PIGMENTS IN THERMOPHILIC FUNGI

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ABSTRACT

Ultraviolet and visible absorption spectra of thermophilic fungi have been obtained by photoacoustic spectroscopy. Based on these data as well as on the chemical properties and infrared spectra, it is suggested that the pigments may be hydroxylated polycyclic quinones.

INTRODUCTION

SOME species of thermophilic fungi show bright pigmentation characteristics, the colours varying from yellow to red[1]. In some species the pigmentation varies considerably, depending upon temperature, age and substratum. Although some of the chemical and other properties of the thermophilic fungus, *Thermomyces lanuginosus* (formerly *Monotospora lanuginosa*), were examined by Crisan[2], little is known about the nature of the pigments. Even the absorption bands in the ultraviolet and visible regions of these fungi have not been documented. We considered it worthwhile to obtain the electronic spectra of a few thermophilic fungi by means of photoacoustic spectroscopy[3] (PAS) which provides a non-destructive means of recording the spectra. Besides obtaining the electronic spectra, we have attempted to derive the nature of the chemical species responsible for the pigmentation of the fungi by making use of the known chemical properties and infrared spectra.

EXPERIMENTAL

The following thermophilic fungi orginally isolated from soil/compost in India were used: *Talaromyces (Pencillium) duponti* (I), *Thermoascus aurantiacus* (II), *Malbranchea pulchella* Var *sulfurea* (III), *Thermomyces lanuginosus* (IV) and *Humicola grisea* Var *thermoidea* (V). The thermophilic fungi were grown on YpSs agar medium[1] in petri dishes at 42–50°C in a humidified incubator. The fungi were removed when the mycelia covered 3/4 of the petri dish. Subsequent growth was prevented due to the low temperature (21°C) at which PA measurements were carried out. PA spectra were recorded shortly after removing from the incubator.

The photoacoustic spectrometer employed in the study was a home-built instrument. The spectrometer was described in detail earlier[3]. In brief, it contains a 450 W xenon lamp capable of operating both in the UV and the visible regions, a chopper with a variable chopping frequency, a sensitive microphone, a tuned lock-in-amplifier, associated optics and a recorder. The chopping freqeuncy was 37 Hz while the scanning speed was 100 nm/min with a time constant of 1 sec. The spectral resolution was 20 nm. The spectra were normalized with respect to pre-recorded carbon black power spectrum.

Agar blocks (1×1 cm) bearing mycelial growth were cut out from petri dish cultures and transferred into the photoacoustic cell in a teflon sample holder.

ELECTRONIC SPECTRA

In figure 1, we show the normalized photoacoustic spectra of the pink coloured *Talaromyces (Pencillium) duponti* (I). The spectra show distinct absorption bands in the UV and visible regions (λ_{max}, 275, 360, 475 and 550 nm). The spectra also show increase in the intensity of the colour with the age of the culture. In figure 2, the normalized photoacoustic spectra of *Thermoascus aurantiacus* (II) are shown (λ_{max}, ca 250 and 375 nm). The spectra of the young orange-buff culture and the older reddish brown culture are given. In figure 3, we show the normalized photoacoustic spectra of *M. pulchella* Var *sulfurea* (III) with λ_{max} around 250 and 390 nm and also of *T. lanuginosus* (IV) with λ_{max} at 270, 350 nm and a broad band extending to 650 nm. III is bright sulphur-yellow in colour whereas IV shows pinkish and greenish bands from the centre to the margin of the colony. *H. grisea* Var *thermoidea* (V) shows a

broad band extending to 650 nm with a λ_{max} in the visible region around 550 nm. The λ_{max} values of the various fungi are listed in table 1. The photoacoustic spectra presented here clearly show that the pigments of the fungi can be characterized in terms of their electronic spectra. *M. pulchella* Var *sulfurea* and *T. lanuginosus* showed gradual changes in colour with age suggesting transformations of the pigments.

Table 1 *λ_{max} values (nm) of the various thermophilic fungi*

Talaromyces (Pencillium) duponti (I)	275, 360, 475, 550
Thermoascus aurantiacus (II)	ca 250, 375
Malbranchea pulchella Var *sulfurea* (III)	ca 250, 390
Thermomyces lanuginosus (IV)	270, 350 (and a broad band extending to 650 nm)
Humicola grisea Var *thermoidea* (V)	~550 (and a broad band extending to 650 nm)

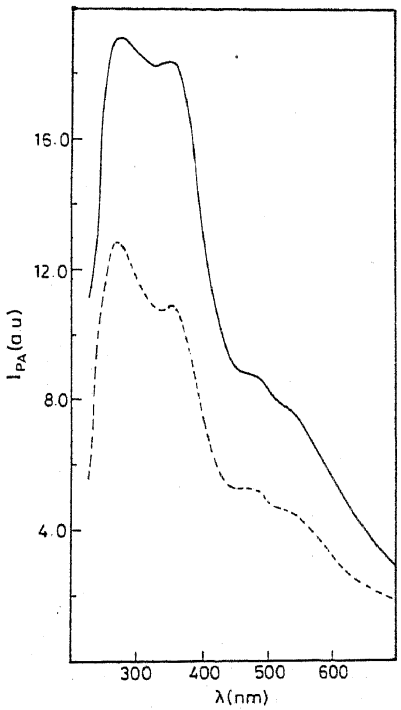

Figure 1. Normalized photoacoustic spectra of the pink coloured fungus *T. (Pencillium) duponti* (I). Full line shows the old culture (5 days) and broken line shows the young culture (3 days).

