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Attachment of the tetraspores of *Padina dubia* HAUCK (Phaeophyta, Dictyotales)

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After liberation, the tetraspores of *Padina dubia* secreted an adhesive which firmly attaches them to suitable substratum. The secretion started immediately after their liberation around the spores and the rhizoids. This secretory substance is of mucopolysaccharide type. It is suggested that the synthesis of the bioadhesive has some ecological and physiological importance. Histochemical studies were carried out to examine the nature of the extracellular bioadhesive by using specific stains like alcian dyes, periodic acid Schiff's (PAS) and toludine blue (TB). From the staining reactions it was found that the spores contain large amounts of both acidic and sulfated polysaccharides, while the extracellular bioadhesive contains only a sulfated polysaccharide, probably fucoidin.

Key Index Words: Bioadhesive; Dictyotales; histochemistry; Padina dubia; *Phaeophyta; polysaccharide; tetraspores.*

Padina dubia HAUCK is a common intertidal alga belonging to the family Dictyotaceae of the order Dictyotales. It is a tropical alginophyte found plenty in the intertidal regions of the Gujarat coast of India. The tetrasporic plants produce enormous number of spores and only a very few of them may get a chance to settle and adhere to suitable substratum. The attachment of these non-motile spores to the substratum against the tidal current is one of the most important events in the life history of an intertidal alga (HARDY and Moss 1979). Only a very little information is available on the nature of the extracellular bioadhesive secreted by the spores at the time of germination.

HARDY and Moss (1978, 1979) studied the attachment of the zygotes and germlings of *Halidrys siliquosa* and *Pelvetia canaliculata* by using specific stains. FORBES and HALLAM (1979) investigated the nature of the bioadhesive secreted by the zygotes of *Hormosira banksii* at the time of germination. The histochemical studies on the extracellular substance in brown algae have been carried out by a few researchers (MCCULLY 1965, 1966, 1970; FULCHER and MCCULLY 1969) and these substances were identified as non-sulfated acidic polysaccharide (alginic acid) and sulfated polysaccharide (fucoidin).

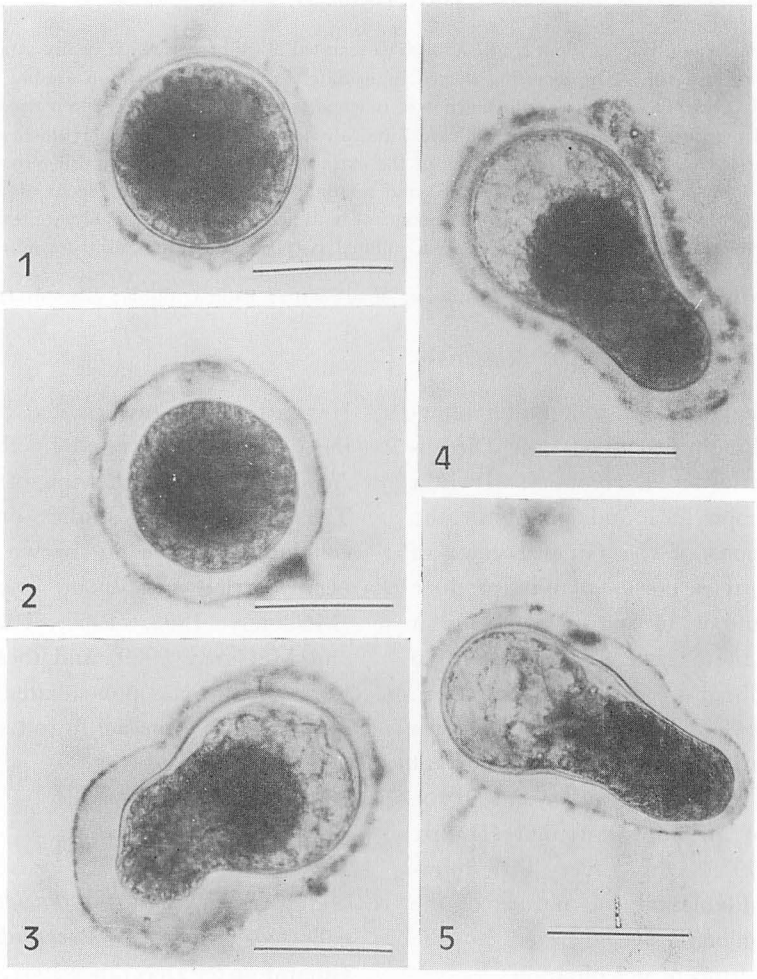
Materials and Methods

Tetrasporic plants of *Padina dubia* were collected from the Porbander coast of Gujarat on 21 December 1982. The plants were transported to the laboratory in plastic buckets containing seawater, and kept in the culture room at 20°C. The next day the fertile portions were removed

