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# THE USE OF PHYCOERYTHRIN ABSORPTION SPECTRA IN THE CLASSIFICATION OF RED ALGAE

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SUMMARY

The phycoerythrins of 25 species of the Rhodophyta have been examined; absorption spectra of the native phycoerythrins of these species have been recorded. In taxonomy only a very limited use of phycoerythrin absorption spectra can be made. As a criterion it is only useful at species level and sometimes at genus level.

## 1. INTRODUCTION

The accessory photosynthetic pigment phycoerythrin in red algae forms large aggregates, the phycobilisomes. These are located on the outer side of the chloroplast lamellae (GANTT & CONTI 1965); their shape is cylindrical (LICHTLÉ & GIRAUD 1970), globular (GIRAUD et al. 1970) or disc-like (GANTT et al. 1968). On disruption of the cells the water-soluble pigment phycoerythrin is liberated easily into the homogenizing medium.

Phycoerythrin has been investigated extensively since the work of KYLIN (1910, 1912 and 1931). It is a glycoprotein (RAFTERY & O'HEOCHA 1965; KIMMEL & SMITH 1958; FUJIWARA 1964) to the polypeptide chains of which chromophores with a tetrapyrrole structure are attached (O'HEOCHA 1965a and 1965b).

Phycoerythrin absorption spectra have been used as criterion in the taxonomy of the Rhodophyta (HIROSE & KUMANO 1966; HIROSE et al. 1969). After the study of the phycoerythrin absorption spectra of a wide variety of red algae it is discussed to what extent these spectra are really useful in taxonomy.

## 2. MATERIAL AND METHODS

### 2.1. Plant material

The names of the species investigated are according to the check-list of British marine algae (PARKE & DIXON 1968).

*Acrochaetium codii* (Crouan) Born., *A. daviesii* (Dillw.) Näg., *A. secundatum* (Lyngb.) Näg., *A. spec.**. *A. virgatulum* (Harv.) J. Ag., *Antithamnion spec.*,

* This species belongs to the *Acrochaetium hallandicum-parvulum* complex.

*Bonnemaisonia hamifera* Hariot, *Erythrocladia spec.*, *Erythrotrichia spec.*, *Goniotrichum elegans* (Chauv.) Le Jol., *Gracilaria confervoides* Grev., *Polysiphonia urceolata* (Lightf. ex. Dillw.) Grev., *Rhodochorton spec.* and *Trailliella intricata* (J. Ag.) Batt. have been cultured in an enriched seawater medium according to PROVASOLI (1964) at a temperature of 12° C, a photoperiod of light: dark = 12:12h and a light intensity of $\pm$ 3 $J.m^{-2}.s^{-1}$.

Several strains of *Acrochaetium virgatulum* have been cultured and investigated. These strains were kindly supplied by drs. W. J. Borsje (Department of Botany, Free University, Amsterdam). The strain number of *Acrochaetium virgatulum* is followed by the place of isolation from the natural habitat: 65, 140A, 140B, 147A and 147B Oosterschelde, Zeeland (The Netherlands); 126A and 126B Ile de Batz, N. Finistère (France); 144 N.I.O.Z. harbour, Texel (The Netherlands) and 152 A and 152B former oyster-basins near Yerseke, Zeeland (The Netherlands). From all strains tetrasporophytes were investigated; from strain numbers 65 and 126A also gametophytes were examined.

*Dasya pedicellata* C. Ag. has been collected in the Gat van Ouwerkerk, Zeeland (The Netherlands) on 21. X.1970 and 8.IX.1971.

*Chondrus crispus* Stackh., *Gigartina stellata* (Stackh.) Batt. and *Porphyra umbilicalis* (L.) J. Ag. have been collected on the western pier of Zierikzee, Zeeland (The Netherlands) on 22.III.1971.

