Red rot of cultivated *Porphyra* ('nori') in Japan

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INTRODUCTION

Porphyra is the world's highest-valued marine crop, constituting a billion-dollar industry in Japan. *Pythium porphyrae* Takahashi et Sasaki a facultative parasite of *Porphyra* spp., is the common microbial agent responsible for red rot disease of this red alga in Japan.¹⁾ To overcome this problem there is an immediate need to develop or breed red rot resistant strains for commercial cultivation in Japan.²⁾ Understanding the host-pathogen interaction is important for breeding and developing disease-resistant strains for cultivation.

Here we present an overview of the recent research efforts made by us in understanding the host-pathogen interactions between *Porphyra* and *Pythium*.^{2,3,4,5, 6)}

Initial stages of host-pathogen interaction between *Py. porphyrae* and *P. yezoensis*: Host infection by this species is initiated by motile biflagellate zoospores. The zoospores come in contact, attached and encyst onto the host thallus. Cysts attached to the host thallus concomitant with cell wall formation, germinate, form appressoria, and penetrate the host surface near the site of cyst attachment (Figs. 1 A-D). Appressoria was observed as an essential prelude of penetration (Fig. 1C).

Carbohydrate regulation of *Py. porphyrae* zoospores on *P. yezoensis*: The zoospores infected only *Porphyra* spp. and *Bangia* thalli, although they attached to and encysted on many other red algae, although not on *Kappaphycus striatum*, the brown alga, as well as Ulvaceae members. *In vitro* and *in vivo* experiments have indicated that encystment and appressorium formation, were only induced by sulfated galactans (Fig. 1C), implicating carbohydrates in species-specific parasitism in *Pythium porphyrae*. Attachment and encystment were induced on agar and agarose films (Figs. 1 E, F), but appressoria were not induced on agarose films (Fig. 1E). Supplementation

Fig. 1A-H. Different stages in the invasion of *Py. porphyrae* zoospores on *P. yezoensis* thallus surfaces and Appressorium formation *in vitro*. **A.** Encysted zoospores. **B.** Cyst germination with formation of appressorium (arrowhead). **C.** Germinated cyst with appressorium (arrowhead) penetrating the thallus surface. Note the dead cells with orange fluorescence. **D.** Colonized thallus. Note the mycelia with blue fluorescence. **E.** Germinating cysts with no appressorium on agarose media. **F-H.** Appressoria (arrowheads) induced on agarose films supplemented with hot water soluble fraction, porphyran and cold water soluble fractions respectively. Scale bar (=150 μ m) for Figs. 1G-H.

of agarose media with both cold and hot water fractions and porphyran from *P. yezoensis* induced appressoria, implicating sulfated galactan (porphyran) in appressorium formation (Figs. 1G,H).

The relative resistance of *Porphyra* species to infection by *Py. porphyrae*: Among the germplasm screened, the cultivated and wild strains of *P. yezoensis* were highly susceptible. The non-cultivated species of *P. suborbiculata* (0.13) and *P. tenuipedalis* (0.19) were most resistant (Fig. 2). Initial steps of host-pathogen interaction observed further delineate the nature of resistance, reflecting that relatively higher numbers of zoospores attached and encysted on the susceptible strains than the partially resistant strains. Only a transient relationship between encystment rate and degree of resistance was observed. The encysted zoospores immediately germinated, formed appressoria and penetrated the algal cells near the site of encystment on the susceptible cultivar strain. While on the partially resistant *P. tenuipedalis* thallus surface, cysts with germ tubes growing on the thallus surface with out

Fig. 2. Resistance levels among *Porphyra* spp. Py: *P. yezoensis* T-14, Pyw: *Py* (wild) Pte: *P. tenera*, Pt: *P. tenuipedalis*, Pl: *P. lacerata*, Ps: *P. suborbiculata*, Pse: *P. seriata*, Pk: *P. kanyakumariensis*, Pp: *P. pseudolinearis*. Encysted zoospore scored after 30 min of exposure to 1×10^3 zoospores mL^{-1} suspension. The infected sites were counted after 24 h of incubation at 20°C . Error bars are means $\pm$ SD ($n = 45$), encystment or infection site values with different letters (above error bars) are significantly different, $p < 0.001$.

Fig. 3 A-B. A schematic representation of initial events in host-pathogen interactions on *P. yezoensis* T-14 (Fig. 3A) and *P. tenuipedalis* (Fig. 3B) thalli.

Fig. 4. Encystment rate and amount of disease produced on on parents *P. yezoensis* T-14 (Py), *P. tenuipedalis* (Pt), and partially resistant interspecific fusion product progeny: PyPt-2 and PyPt-5. Experimental conditions were similar to those explained in Fig. 2. values with different letters (above error bars) are significantly different, $p < 0.001$.

host penetration was observed. Rarely very few long germ tubes penetrating the algal cells, with delayed appressoria formation was seen on *P. tenuipedalis* thalli (Figs. 3A,B).

Transfer of red rot resistance through interspecific protoplast fusion: Protoplast fusion was attempted to transfer resistance traits from *P. tenuipedalis* in to the cultivated *P. yezoensis*. This combination is difficult

to achieve with conventional sexual hybridization. Nine primary regenerants (FPP) were recovered by interspecific protoplast fusion. Intermediate resistance between *P. yezoensis* T-14 (1.00) and *P. tenuipedalis* (0.13) was observed in two of the Py-type FPP, PyPt-2F₂ (0.25) and PyPt-5F₂ (0.23) (Fig. 4). Stable inheritance of resistance was observed through two subsequent generations. The morphological and reproductive characteristics of these regenerated foliose thalli were like *P. yezoensis*, while they carried similar resistance nature as *P. tenuipedalis*.

In conclusion, 1. Sulfated porphyrin on the host thallus surface induces signals that trigger encystment and appressorium formation by *Py. porphyrae* zoospores. Specific moieties in porphyrin may trigger these signals: 2. Screening natural populations of *Porphyra* is an important initial step towards breeding resistant strains for cultivation: 3. The approach of protoplast fusion to incorporate the red rot resistance may complement other novel methods to incorporate genetic variability in *Porphyra* species.

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