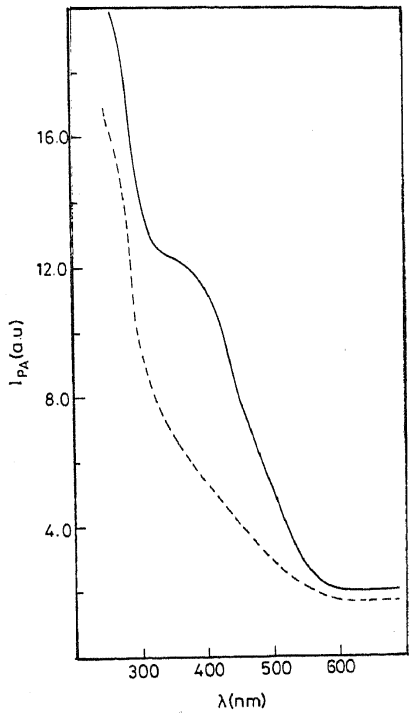

Figure 2. Normalized photoacoustic spectra of the brown coloured fungus *T. aurantiacus* (II). Full line shows the old culture and broken line shows the freshly growing culture.

CHEMICAL PROPERTIES AND INFRARED SPECTRA

The pigments of *T. lanuginosus* are soluble in NaOH and are precipitated by the addition of acid[2] suggesting the presence of phenolic groups. The infrared spectra[4] show the presence of hydrogen-bonded OH groups (3300–3400 cm^{-1}) and of the carbonyl function. The carbonyl stretching vibration frequency of the pigments is in the region 1690–1710 cm^{-1}. This has earlier been ascribed to the carboxylate anion but this may not be correct since we do not clearly see the corresponding symmetric stretching frequency of the carboxylate ion which should appear in the region 1300–1400 cm^{-1}. The observed stretching frequencies are however close to those found in quinones[4]. Furthermore, the C-H stretching bands in the infrared spectra are absent or very weak showing that there are very few C-H groups[2]. These infrared spectral features together with the fact that there are aromatic C-C skeletal vibration frequencies in the 1600–1400 cm^{-1} region, suggest that these pigments may be polyhydroxy-extended quinones. This would explain the solubility of the pigments in alkali. The pigments cannot

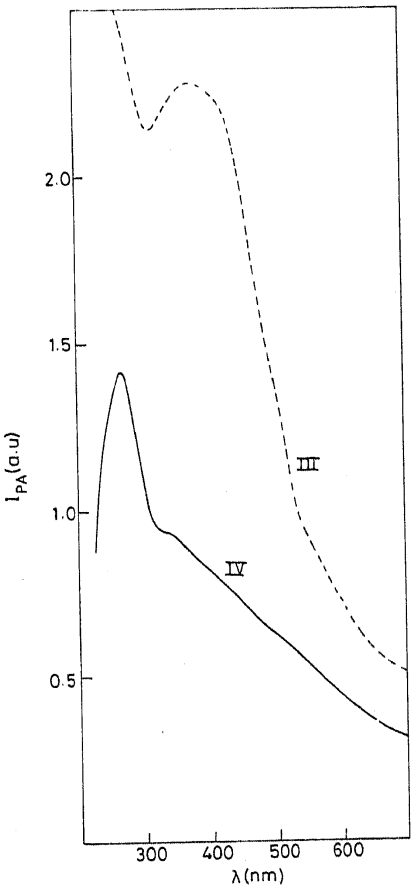

Figure 3. Normalized photoacoustic spectra of the fungi *M. pulchella* Var *sulfurea* (III) and *Thermomyces lanuginosus* (IV).

be polyenes since they do not take up hydrogen, on catalytic hydrogenation[2]. The polycyclic polyhydroxy quinone structure therefore seems to provide an attractive possibility for these pigments.

