participate in cell wall cross-linking.

Several recent investigations indicate that many of these concepts can also be used to study defence mechanisms in the distant lineages found in the sea (see Boyen et al., this volume). A few model species of red, brown and green algae have provided new insights in our understanding of cell-cell recognition mechanisms in marine plant-microbe interactions.

Host-pathogen recognition in marine algae involves cell wall oligosaccharides

Most of the recently published data point at the involvement of oligosaccharides as elicitors of defence responses in marine macroalgae. Marine multicellular algae contain large amounts of extracellular matrix polymers, with unique structural features (Kloareg & Quatrano, 1988). They consist of a variety of carboxylated and/or sulfated, linear or

Figure 1. Major events of the signal transduction chain from pathogen recognition to defence gene activation in higher plant cells. Cell-wall fragments or other elicitors (peptides, lipids, …) from either the host or the pathogen or both, are released during the first stages of the infection. In the host, these molecules are likely to be perceived, as warning signals, by specific receptors at the plasma membrane, initiating a cascade of transduction events which activate defence gene expression. Early events are the activation of transient Ca^{2+} and H^+ influxes and K^+ and Cl^- effluxes. In several cell-culture systems, these ion fluxes are a prerequisite for the activation of specific Protein Kinases (PK) and for the massive release of AOS (the oxidative burst) involving membrane-associated NAD(P)H oxidases. Activation of PKs from the MAP (Mitogen Activated Protein) kinase family results in their translocation into the nucleus. A number of proteins are also phosphorylated or dephosphorylated upon elicitor recognition. The generation of AOS is required for the activation of several genes involved in the synthesis of defence compounds, for subsequent peroxidase mediated cross-linking of cell-wall components, and also for direct killing of pathogens, thus containing its growth and/or penetration. Elicitor recognition also activates membrane-bound phospholipases and results via the octadecanoic pathway in the synthesis of jasmonic acid and in the consecutive induction of some other responsive genes.

Figure 1. Principaux événements de la chaîne de transduction du signal de la reconnaissance du pathogène à l'activation des gènes de défense chez les cellules des plantes supérieures. Des fragments de parois ou d'autres éliciteurs (peptides, lipides…) provenant de l'hôte ou bien du pathogène ou des deux sont libérés dans les premières étapes de l'infection. Chez l'hôte, ces molécules sont probablement perçues comme des signaux d'alerte par des récepteurs spécifiques situés au niveau de la membrane plasmique. Elles initient une cascade d'événements de transduction qui active l'expression des gènes de défense. Les événements précoces sont l'activation d'influx transitoires de Ca^{2+} et de H^+ et des influx de K^+ et de Cl^-. Dans plusieurs systèmes de cultures cellulaires, ces flux ioniques sont un pré-requis pour l'activation de Protéine Kinases (PK) spécifiques et pour le relargage massif d'AOS (le burst oxydatif) impliquant des NAD(P)H oxydases associées aux membranes. L'activation des PKs de la famille des MAP (Mitogen Activated Protein) kinases aboutit à leur translocation dans le noyau. De nombreuses protéines sont aussi phosphorylées ou déphosphorylées suite à la reconnaissance de l'éliciteur. L'émission d'AOS est requise pour l'activation de plusieurs gènes impliqués dans la synthèse de composés de défense, pour le pontage oxydatif de composés pariétaux catalysé par des peroxydases, pour l'élimination directe des pathogènes, contribuant ainsi à la limitation de leur croissance et/ou de leur pénétration. La reconnaissance de l'éliciteur active aussi des phospholipases membranaires et induit la synthèse de l'acide jasmonique, via la voie des octadécanoides et l'expression consécutive de quelques autres gènes.

branched polysaccharides (Lahaye, this volume). Various polysaccharidases are now available for the specific degradation of marine algal polysaccharides (Barbeyron et al., this volume), that allow to enzymatically prepare oligosaccharide fractions of reproducible structure and to explore in detail their biological activity on various plant-, animal- and human-cell models.

