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Ecotypic Variation in Salinity Responses of *Ulva pertusa* (Chlorophyta) from the Korean Coast

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Salinity ecotypes in *Ulva pertusa* Kjellman were examined for the growth responses of the three isolates taken from different salinity regimes. All isolates showed a broad salinity tolerance, but growth patterns were correlated with the salinity regime of their original habitat. The germlings from Anin exhibited optimum growth at the native salinity of 32‰. The germlings from Yongyon which had hypersaline habitats were tolerable to high salinity, i.e. growth rates peaked at 40‰, whereas those from Samgando which had low salinities achieved maximum growth rate at 24‰. The germlings of inter-isolate cross demonstrated intermediate growth response between that of their respective parents. Our data also clearly indicated intraspecific differences among the three isolates, which was interpreted as development of different physiological ecotypes. We conclude that *U. pertusa* may consist of several ecotypes, each of which has some capacity for physiological adaptation to salinity variations.

INTRODUCTION

The littoral benthicalgae experience wide fluctuations in osmotic condition and desiccation. To cope with the abiotic extremes the micro- and macroalgae adjust their cytoplasmic concentrations of inorganic ions and certain organic osmolytes in response to salinity changes to maintain nearly constant turgor pressure (Kirst, 1990; Reed, 1990). Reed (1984) has used the term "osmoadaptation" to describe the genetic modification of the osmotic response of algae under conditions of osmotic stress, i.e., the formation of possible "osmotic strategies" in genetically based ecotypes. The particularly wide distribution of algae within a number of saline regimes may suggest that an alga might well benefit from possessing different osmotic strategies within those differing saline environments. Inter- and intraspecific differences may occur, the latter through the existence of ecotypes.

Membranous green algal mats are important contributors to coastal primary production (Shellem and Josselyn, 1982; Pregnall and Rudy, 1985) and their proliferation in eutrophic areas makes them important to nutrient cycling (Kautsky, 1982; Uno *et al.*, 1983; Fong *et al.*, 1993). *Ulva* species are common components of the marine littoral flora of Korea, and at least 6 species has been recognized in Korea (Lee and Kang, 1986). Of these, *U. pertusa* is abundant throughout the year and widely distributed in Korea.

In Japan, *Ulva pertusa* occurs on the western Pacific from the cold temperate waters of Hokkaido to the warm temperate waters of both eastern and western coasts of Kyushu. Habitats for this species include protected estuaries with a wide range of salinities and exposed coasts with stable salinities (Taniguti, 1962, 1983). Morphology of *U. pertusa* on the coast of Far Eastern Asia varies from several times branched and twisted to sheet-like and flattened plants (Lee *et al.*, 1986; Kamiya *et al.*, 1993). While the euryhaline nature of *Ulva pertusa* with respect to growth and photosynthesis is documented (Floreto *et al.*, 1993, 1994), further data on the physiological variability are still lacking. We have also confidence that this species live in a large number of saline habitats of Korean coast (e.g. estuaries with a wide range of salinities, exposed coast with

stable salinities and highly saline rock pools).

Therefore, the present study examined the effect of different salt treatments on the germlings growth. Three culture strains of *U. pertusa* from the coast of Korea were isolated, and the germlings were raised in the laboratory from zoospores and gametes. The main objective of this study was to determine the different growth responses of each isolate, which may point to the development of ecotypes within *U. pertusa*.

MATERIALS AND METHODS

Populations of *Ulva pertusa* from three locations on the coast of Korea were selected on the basis of geographic isolation and difference in salinity (Table 1). The northernmost population was located in the Anin, a stenohaline environment in which salinities were relatively constant (32‰). The salinity regime of the Yongyon population varied from 32 to 49‰ and that of the Samgando varied from 8 to 28‰.

The plants were transported to the laboratory, rinsed several times with sterile seawater to remove silt and detritus, and transferred to Pyrex Petri dishes containing sterile seawater. Stock cultures were kept in a growth chamber in Pyrex dishes containing 200 ml PES (Provasoli, 1968) at 15°C, 32‰, 35 μmol · m^{-2} s^{-1} and 12 h daily photoperiod. Isolates were grown for a minimum period of 3 - 6 months under these culture conditions before the experiments were conducted. Inter-isolate cross was performed reciprocally between the Yongyon and Samgando isolates cultured.