CONCLUDING REMARKS

The electronic spectra (figures 1–3), the infrared spectra and the chemical properties of the pigments in the thermophilic fungi are similar to those of aphins[5], the hydroxylated quinoid pigments found in some insects (aphids). Thus, protaphin (figure 4a), investigated by Lord Todd[6], is yellow (λ_{max}, 270, 360 and 460 nm) with infrared bands around 3470, 1670, 1613 and 1590 cm^{-1}. It readily transforms to xanthophin (figure 4b) which is also yellow in colour with absorption bands in the 320–450 nm region, then to the orange-coloured chrysoaphin (figure 4c) with absorption bands in the 350–500 nm region and finally to the red coloured erythroaphin (figure 4d)

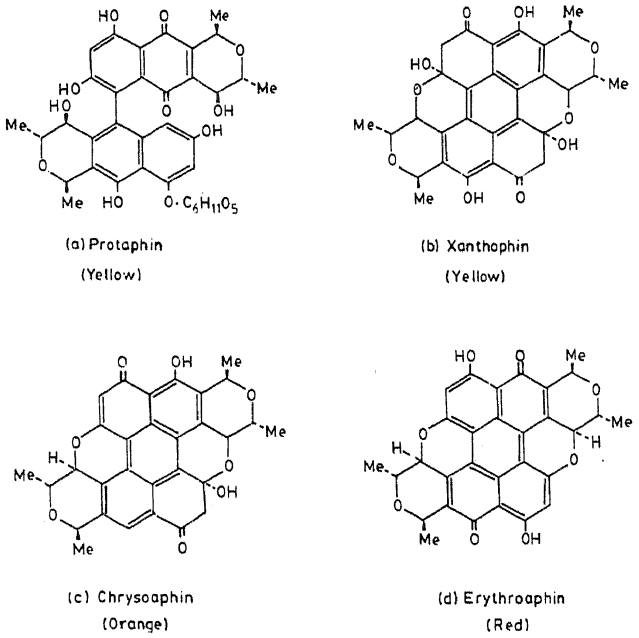

Figure 4. (**a**) Protaphin, (**b**) xanthophin, (**c**) chrysoaphin and (**d**) erythroaphin.

which has absorption bands in the 400–600 nm region and infrared bands around 3400, 1715 and 1625 cm^{-1}. The infrared bands of 4(a) and 4(d) are due to the stretching of hydrogen bonded OH groups, the quinoid carbonyl stretching mode and the aromatic skeletal vibrations. The frequency ranges are close to those found in the pigments of the fungi. The facile transformation found among these aphin pigments, their acidic character and easy solubility in NaOH (due to the presence of phenolic hydroxy groups) are specially noteworthy. We, therefore, suggest that the pigments in the thermophilic fungi studied by us are likely to be polyphenolic, polycyclic quinones akin to the aphins.

ACKNOWLEDGEMENTS

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ANNOUNCEMENTS

FIFTY-SECOND ANNUAL MEETING OF THE INDIAN ACADEMY OF SCIENCES

The Fifty-second annual meeting of the Indian Academy of Sciences, Bangalore, will be held at Varanasi, from Friday 7th November to Monday 10th November 1986.

The scientific programme for the meeting consists of scientific symposia, evening lectures and a series of lecture presentation by Fellows and Young Associates of the Academy.

The two specialized symposia are on 'Crustal Evolution and Metallogeny in the Indian Subcontinent'; and 'X-ray Crystallography of Biopolymers'.

Further information and details may be had from: The Secretary, Indian Academy of Sciences, Bangalore 560 080.

INTERNATIONAL SYMPOSIUM ON PALAEOCLIMATIC AND PALAEOENVIRONMENTAL CHANGES IN ASIA DURING LAST FOUR MILLION YEARS

The above symposium (ISPPCA) will be held from 15–21 December 1986 at Ahmedabad, Gujarat, India, instead of the original proposal for organizing at Srinagar in September 1986.

The main themes of the technical programme are:

1. Palaeoclimatic and Palaeoenvironmental Data,
2. Environment and Hominid Evolution.

Further particulars may be had from the Convener: Prof. D. P. Agrawal, Physical Research Laboratory, Navrangpura, Ahmedabad 380 009.

SERC WINTER SCHOOL ON 'SOLITONS'

(Department of Science and Technology, Government of India)

The Science and Engineering Research Council (SERC) has formulated a programme of annual Summer/Winter School to encourage research by younger scientists in the frontier areas of Non-linear Phenomena. A Winter School in the series on the topic 'Solitons' will be held at the Bharathidasan University, Tiruchirapalli, from January 5–17, 1987.

It is proposed to cover in the school both the mathematical theory and physical (and biological) applications of solitons. The Winter School will admit participants on an all India basis. Preference will be given to fresh Ph.D.s. and those working for Ph.D. Degree in soliton theory and its applications and other related areas of non-linear dynamics below the age of 35. Financial support for train travel, board and lodging will be provided to 25 participants; few others who are able to make their own travel and stay arrangement will be admitted.

For further details and application forms, contact: Professor M. Lakshmanan, Department of Physics, Bharathidasan University, Tiruchirapalli 620 023.