*Chondrus crispus* Stackh., *Corallina officinalis* L., *Gigartina stellata* (Stackh.) Batt., *Gracilaria confervoides* Grev., *Laurencia pinnatifida* (Huds.) Lamour., *Lomentaria articulata* (Huds.) Lyngb., *Plocamium cartilagineum* (L.) Dixon, *Polysiphonia nigrescens* (Huds.) Grev., *Porphyra umbilicalis* (L.) J. Ag. and *Rhodymenia palmata* (L.) Grev. have been collected on the coast between Audresselles and Cap Gris-Nez and on Cap Gris-Nez, Pas-de-Calais (France) on 17 and 18.VI.1971.

Finally *Hypoglossum woodwardii* Kütz. has been collected in the former oyster-basins outside the dikes, west of Yerseke, Zeeland (The Netherlands) on 4.VI.1972.

All collected algae have been kept frozen at –25° C.

## 2.2. Extraction of algae

Both the cultured algae and those collected at natural habitats were homogenized in 0.05 M K-phosphate buffer (pH 6.3). The homogenate was pressed through a double layer of cheese cloth and the resulting filtrate was centrifuged (20 min.; 25,000 × g) in a MSE 18 high speed centrifuge: the resulting supernatant was used as crude extract.

## 2.3. Purification of phycoerythrin

Crude extracts were chromatographed on a column of Sephadex G-200 (Pharmacia), equilibrated with 0.05 M K-phosphate buffer (pH 6.3) which was also used as elution buffer. Fractions were collected with a 7000. ultrarac automatic fractioncollector (LKB). From all coloured fractions the absorbance was measured at 565 and 615 nm with a Zeiss spectrophotometer PMQ II.

### 2.4. Absorption spectra

Absorption spectra were recorded at 25° C in cells with a light path of 10 mm using a Unicam SP.800A spectrophotometer, with the aid of a scale expansion set combined with a Servogor RE 511 Kompensationsschreiber.

Unless stated otherwise, all procedures were performed at a temperature of + 5° C.

## 3. RESULTS

By the gelfiltration method the native phycoerythrin could be separated from other proteins (structural proteins, phycocyanin and the phycoerythrin subunit). A typical elution pattern of a crude red algal extract with a Sephadex G-200 column is shown in *fig. 1.*

Absorption spectra of chromatographically pure native phycoerythrin (PE-I in *fig. 1*) are available in the literature for the species: *Acrochaetium virgatulum* (BONEY & WHITE 1968), *Bonnemaisonia hamifera* (HIROSE & KUMANO 1966), *Polysiphonia urceolata* (HIROSE & KUMANO 1966; SVEDBERG & ERIKSSON 1932; HATTORI & FUJITA 1959) and *Rhodymenia palmata* (DALE & TEALE 1970); these four species were also studied by the present author, with identical results. The absorption spectrum of the native phycoerythrin from *Acrochaetium virgatulum* is shown in *fig. 2A.*

Absorption spectra of the native phycoerythrin from the species that had not been investigated by other authors, are shown in *fig. 2B–2F.*

The phycoerythrins from all species investigated exhibit three maxima in the visible region: one near 495 nm, one near 535 nm and one near 565 nm. For each species the isolation of the native phycoerythrin and the recording of its absorption spectrum were performed at least three times: all experiments yielded the same result.