and rinsed with sterilised seawater several times. The upper portions of the thallus with mature bands were cut into pieces and they were placed on glass slides in petri dishes containing sterilised seawater. The slides with the spores were fixed in 5% formaldehyde at 10 minute intervals for two days. The spores were stained by using different stains for histochemical studies. Alcian dyes were prepared according to PARKER and DIBOLL (1966). Periodic acid Schiff's (PAS) stain was used after the method of MCMANUS (1948). The spores were also stained with toluidine blue (MCCULLY 1970). Cultures were maintained at 20°C, 14:10 hr LD cycle and 1500 lux. The stained spores and the germlings were observed under a light microscope.

Results

The spores started secreting an extracellular substance after 1–2 hrs of libera-



Figs. 1–5. *Padina dubia* tetraspores stained in toluidine blue.
Fig. 1. One hr old spore. Fig. 2. Three hrs old spore with bioadhesive of extracellular polysaccharide. Fig. 3. Five hrs old germinating spore. Fig. 4. Six hrs old germinating spore. Fig. 5. Eight hrs old germinating spore. All with extracellular polysaccharide around the spore and the rhizoid. Bar=25 μm.

Table 1. Different stains, their staining reactions on the tetraspores of *Padina dubia* and the polysaccharides identified.

Stains	Regions stained	Reaction colour	Staining reaction	Polysaccharides identified
Alcian Yellow	SP	Yellow	+	Non-sulfated
(AY) pH 0.5	EM	—	−	————
Alcian blue	SP	Blue	+	Sulfated
(AB) pH 2.5	EM	Blue	+	Sulfated
AY and AB	SP	Green	+	Carboxylated and sulfated
	EM	Blue	+	Sulfated (PARKER and DIBOLL 1966)
Peridic acid	SP	Red	+	With hydroxyl groups
Schiff's (PAS)				(MCCULLY 1965)
	EM	—	−	————
Toludine blue	SP	Red	+	Carboxylated and sulfated
	EM	Pink	+	Sulfated (MCCULLY 1965)

SP=spore, EM=extracellular material, +=positive, −=negative.

tion (Fig. 1). They adhere to the slide firmly after 3–4 hrs and the secretory substance was about 2 μm in thickness (Fig. 2). Their attachment was so firm that it was difficult to remove them even by applying a jet of water. After 5–6 hrs, the spores started producing a lateral rhizoid (Fig. 3). Gradually the rhizoid elongated and firmly attached on the slides (Figs. 4 & 5) by secreting an adhesive around it. The results of the staining reactions of different stains are presented in Table 1. The accumulation of alcian yellow (AY) was found only in the spore. The blue colour of alcian blue (AB) was found both in the extracellular bioadhesive and the spore. When stained with AY and AB the spores remained greenish, while the bioadhesive was bluish in colour. When the spores were treated with 1% $NaCO_3$ to remove the carboxylated alginic acid, prior to staining AB accumulation was found in the extracellular bioadhesive and the spore. Metachromasia with TB occurred both in the spore and the bioadhesive, the former being reddish and the latter pinkish in colour. An intense PAS positive reaction was found only in the spore, while the bioadhesive remained PAS negative.

Discussion

The spore alone was stained bright yellow with AY. This is due to the presence of a non-sulfated acidic polysaccharide (alginic acid). The AY at pH 2.5 readily complexes only with a non-sulfated polysaccharide. The AB at pH 0.5 complexes only with sulfated polysaccharide, its accumulation and blue staining both the spore and extracellular substance indicating the presence of polysaccharides with sulfate groups. Histochemical studies have already shown that sulfated polysaccharide in the brown algal tissue is fucoidin (PARKER and DIBOLL 1966, MCCULLY 1970, FORBES and HALLAM 1979). When the stains AY and AB were used simultaneously the spore was stained green, showing the occurrence of both sulfated and carboxylated polysaccharides, and the blue colour of the extracellular substance further confirms that it is a sulfated polysaccharide. When the germinating spores were treated with $NaCO_3$ solution to remove the non-

