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An examination of the population genetics of *Laminaria* and other brown algae in the laminariales using starch gel electrophoresis

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Abstract

While some investigators have attempted to use isozyme electrophoresis to gain information on the genetics of brown algae, most have reported unsatisfactory results. Through exhaustive screening and modification of sample preparation techniques, gel and tray buffers systems, plus staining recipes, we have developed procedures that consistently provide scorable bands for over 20 enzyme systems in several laminarian algae. We have used our procedures to examine geographically diverse populations of *Laminaria saccharina* and *L. longicruris*, as well as *L. digitata*, *L. groenlandica*, *Agarum cribrosum*, *Alaria esculenta*, *Chorda tomentosa*, and *Macrocystis pyrifera*. Overall, these kelp species seem to have an extremely low degree of enzyme polymorphism, both within and between populations. While some 'rare alleles' occurred in several enzyme systems, only 3–5 loci were found to be polymorphic. Our results are consistent with the few reported studies that have used molecular genetic techniques to look at the intraspecific variability of laminarian algae. We suggest that at the species level the Laminariales, and perhaps other groups of brown algae, are genetically extremely conservative as compared to other divisions of plants. We further suggest that isozyme electrophoresis provides a quick and useful tool for algal population genetic studies.

Introduction

The Laminariales (Phaeophyta) is an ecologically and commercially important group of marine macrophytes. The large brown algae, collectively known as kelps, are a major source of primary production in temperate coastal waters and they provide substratum and cover for a host of other marine organisms (Mann, 1972; Kain, 1979). Organic matter from kelp enters the food chain through grazing organisms, detrital cycles, and the release of dissolved organic matter (Laycock, 1974; Griffiths & Stenton-Dozey, 1981; Robinson *et al.*, 1982). Kelps are used commercially as an important source of the phycocolloid algin, as liquid seaweed agricultural fertilizers, as livestock feed supplements, and as a direct source of human food (Waaland, 1981).

Until recently the taxonomic and evolutionary relationships of laminarian algae have been poorly understood (Kain, 1979). That is, due to their morphological plasticity and interfertility, taxonomic delineations of several kelp species have been confusing (Chapman, 1974; Mathieson

et al., 1980; Yarish *et al.*, 1990). Recent interfertility studies have demonstrated that interspecific and possibly intergeneric hybridization may occur (Lüning *et al.*, 1978; Mathieson *et al.*, 1981; Bolton *et al.*, 1983; Innes, 1984; Egan *et al.*, 1989; Neushul, 1989). For example, Cosson & Olivari (1982) describe interspecific hybrids of *Laminaria digitata* with *L. saccharina* and *L. ochroleuca* and between each of these and *Saccorhiza polyschides*. Cosson (1987) notes that these hybrids are incapable of producing another generation of normal sporophytes. In discussing such intergeneric kelp hybrids Neushul (1989) suggests that it is possible that seemingly major morphological differences between genera may be due to relatively minor genetic differences.

Several recent molecular investigations of kelps have been conducted. Stam *et al.* (1988) employed DNA-DNA hybridizations between single-copy nuclear DNA from *Laminaria digitata*, as well as total DNA from *L. saccharina*, *L. hyperborea*, *L. rodriguezii*, *L. ochroleuca* and *Chorda filum*. They found that the various *Laminaria* species were closely related genetically, while *C. filum* was only distantly related to *L. digitata*. Druehl (1989) employed restriction fragment length polymorphism (RFLP) of kelp chloroplast DNA to evaluate phylogenetic relationships within the Laminariales. Saunders & Druehl (1991) used analogous RFLP techniques on eight Northeast Pacific kelps and found that they were highly conserved, as only three different restriction-site differences were observed among all eight taxa. Using RFLP Bhattacharya & Druehl (1988, 1989) found only one restriction-site difference between two morphologically distinct populations of *Costaria costata*. In two other molecular DNA studies Lim *et al.* (1986) and Hori & Osawa (1987) compared the ribosomal RNA contents of *Eisenia bicyclis* to other brown algae, plus other divisions and phyla.

Although DNA extraction techniques (cf. Stam *et al.*, 1988; Saunders & Druehl, 1991) may give higher genetic resolution than starch gel electrophoresis, the latter method has many advantages, particularly if large numbers of individuals and enzymes are to analyzed (Kephart, 1990). As a consequence, electrophoresis has been extensively used to measure both intra- and interspecific genetic variability within diverse populations of animals (Meizel & Markert, 1967; Lewontin, 1974; Avise, 1975) and vascular plants (Mitton *et al.*, 1977; Soltis *et al.*, 1983; Tansley & Orton, 1983). Isozyme electrophoresis has been used less frequently to investigate the genetic similarity between algal species (Mathieson *et al.*, 1981). Blair *et al.* (1982), Innes (1984, 1987), and Innes & Yarish (1984) investigated genetic variation between species of green algae, while Cheney and Babbel (1978) and Cheney & Mathieson (1979) examined species of red algae. Marsden *et al.* (1981, 1984) and Rice & Crowden (1987) have described polyacrylamide gel electrophoretic techniques (PAGE) for use with brown macrophytes.

Like DNA extraction techniques, sample preparation and extraction procedures for PAGE makes it difficult to use for large scale population assessments. The primary advantages of starch versus polyacrylamide gel electrophoresis are five-fold: 1) simplicity of starch gel preparation; 2) use of less toxic chemicals (the acrylamide in PAGE is a neurotoxin); 3) reduced costs of equipment and supplies; 4) ease of sample loading; and 5) a greater amount of data obtainable per gel. That is, each gel can be cut into 5 horizontal slices, allowing a gel to be stained for 5 enzymes. As a result of these advantages, starch gel isozyme electrophoretic techniques have recently been perfected with macroalgae (Penniman *et al.*, 1985; Penniman, 1987). Typically extraction of enzymes from brown seaweeds is difficult due to the presence of high levels of polyanionic polysaccharides (*i.e.* algin and fucoidan) and polyphenolics that freely bind proteins. A protective extraction buffer used for conifer studies (Gagnon *et al.*, 1988; Mitton *et al.*, 1977, 1979) has been successfully modified for use with brown macrophytes (Penniman *et al.*, 1985), including kelp tissues.

The overall objectives of the present study were to further refine starch gel electrophoresis techniques for kelp, increase the number of enzyme systems that can be scored, and to use these pro-