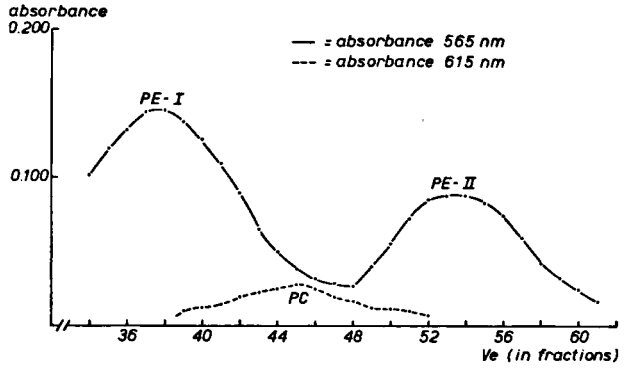
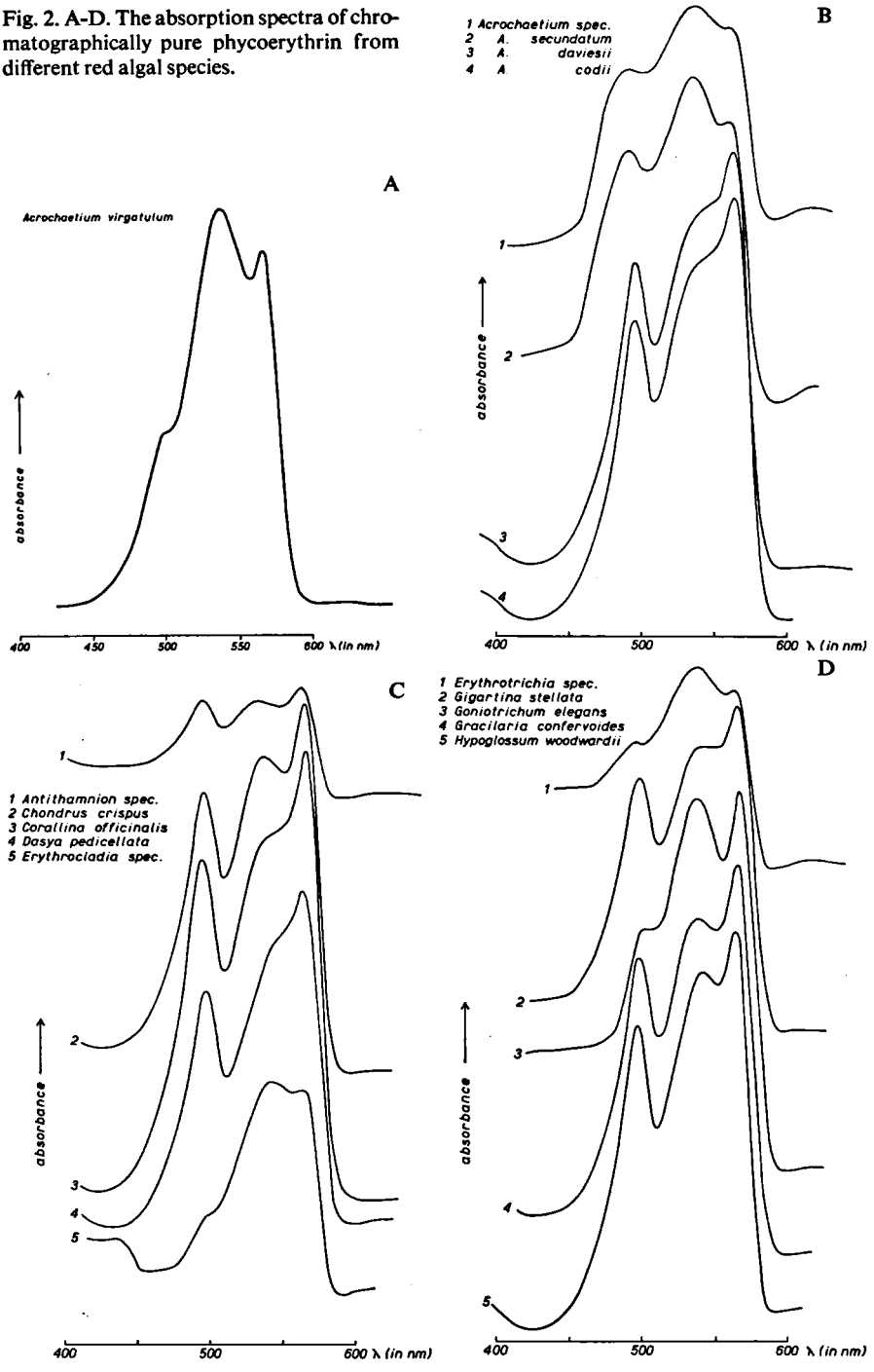



Fig. 1. Elution pattern of a crude extract of *Acrochaetium virgatulum* chromatographed on a Sephadex G-200 column (PE-I = native phycoerythrin; PE-II = phycoerythrin subunit; PC = phycocyanin).