The maricultured agarophyte *Gracilaria conferta* provided the first demonstration in a marine plant that oligosaccharides (namely agar fragments) are recognized as defence elicitors (Weinberger et al., 1999). The bacterial communities and several bacterial diseases associated with the intensive cultivation of this species have been documented and it may serve as a model of plant-pathogen interactions in aquacultured algae (Weinberger et al., 1994). More recently, new strains were characterized as necrosis inducers and are devoid of agarolytic activity (Weinberger et al., 1997). Small secreted molecules, such as toxins or peptides, represent good candidates of necrotizing agents and may act as exogenous elicitors involved in the molecular recognition of the pathogen by the host (Weinberger & Friedlander, 2000). More remarkably, oligoagars, cell-wall fragments that are likely released upon infection by agarolytic pathogenic bacteria, trigger an oxidative burst in *G. conferta* (Weinberger et al., 1999). A six-fold increase in respiration was observed as soon as three minutes after the addition of neoagarohexaose in micromolar concentrations and the emission of hydrogen peroxide was recorded within less than 15 minutes. Interestingly, among the agarophytes, recognition of this signal as a defence elicitor apparently is restricted to the order Gracilariales.

The brown algal kelp *Laminaria digitata* recognizes alginate fragments as potent defence signals, probably because they are generated during infections with cell wall degrading bacteria. They also are likely to be generated by the digestive enzymes of grazers. Alginate blocks prepared by either enzymatic digestion or mild acid hydrolysis induce a dramatic increase in oxygen consumption and concomitant H_2O_2 emission, as well as marked effluxes of potassium and iodine by *L. digitata*. Homo-oligomeric fragments composed of guluronate residues are active when added at µmolar concentrations or less, whereas poly-mannuronate blocks do not elicit significant effects (Küpper et al., 2001).

In contrast to Gracilariales or Laminariales, *Chondrus crispus* does not recognize its own cell-wall oligosaccharides as defence elicitors, but oligo-carrageenans instead behave as signalling molecules that modulate the virulence of its green algal endophyte *Acrochaete operculata* (Bouarab et al., 1999). This pathosystem, therefore, provides a very interesting model to address both the biochemistry and the ecological significance of oligosaccharide signals in marine plants (reviewed in Bouarab, 2000). Modulation of specificity, susceptibility and pathogenicity was investigated, based on the well known phenomenon of differential patterns of sulphate substitution present in the extracellular

carrageenans of the two isomorphic life history phases of *C. crispus* (Fig. 2). Consistent with the cell wall composition of the tetrasporophytic susceptible generation, λ-carrageenan oligosaccharides stimulated new protein synthesis and elicited the production of specific polypeptides in the pathogen, resulting in a markedly increased pathogenicity. In contrast, incubation of the pathogen in the presence of κ-carrageenan oligosaccharides enhanced its recognition by the host and reduced its virulence (Bouarab et al., 1999). Similarly, the specific recognition of the agaroid porphyran motif enhances apressorium formation in *Pythium porphyrae*, the agent of red rot disease in *Porphyra yezoensis* cultures (Uppalapati & Fujita, 2000). The defence mechanisms of *C. crispus* sporophytes and gametophytes are also different. Challenging the host with cell-free extracts of *A. operculata* triggered a substantial release of hydrogen peroxide in gametophytes, whereas sporophytes displayed weaker responses. Identification of the compounds which in *Acrochaete operculata* cell-free extracts are recognized by *Chondrus crispus* gametophytes is under way, using the oxidative burst as a bioassay (Weinberger F, Bouarab K, Kloareg B, Potin P., unpublished data).

We now, for the first time, have the possibility of experimentally mimicking attacks by pathogens in marine algae and of exploring the nature and the transduction pathways of the induced defences.

Signal transduction and active oxygen species (AOS) sources

In higher plants, changes in ion fluxes and protein phosphorylation constitute an early inducible response, occurring within minutes after elicitor application and regulating both the oxidative burst and the expression of defence genes (Yang et al., 1997). In *Chondrus crispus* gametophytes, pharmacological inhibitor studies suggest that ion fluxes, activation of protein kinases and phospholipases control AOS production, whilst pathogen recognition also likely induced convergent signal pathways downstream or independent from the oxidative burst leading to the establishment of resistance to *Acrochaete operculata* (Bouarab, 2000). In *Laminaria digitata* also, inhibitor studies suggest that the perception of oligoguluronates activates ion fluxes, protein kinases and phospholipases, which control key events in the transduction pathway leading to H_2O_2 emission (Küpper et al., 2000).