Qudriflagellated zoospores and biflagellated gametes were obtained from apical fragments of adult plants. The suspension of zoospores and gametes was inoculated into culture vessels (Corning, 25810-6) containing 22 mm × 22 mm cover glass and 15 ml PES medium. After 3 days, the resulting germlings (ca. 0.1 mm in length) were used (ca. 100 plants in each culture vessel) for the experiments. Three-day old germlings were introduced to the lower or higher salinities by gradually passing them through decreasing or increasing salinity gradients. Generally, germlings were held in each gradient for one day prior to any subsequent experiment.

The experiments employed salinities of 8, 16, 24, 32, 40 and 48‰ at 15°C, 60 μmol · m^{-2} · s^{-1} and 16 h daily photoperiod. Salinities were obtained by diluting seawater with distilled water or concentrating natural seawater at 60°C before the enrichment. Irradiance was provided by ceiling light in set of three 40W cool-white fluorescent lamps (Westinghouse). Temperature was controlled within ±0.5°C accuracy at 15°C.

The length of germlings under was measured every 48 h for 14 days experimental periods with three replicates for each isolate and treatment, using a light microscope and an eyepiece graticule. Throughout these incubation periods at optimum salinities, steady exponential growth was observed. The length measurement of germlings excluded rhizoid. The culture medium was replenished at intervals of 2 - 4 days to minimize the change in salinity and pH, and to prevent depletion of the nutrients. Specific growth rate (μ) was calculated for each plant during the exponential growth phase using the equation:

$$\mu = \ln (L_2/L_1) \times T^{-1}$$

where T is the time in days, L_1 is the initial length,

Table 1. Collection locations, dates, habitats and salinity conditions for the three populations of *Ulva pertusa*

Location and date	Date	Habitat	Salinity(‰) regime
Anin, Kangreung (37° 42' N: 129° 02' E)	15 Feb. 1989	lower eulittoral (rocky shore)	32
Yongyon, Ulsan (35° 28' N: 129° 23' E)	10 Feb. 1989	fringe rock pool (rocky shore)	32-49
Samgando, Kwangyang (34° 50' N: 129° 39' E)	19 Feb. 1989	upper eulittoral (gravelly drainage)	8-28

and L_2 is the length on day T.

RESULTS

The specific growth rates of gametophytic and parthenogenetic germlings from the isolates and inter-isolate cross grown at the various salinities are shown in Fig. 1. All isolates of *U. pertusa* grew in the range of salinities tested, i.e. between 8 and 48‰, but with quite different rates. The growth rates increased generally as salinity level increases, reaching the maximum at 24, 32 or 40‰, then decreased to the highest salinity tested (48‰). Under the lowest salinity (8‰), no difference between isolates was occurred. However, highly differences existed between the isolates, particularly with respect to responses under the highest salinities tested. On the other hand, the growth patterns of all isolates were similar between gametophytic and parthenogenetic germlings.

Isolates from the Anin exhibited optimum growth in native salinity condition (32‰) and good growth over a broad range from 16 to 40‰. They showed poor growth under the lowest salinity tested (8‰). The maximal growth rate of gametophytic and parthenogenetic germlings was 0.31 and 0.29 d^{-1}, respectively (Fig. 1A).

The Yongyon isolates originating from hypersaline habitat grew optimally at 40‰, and salinity of 8‰ was lethal. An increase in salinity was accompanied by a linear increase of growth rate, i.e. the rates increased abruptly from 0.01 d^{-1} at 8‰ up to 0.26 (gametophyte) and 0.24 d^{-1} (parthenogenesis) at 40‰. Under the highest salinity tested, however, the growth rate slightly decreased (Fig. 1B).