Fig. 2. A-D. The absorption spectra of chromatographically pure phycoerythrin from different red algal species.



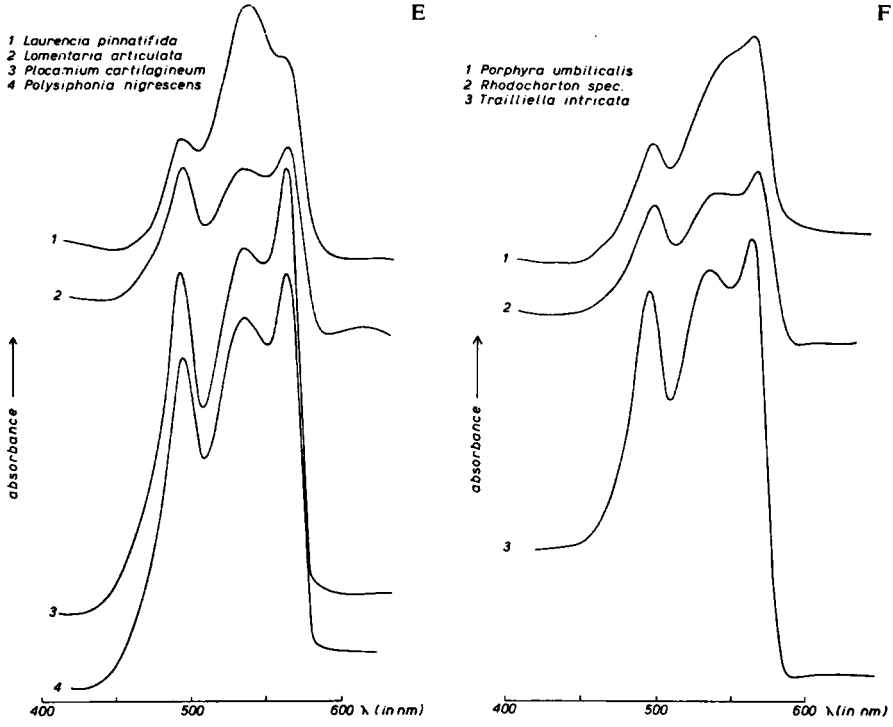



Fig. 2. E–F. The absorbtion spectra of chromatographically pure phycoerythrin from different red algae species.

## 4. DISCUSSION

### 4.1. General remarks

Algal classification is principally based on the differences in the accessory photosynthetic chromoproteins in the algae. Red algae contain chlorophyll a, carotenoids, phycoerythrin and phycocyanin.

It has been attempted to divide the red algae into groups following the spectral properties of their phycoerythrins; two types were distinguished: B-phycoerythrin (from Bangiophycidae) and R-phycoerythrin (the rest of the Rhodophyta) (HAXO & O'HEOCHA 1960).

Some authors tried to make a more detailed subdivision of the Rhodophyta according to the spectral properties of their phycoerythrins (HIROSE & KUMANO 1966; HIROSE et al. 1969). Six types of phycoerythrin occur in the Rhodophyta (see *table 1*).

Reviewing the phycoerythrin spectra published in literature and those given in this paper, it is to be concluded that the phycoerythrin absorption spectrum is not a useful taxonomic character in higher order systematics. Within an order, a family and even within a genus different types of spectra occur. Mostly within a genus all species show the same phycoerythrin absorption spectrum.