The identity of the AOS-producing machinery is still controversial in higher plants (Bestwick et al., 1999, Bolwell, 1999). It is noteworthy that in the three algal species investigated so far (*Gracilaria conferta*, *L. digitata* and *C. crispus*), the H_2O_2 emission is associated with a sudden increase in the consumption of oxygen, resistant to azide and cyanide (Weinberger, 1999, Küpper et al., 2001; Bouarab et al., unpublished results). Therefore, it is likely that the oxidative burst in seaweeds shares very close mechanisms with the original definition of the oxidative burst in mammalian systems. Furthermore, the phenomenon in these three species is completely inhibited by treatment with diphenyleneiodonium (DPI), a suicide inhibitor of the mammalian NADPH oxidase and a potent inhibitor of nitric oxide synthase (NOS) in mammalian systems (Stuehr et al., 1991). Despite we could not exclude that AOS generation in seaweeds in response to oligosaccharide elicitors may come from multiple mechanisms, the current data emphasize the involvement of NADPH oxidase-like system.

Defence reactions in marine algae

That AOS are an essential component of the defence system in the red alga *Chondrus crispus* can be indirectly inferred from the fact that, contrarily to the gametophytes, the susceptible tetrasporophytic host life-history phase exhibited a weak oxidative burst when challenged by pathogen extracts. More directly, treatment of *C. crispus* gametophytes with DPI completely abolished the emission of H_2O_2 and also made possible the invasion of the host resistant phase by *Acrochaete operculata* filaments (Bouarab et al., 1999). AOS generated during an oligosaccharide-induced oxidative burst were also recently shown to kill pathogenic microrganisms of algae. The oligoagar-induced oxidative burst in *Gracilaria conferta* resulted in the elimination of epiphytic agarolytic bacteria (Weinberger & Friedlander, 2000). Peroxide levels of ca. 50 μM proved also sufficient to inhibit the growth of the associated flora of *Laminaria digitata* as well as to kill a *L. japonica* pathogen, *Pseudoalteromonas elyakovii* (Küpper et al., 2001).

A rapid response likely to constitute a chemical defence in seaweeds is the emission of volatile halocarbons (VHCs). An increased production of such iodinated, brominated or chlorinated carbon skeletons is associated with oxidative stress from various origins, whether caused by carbon privation, high light or mechanical injuries and VHCs may therefore be seen as AOS scavenging products (Mtolera et al., 1996; Collen et al., 1995). Their biogenesis involves vanadium-haloperoxidases, which catalyse the oxidation of halides (X-) into hypohalous acids (HXO). As highly electrophilic agents, hypohalous acids can react with a variety of organic compounds and they are considered as potent natural biocides (Wever et al., 1991). Recent data indicate that incubation of *L. digitata* in the presence of oligoalginates drastically increases the apoplastic levels of both H_2O_2 (Küpper et al., 2001) and iodine (Baker et al., unpublished), concomitantly enhancing the production of such iodine-containing di- and tri-halomethanes as CH_2I_2,