The plants from the Samgando had a sharply pronounced maximum at 24‰ within the range of the original habitat, but decreased steeply in growth rate beyond the level. They showed a slower growth rate under the lowest and highest salinities tested. With increasing salinities up to 24‰ the specific growth rate increased abruptly from 0.05 (gametophyte) and 0.06 d^{-1} (parthenogenesis) at 8‰ up to 0.30 (gametophyte) and 0.29 d^{-1} (parthenogenesis) at 24‰. A further increase in salinity was accompanied by a strong inhibition in growth: 0.23 (gametophyte) and 0.22 d^{-1} (parthenogenesis) at 32‰ and only 0.05 (gametophyte) and 0.02 d^{-1} (parthenogenesis) at 48‰ (Fig. 1C).

A marked reduced growth rates under lowest and highest salinities were evident in the Yongyon and Samgando isolates cross. With increasing salinities up to 32‰ the specific growth rate increased from 0.01 d^{-1} at 8‰ up to 0.21 d^{-1} at 32‰, but due to the relatively high standard error the mean values between 24 and 32‰ were not significantly different. At 40% the specific growth rate decreased slightly to 0.18 d^{-1} (Fig. 1D). The plants of inter-isolate cross were intermediate in their pattern of responses to the salinities as compared with their respective parents, through they were less euryhaline and had lower growth rate than parents.

DISCUSSION

Variation in salinity responses between populations of a certain species has been demonstrated in a number of algae (Young *et al.*, 1987; Rueness and Kornfeldt, 1992; Dawes, 1994). More information on the physiological mechanisms has been made at the levels of cell and organism (Reed and Russell, 1980; Kirst, 1990). Survival range, as indicators of physiological tolerance, is generally investigated using photosynthesis, growth rate and cell viability. However, some variation may be attributable to methodological problems, such as conditioning and age of algae examined. In *Ulva* species there are also problems in describing the growth rates on the basis of germling length, because variations in cell volume and the number of adventitious branches are produced at various salinities (Koeman and van den Hoek, 1981). In this study, the inoculum was as homogenous as possible (cohorts of germlings of approximately equal size and age). Furthermore, attempts to acclimate all the isolates were held in identical salinity conditions. We therefore suggest that the differences observed reflect a long-term genetic adaptation to the salinity regimes of the original habitats.