sulfated polysaccharide, the spore and the adhesive were stained blue, indicating the presence of sulfated polysaccharides. The AY staining substance was removed from the spore, which is a carboxylated polysaccharide (alginic acid). The PAS positive reaction occurred in the spore, while the bioadhesive remained PAS negative. The PAS positive reaction is quite specific for polysaccharide having free hydroxyl groups on 2 vicinal carbon atoms (JENSEN 1962). The alginic acid has free hydroxyl groups and therefore PAS positive, however fucoidin lacks hydroxyl groups and it is PAS negative (MCCULLY 1966). From these staining reactions it is clear that the bioadhesive may be a sulfated ester of fucose. The bioadhesive stained with TB was intense pink, while the spore was reddish. The metachromatic pink colour of TB is very characteristic of sulfated polysaccharide (LISON 1936), while the reddish and blue colour are generally produced by reaction with carboxyl groups (MCCULLY 1965). This staining reaction further confirms that the bioadhesive is a sulfated polysaccharide (fucoidin). The spore contains both sulfated and non-sulfated polysaccharides.

At the time of germination the zygotes of brown algae secrete a sulfated polysaccharide for firm attachment (Moss 1974, HARDY and Moss 1978, FORBES and HALLAM 1979). This bioadhesive is secreted by the spores and the rhizoids. FORBES and HALLAM (1979) observed the presence of polyphenols during the growth of the rhizoids of *Hormosira* zygotes. Such a secretion during germination may have some ecological and physiological significance in the establishment of benthic marine algae. The extracellular bioadhesive probably suppresses the growth of bacteria and other microorganisms (CRAIGIE and MCLACHLAN 1964). From the histochemical studies it is clear that the bioadhesive may be fucoidin and it may possibly contain some polyphenolic materials having antibacterial activities. Thus the bioadhesive is meant for adhesion and protection of the developing germling.

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Ampili, P. · Panikker, M.V.N. · Chauhan, V.D.: *Padina dubia*（褐藻植物門　アミジグサ目）の四分胞子の付着

Padina dubia の四分胞子は放出された後，粘着性の物質を分泌し，それによって基質にしっかり付着する。この分泌は四分胞子放出直後から胞子と仮根のまわりで始まる。この分泌物はムコ多糖類型のものである。この生物粘着物質(bioadhesive)の合成は何等かの生態的生理的重要性をもつことが示唆される。この細胞外に産生される生物粘着物質の性質を調べるため，アルシアン・イエロー，アルシアン・ブルー，PAS，トルイジン・ブルーなどを用いて組織化学的研究を行なった。染色反応から，四分胞子には酸性多糖類と硫酸含有多糖類とが共に多量に含まれるが，細胞外産生の生物粘着物質には硫酸根をもつ多糖類（恐らくフコイジン）のみが含まれることが判明した。

新　刊　紹　介

徳田　廣・大野正夫・小河久朗著　海藻資源養殖学。水産養殖学講座第10巻。緑書房，東京，v＋2＋354 pp.（定価 5,500円）

本書は9章からなる。第Ⅰ章は地球の生態系としての海藻；第Ⅱ章は光・温度・塩分等の環境要因と生長・垂直及び地理的分布；第Ⅲ章は食用・飼料・肥料・医薬用・化学工業用となる海藻の種類・利用法，その特性；第Ⅳ章は漁業区別にみた世界の海藻の資源と生産量，我が国への原藻の輸入とその製品の輸出量；第Ⅴ章は種毎にその生活史及びその生態特性を解説して，採苗から収穫までの養殖の理論と技術，その製品の品質と出荷；第Ⅵ章は種毎の藻場特性に基づいた藻場造成法；第Ⅶ章は世界の主要地区別または国別にみた養殖の現状；第Ⅷ章は主要海藻類の養殖への問題点と展望；第Ⅸ章は品種改良・品種保存等の将来配慮すべき問題点。

本書は日本及び世界の海藻資源と養殖の現状を系統的且つ理論的に纏めた世界に類のない専門書であり，大学の教科書として，また養殖に携わる漁業者や水産行政担当者の必携の良書である。海外の最近の多くの文献及び統計資料が引用され，また多くの写真と図表があるし，巻末には専門術語を解説した用語集があって読者の理解を援けている。

梅崎　勇（京大・農・熱農）