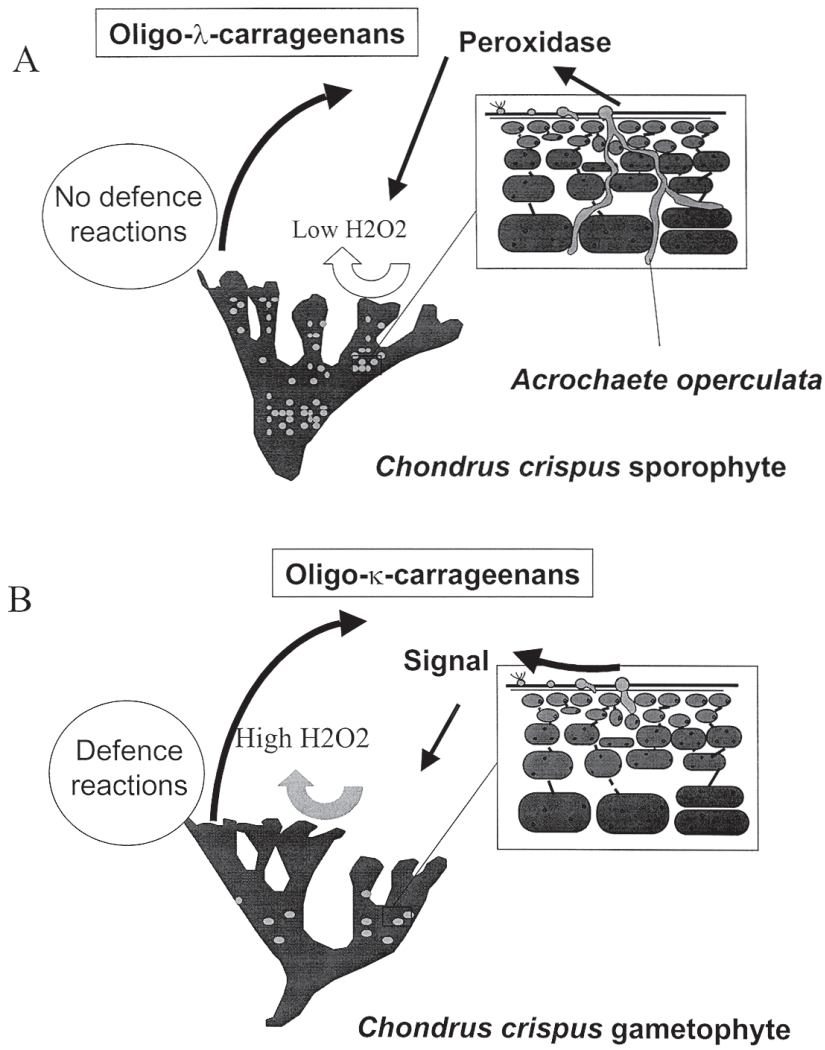


Figure 2. A synthetic model of sulphated oligosaccharide signalling in the *Chondrus crispus-Acrochaete operculata* host-pathogen association. In the red algal diploid sporophytic phase (A), oligo-λ-carrageenans released from the extracellular matrix signal the pathogen to avoid or control the host defence reactions. Oligo-λ-carrageenan signalling is amplified by the enhancement of a carragenolytic activity in the pathogen, which efficiently cleaves the carrageenans from the sporophytic phase. Fragments of λ-carrageenan enhance new protein synthesis in *A. operculata* and remarkably induce a specific peroxidase isoform, which is likely involved in the quenching of AOS emission by the host. In the haploid plants (B), κ-carrageenan fragments both inhibit new protein synthesis and virulence in the pathogen. Furthermore, AOS production by the host is enhanced, thus likely participating in oxidative cross-linking of cell wall components and preventing further invasion by the pathogen. Upon binding to a putative receptor, compounds which in *A. operculata* cell-free extracts are recognized as signals by *C. crispus* gametophytes, initiate a signal transduction cascade, leading to the establishment of resistance towards penetration of *A. operculata* filaments.

Figure 2. Modèle synthétique de la signalisation par les oligosaccharides dans l'association hôte-pathogène *Chondrus crispus-Acrochaete operculata*. Dans la phase sporophytique de l'algue rouge (A), les oligo-λ-carraghénanes libérés de la matrice extracellulaire signalent au pathogène d'éviter ou de contrôler les réactions de défense de l'hôte. La signalisation par les oligo-λ-carraghénanes est amplifiée par l'activité carraghénolytique présente chez le pathogène, qui dégrade efficacement les carraghénanes de la phase sporophytique. Les fragments de λ-carraghénane stimulent la néosynthèse de protéines chez *A. operculata* et induisent une isoforme spécifique de peroxydase, qui est probablement impliquée dans la consommation des AOS émis par l'hôte. Dans les algues haploïdes (B), les fragments de κ-carraghénane inhibent à la fois la néosynthèse de protéines et la virulence chez le pathogène. De plus, la production d'AOS par l'hôte est accrue, participant probablement au pontage oxydatif de composés pariétaux et limitant l'invasion du pathogène. Après liaison à un récepteur hypothétique, les composés provenant d'extraits acellulaires d'*A. operculata* sont reconnus comme des signaux par les gamétophytes de *C. crispus*, initiant une cascade de transduction du signal, menant à l'établissement de la résistance à la pénétration par les filaments d'*A. operculata*.