As shown in Fig. 1, the effects of salinity on

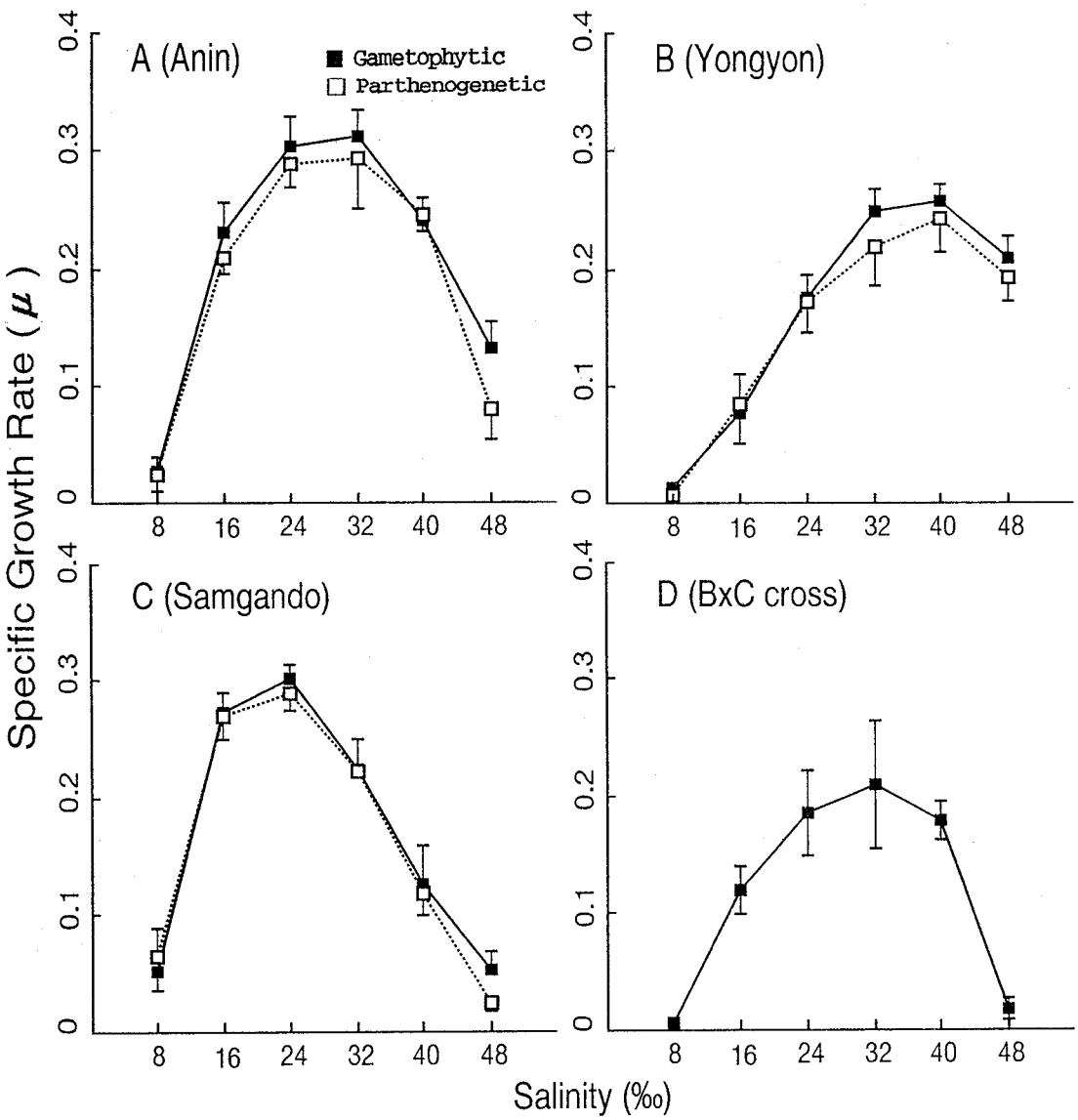

Fig. 1. The specific growth rates (μ) of gametophytic and parthenogenetic germlings of three isolates and an inter-isolate cross of *Ulva pertusa* as a function of salinity. Vertical bars are standard error.

germlings growth between the isolates of *Ulva pertusa* were significantly different. The results were consistent with the saline differences at the original habitats (Table 1). The Yongyon isolates appear to have a significant advantage under conditions of high salinity, being typical of its native habitat, whereas the Samgando isolates originating from low salinities show optimal growth under condition of low salinity. This, together with the intermediate responses of inter-isolate cross, suggests that these characteristics are genetically determined and that ecotypic differentiation has taken place. The presence of ecotypes within algal species has been demonstrated in many taxa, including marine phytoplankton (Gallagher *et al.*, 1984) and marine benthic algae (Innes, 1984; Rietema, 1993; Breeman and Pakker, 1994). Populations may retain a common genotype while phenotypic expression

changes across environmental gradients. Such phenotypic changes may be reversible plastic modification in response to environmental changes, or those may be irreversible developmental modification of gene expression such that individuals with identical genotypes differ phenotypically (Mayr, 1970). The optimum strategy for any given population probably depends on the specific trait, the intensity of natural selection, and the scales of environmental variability (Slatkin, 1987).

Resently, Rietema (1991) reported consistent differences, both physiologically and morphologically, between *Phycodrys rubens* (L.) Batters from the North Sea and the Baltic Sea. Both growth and survival rate examined in the laboratory at various salinities could be correlated with the salinity regimes of the original habitats. Russel and Bolton (1975) also showed in culture experiments that three isolates of *Ectocarpus siliculosus* (Dillw.) Lyngb., taken from different salinity regimes reveal different responses to change salinity, suggested that responses this species under culture condition may be linked with their original habitats. Differences in responses were maintained after four years of cultivation under identical conditions, thus suggesting genetic differences rather than the effect of acclimation or non-genetic adaptation. Thomas and Kirst (1991) described the salt tolerance of forty different isolates of *E. siliculosus* taken from different geographical origin. This work was probably the most extensive study of intraspecific variation in the salt tolerance of a marine macroalga. Ecotypic differences of gametophytes and sporophytes in salinity responses were also found. However, this seems not always the case, even between gametophyic and parthenogenetic germlings within the species of *U. pertusa*.

