Dissection of Two Distinct Defense-Related Responses to Agar Oligosaccharides in *Gracilaria Chilensis* (Rhodophyta) and *Gracilaria Conferta* (Rhodophyta)

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Abstract

The two agar-producing red algae, *Gracilaria chilensis* C. J. Bird, McLachlan & E. C. Oliveira and *Gracilaria conferta* (Schousboe ex Montagne) Montagne, responded with hydrogen peroxide (H_2O_2) release when agar oligosaccharides were added to the medium. In *G. conferta*, a transient release was observed, followed by a refractory state of 6 h. This response was sensitive to chemical inhibitors of NADPH oxidase, protein kinases, protein phosphatases, and calcium translocation in the cell, whereas it was insensitive to inhibitors of metalloenzymes. Transmission electron microscopic observations of the H_2O_2-dependent formation of cerium peroxide from cerium chloride indicated oxygen activation at the plasma membrane of *G. conferta*. A putative system, consisting of a receptor specific to agar oligosaccharides and a plasma membrane-located NADPH oxidase, appears to be responsible for the release of H_2O_2 in *G. conferta*. Subcellular examination of *G. chilensis* showed that the H_2O_2 release was located in the cell wall. It was sensitive to inhibitors of metalloenzymes and flavoenzymes, and no refractory state was observed. The release was correlated with accumulation of an aldehyde in the algal medium, suggesting that an agar oligosaccharide oxidase is present in the apoplast of *G. chilensis*. The presence of this enzyme could also be demonstrated by polyacrylamide electrophoresis under nondenaturating conditions and proven to be variable. Cultivation of *G. chilensis* at 16 to 17°C resulted in significantly stronger expression of agar oligosaccharide oxidase than cultivation at 12°C, which indicates that the enzyme is used under conditions that generally favor microbial agar macerating activity.