CH_2ClI, CH_2BrI, $CHBrI_2$ and CHI_3 (Malin et al., unpublished). *C. crispus* gametophytes also exhibited an increased emission of VHCs and an activation of a haloperoxidase isoform when challenged by pathogen extracts (Bouarab, 2000), while an increase in brominating activity was reported in oligoagar-elicited *G. conferta* (Weinberger et al., 1999).

In terrestrial plants, the first biochemical defence response, which was identified upon pathogen attack or elicitor treatment was the induction of a class of antimicrobial secondary metabolites, termed phytoalexins (Keen, 1975), which derive from the phenylpropanoid metabolism, via the shikimic acid pathway (Somssich & Hahlbrock, 1998). This metabolic pathway was recently shown to be a central element during defence activation in *C. crispus* (Bouarab et al., unpublished). Challenging *C. crispus* gametophytes, the generation resistant to *A. operculata*, induced accumulation of UV-fluorescent compounds at the sites of attempted penetration by the pathogen zoospores, whereas no accumulation was observed in the susceptible sporophytic phase. This response was correlated with an enhancement of shikimate dehydrogenase activity. Both the accumulation of UV-fluorescent compounds and the activation of shikimate dehydrogenase were prevented by pretreatment with glyphosate, an inhibitor of the shikimic acid pathway resulting in the suppression of the natural resistance of *C. crispus* gametophytes to *A. operculata*.

In contrast to land plants, the molecular basis of host-pathogen interactions have hardly been studied in marine algae. With the possible exceptions of the haloperoxidases from the red alga *Corallina pilulifera* (Shimonishi et al., 1998) and from the brown alga *Ascophyllum nodosum* (Weyand et al., 1999), no gene involved into defence reactions is known in marine algae. A preliminary account of the genes specifically expressed in response to bacterial infection in *Gracilaria gracilis* (Jaffray & Coyne, 1997, 1999) refers to proteins with no obvious or little relevance to defence reactions (such as nitrate reductase, HMG-CoA reductase, a starch-branching enzyme, phosphoglycerate deshydrogenase, a disease resistance protein homologue of soybean and 3-hydroxy-3-methylglutaryl-coenzyme A). Systematic investigation of gene expression in the presence or absence of oligosaccharide signals seems likely a more promising approach for identifying defence genes induced upon pathogen attack.

Conclusion

From an ecological perspective, the role of pathogens on algal populations is still poorly understood and the information available is limited or non-existent. The few well-studied cases in algae indicate that populations of these organisms tolerate higher rates of infection than their terrestrial counterparts. These high rates of infection seem to have effects on the reproductive output of some species, as it was reported for the endophytic infections of the rhodophycean host *Mazzaella laminarioides*, where carpospore germination was significantly reduced. Another aspect requiring additional research is the individualization of the factors determining the expression of a disease. Micro-environmental conditions were suggested as responsible for determining the degree of disease expression of the deformative disease in *Mazzaella laminarioides*. There are also strong suggestions indicating that patchy spatial expression of the deformative disease could reflect a genetically structured host population, similar to what has been reported for some terrestrial pathosystems.

Regarding the biochemical aspects of the algal-pathogen interactions, more and more elements of the perception mechanisms and defence pathways operating in higher plants are identified in several models of red and brown algae. Pathogen recognition by marine algae involves oligosaccharide signals and, like in terrestrial systems, is followed by an oxidative burst in the host. We have also gained much more knowledge on the biochemical basis of recognition and virulence in green algal and oomycete pathogenic endophytes, which is relevant for the general understanding of plant-pathogen interactions. In spite of these huge progress, the molecular basis of marine plant-microbe interactions remain unknown. We can not yet conclude on the genetic basis of some associations, nor generalize the use of oligosaccharides as universal recognition molecules in the sea.

In order to establish whether the key points underlined above are actually involved in chemical defence against pathogens, oligosaccharides can be a powerful tool. Elicitation with such defined signals, followed by monitoring the induced responses, may be helpful to identify the pathways through which seaweeds acquire resistance. This may be of great interest in the management of diseases of tank cultivated algal crops and in nurseries.

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