Populations from brackish water have smaller cell dimensions than those from full salinity water (Reed, 1983; Thomas *et al.*, 1990). Algae of small-celled forms may be found from brackish waters, suggesting an adjustment of turgor in response to lower salinity. Although cell dimensions of the various isolates in this study were not measured, the abnormal shaped cells of the Yongyon isolate may represent an adaptation to the lower salinities.

Ulva pertusa occurs in a broad range of salinity, from estuaries to relatively exposed coasts, suggesting a tolerance of large salt fluctuations. The ability of *U. pertusa* to thrive under a broad range of salinity argues for the existence of a diversity of ecotypes, some of which may be highly adapted to local conditions. In fact, genetic structure among populations of *U. pertusa* appears to be maintained by factors operating on a microgeographic scale (Doi et al., 1993). The evidence presented here suggests that ecotypic variation in salinity responses of *U. pertusa* provides an important mechanism by which can occupy a wide range of salinity. Further physiological studies should consider enzymatic (isozymic) features of differing populations to determine their importance in acclimative ability and adaptation tolerances.

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REFERENCES

Breeman, A.M. and H. Pakker, 1994. Temperature ecotypes in seaweed: adaptive significance and biogeographic implications. *Bot. Mar.*, **37**: 171-180.

Dawes, C., 1994. Physiological differentiations of the red seaweed *Gracilaria tikvahiae* from a mangel estuary, exposed coast, and culture. *Bull. Mar. Sci.*, **54**: 361-366.

Doi, K., M. Kamiya, Y. Hara and M. Chihara, 1993. Taxonomic studies of *Ulva pertusa* (Ulvophyceae). II. Preliminary isozyme analysis. *Jpn. J. Phycol.*, **41**: 199-206.

Floreto, E.A.T., H. Hirata, S. Ando and S. Yamasaki, 1993. Effect of temperature, light intensity, salinity and source of nitrogen on the growth, total lipid and fatty acid composition of *Ulva pertusa* Kjellman (Chlorophyta). *Bot. Mar.*, **36**: 149-158.

Floreto, E.A.T., H. Hirata, S. Yamasaki and S.C. Castro, 1994. Effect of salinity on the growth and fatty acid

composition of *Ulva pertusa* Kjellman (Chlorophyta). *Bot. Mar.*, **37**: 151-155.

Fong, P., J.B. Zedler and R.M. Donohoe, 1993. Nitrogen vs phosphorus limitations of algal biomass in shallow coastal lagoons. *Limnol. Oceanogr.*, **38**: 906-923.

Gallagher, J.C., A.M. Wood and R.S. Alberte, 1984. Ecotype differentiation in the marine diatom *Skeletonema costatum*: influence of light intensity on the photosynthetic apparatus. *Mar. Biol.*, **82**: 121-134.

Innes, D.J., 1984. Genetic differentiation among populations of marine algae. *Helgol. Meersunters.*, **38**: 401-417.

Kamiya, M., K. Doi, Y. Hara and M. Chihara, 1993. Taxonomic studies of *Ulva pertusa* (Ulvophyceae). I. Morphological analysis. *Jpn. J. Phycol.*, **41**: 191-198.

Kautsky, L., 1982. Primary production and uptake kinetics of ammonium and phosphate by *Enteromorpha compressa* in an ammonium sulfate industry outlet area. *Aquat. Bot.*, **12**: 23-40.

Kirst, G.O., 1990. Salinity tolerance of eukaryotic marine algae. *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, **41**: 21-53.

Koeman, R.P.T. and C. van den Hoek, 1981. The taxonomy of *Ulva* (Chlorophyceae) in the Netherlands. *Br. phycol. J.*, **16**: 9-35.