Table 1. Types of phycoerythrin absorption spectra. The place of the maxima is given in nm; the band with maximal absorbance is underlined; shoulders are in parentheses.

| Type number | Absorption bands | | |
|---|---|---|---|
| 1 | 495 | 535 | 565 |
| 2 | (495) | 535 | 565 |
| 3 | 495 | 535 | 565 |
| 4 | 495 | (535) | 565 |
| 5 | 495 | 535 | 565 |
| 6 | 495 | (535) | 565 |

Exceptions are *Acrochaetium* (types 1, 3 and 4 – see paragraph 4.2.), *Gracilaria* (types 3 and 5), *Rhodymenia* (types 3 and 4) and *Laurencia* (types 1 and 3) (see *table 2*).

## 4.2. The Acrochaetium species

Within the genus *Acrochaetium* so far three types of phycoerythrin absorption spectra could be detected (see *table 2*). The yet undetermined species belongs to the *Acrochaetium hallandicum-parvulum* complex.

The fact that the phycoerythrin absorption spectra of *Acrochaetium codii* and of *A. daviesii* are similar, forms a confirmation for the hypothesis (on morphological grounds) that these two organisms belong to one and the same species. All strains of *Acrochaetium virgatulum* tested show the same phycoerythrin absorption spectrum (see *fig. 2A*).

## 4.3. Bonnemaisonia hamifera – Trailliella intricata

The fact that the phycoerythrin absorption spectra of *Bonnemaisonia hamifera* and of *Trailliella intricata* are similar, forms a confirmation for the statement that both organisms belong to one and the same species (culture experiments by Koch, cited from KYLIN 1956).

## 4.4. Conclusions

4.4.1. Each species shows a typical phycoerythrin absorption spectrum, which can be used as a species characteristic: each species forms its own proteins and phycoerythrin is a chromoprotein, the absorption spectrum of which – at least partially – is determined by its protein structure (JONES & FUJIMORI 1961). Therefore it is necessary to record absorption spectra of phycoerythrin solutions which have been prevented from denaturation during their preparation.

4.4.2. Taxonomically phycoerythrin absorption spectra have only limited value: a phycoerythrin absorption spectrum is a species characteristic and sometimes it may be a genus characteristic, but for higher order systematics in the Rhodophyta extreme caution is to be taken in using the form of the phycoerythrin absorption spectra as a criterion in taxonomy.

Table 2. Differences in phycoerythrin absorption spectrum between species from one genus. The place of the three absorption bands is given in nm; the band with maximal absorbance is underlined; shoulders are in parentheses.

| Species | Absorption bands | | | References |
|---|---|---|---|---|
| *Acrochaetium* | | | | |
| *A. spec.** | 492 | 536 | 562 | this paper |
| *A. asparagopsis* | 498 | 550 | 570 | Boney & White 1968 |
| *A. codii* | 496 | (536) | 564 | this paper |
| *A. daviesii* | 497 | (536) | 565 | this paper |
| *A. endophyticum* | 498 | (±535) | 570 | Boney & White 1968 |
| *A. infestans* | 495 | 545 | 568 | Boney & White 1968 |
| *A. secundatum* | 490 | 534 | 562 | this paper |
| *A. virgatulum* | 496 | 537 | 565 | Boney & White 1968; this paper |
| *Gracilaria* | | | | |
| *G. compressa* | 495 | 540 | 565 | Hirose & Kumano 1966 |
| *G. confervoides* | 496 | 536 | 565 | this paper |
| *Rhodymenia* | | | | |
| *Rh. spec.* | ±495 | ±535 | ±565 | Dale & Teale 1970 |
| *Rh. pacifica* | 497 | 537 | 564 | Haxo et al. 1955 |
| *Rh. palmata* | 497 | (541) | 566 | this paper |
| *Laurencia* | | | | |
| *L. pinnatifida* | 494 | 539 | 563 | this paper |
| *L. intermedia* | 500 | 545 | 565 | Hirose & Kumano 1966 |
| *L. obtusa* | 495 | 535 | 565 | Hirose et al. 1969 |
| *L. okamurae* | 495 | 545 | 565 | Hirose & Kumano 1966 |

* See footnote on p. 92.

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