Lee, I.K. and J.W. Kang, 1986. A check list of marine algae in Korea. *Korean. J. Phycol.*, **1**: 311-325. (in Korean)

Lee, I.K., Y.P. Lee and Y.S. Ahn, 1986. Flora of marine algae in Cheju Island. 1. Ulvaceae. *Korean J. Phycol.*, **1**: 157-167. (in Korean)

Mayr, E., 1970. Populations, Species and Evolution. Harvard University Press, Cambridge. 453 pp.

Pregnall, A.M. and P.P. Rudy, 1985. Contribution of green macroalgal mats (*Enteromorpha* spp.) to seasonal production in an estuary. *Mar. Ecol. Prog. Ser.*, **24**: 167-176.

Provasoli L., 1968. Media and prospects for the cultivation of marine algae. In: Cultures and Collections of Algae, edited by A. Watanabe and A. Hottori, Japanese Society of Plant Physiology, Tokyo. 47-74 pp.

Reed, R.H., 1983. The osmotic responses of *Polysiphonia lanosa* (L.) Tandy from marine and estuarine sites: Evidence for incomplete recovery of turgor. *J. Exp. Mar. Biol. Ecol.*, **68**: 169-193.

Reed, R.H., 1984. Use and abuse of osmo-terminology. *Plant, Cell & Environ.*, **7**: 165-170.

Reed, R.H., 1990. Solute accumulation and osmotic acclimation. In: Biology of the Red Algae, edited by K. M. Cole and R.G. Sheath, Cambridge University Press, Cambridge. 147-170 pp.

Reed, R.H. and G. Russell, 1980. The effects of salinity upon celluar volume of the marine red algae *Porphyra purpurea* (Roth.) C. Ag. *J. Exp. Bot.*, **31**: 1521-11537.

Rietema, H., 1991. Evidence for ecotypic divergence between *Phycodrys rubens populations* from the Baltic Sea and North Sea. *Bot. Mar.*, **34**: 357-381.

Rietema, H., 1993. Ecotypic differences between Baltic and North Sea populations of *Delesseria sanguinea* and *Membranoptera alata. Bot. Mar.*, **36**: 15-21.

Rueness, J. and R. Kornfeldt, 1992. Ecotypic differentiation in salinity responses of *Ceramium strictum* (Rhodophyta) from Scandinavian waters. *Sarsia*, **77**: 207-212.

Russel, G. and J.J. Bolton, 1975. Euryhaline ecotypes of *Ectocarpus siliculosus* (Dillw.) Lyngb. *Estur. Coast. Mar. Sci.*, **3**: 91-94.

Shellem, B.H. and M.N. Josselyn, 1982. Physiological ecology of *Enteromorpha clathrata* on a salt marsh mudflat. *Bot. Mar.*, **25**: 541-549.

Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. *Science*, **236**: 787-792.

Taniguti, M., 1962. Phytosociological study of marine algae in Japan. Inoue & Co. Ltd., Tokyo, 129 pp.

Taniguti, M., 1983. The vegetation area of marine algae in the far east Asia. *Bull. Fac. Edu. Mie Univ. Nat. Sci.*, **34**: 29-33.

Thomas, D.N. and G.O. Kirst, 1991. Salt tolerance of *Ectocarpus siliculosus* (Dillw.) Lyngb: comparison of gametophytes, sporophytes and isolates of different geographic origin. *Bot. Act.*, **104**: 26-36.

Thomas, D.N., J.C. Collins and Russell, 1990. Interpopulation differences in the salt tolerance of two *Cladophora species. Estur. Coast. Shel. Sci.*, **30**: 201-206.

Uno, S., Y. Sakai and K. Yoshikawa, 1983. Distribution of *Ulva pertusa* and amount of nitrogen in Yamaguchi *Bay. Jap. J. Phycol.*, **31**: 148-155.

Young, A.J., J.C. Collins and G. Russel, 1987. Ecotypic variation in the Osmotic response of *Enteromorpha intestinalis* (L.) Link. *J. Exp. Bot.*, **38**: 1309-1324.