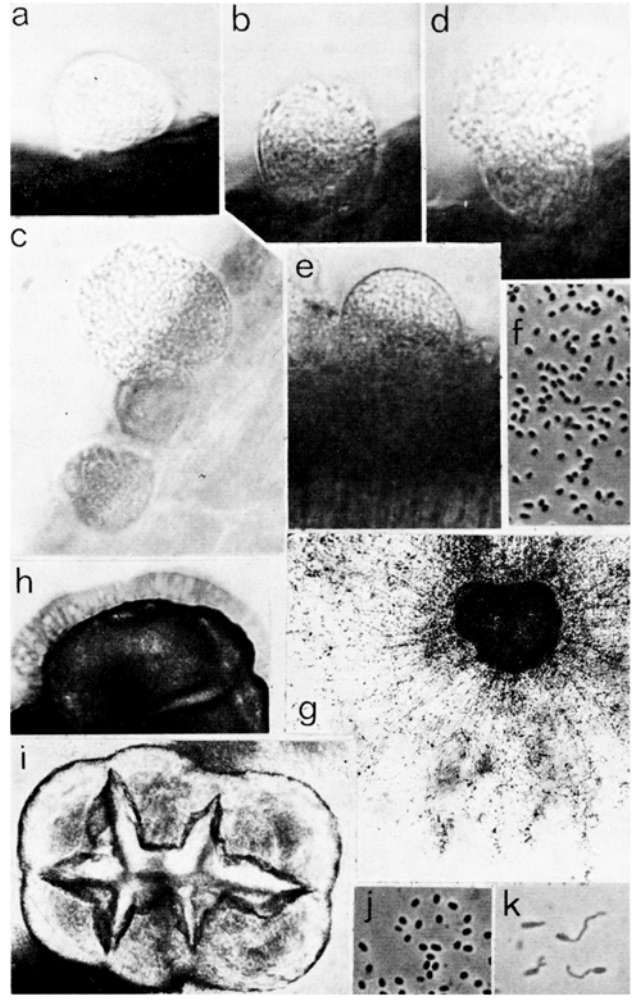
Sporangium Formation in the Actinoplanaceae Induced by Humic Acids

In a study of the ecology of aquatic Actinomycetales particular attention is being given to forms growing on allochthonous leaf material cast up on the foreshore of Windermere. In sampling, portions of such leaves (usually oak) have been subjected to washing procedures and the washings plated onto chitin plus actidione agar. Of the Actinoplanaceae recovered *Actinoplanes* almost always form sporangia directly on the original isolation dish, but *Ampullariella* does this rarely and *Amorphosporangium* hardly ever. Sampling by a second method, namely by damp incubation of the collected leaf material, has repeat edly given *Actinoplanes* and in addition sporangia of an unidentified form which may be related to *Pilimelia*[1] (Figure a–d). The sub-culture of pure isolates of this unidentified form has re-emphasized our lack of knowledge of fruiting requirements of the Actinoplanaceae. Representative isolates, S23 and S25, were sterile on chitin[2], chitin-yeast (0.2% w/v) extract and starch-casein[3] agars both dry and immersed in water. Again, material first grown in liquid culture as pellets and then transferred to sterile filtered lake water, a classical method for sporangium induction in the aquatic Phycomycetes, was also unproductive. The choice of yeast extract as an addition to the agar was not entirely without reason. During the course of this work, leaf washings onto chitin plus actidione agar have yielded a number of motile spored aquatic Actinomycetales isolates designated 'spore dome' types. They respond dramatically to yeast extract addition by exhibiting greatly enhanced sporulation on solidified media. Under these conditions the central elevated portion of the colony (Figure g), and indeed the whole colony itself, may transform to a crustose spore mass (Figure h, i). In the case of isolates S23 and S25 however, as in the case of other presumptive Actinoplanaceae isolates which have been manipulated, we have gained the impression that although vegetative growth is enhanced by such enrichments success in inducing sporangium formation might well depend on dilution or debilitation of the growth medium rather than the reverse. In accord with this reasoning has been the generally improved fruiting performance on chitin agar as compared with starch-casein or Czapek-Dox[4] agars. Considering some of the more refractory elements of the natural environment, cellulose and lignin are important components and in addition the derived humic acid complex resulting from the decomposition and degradation of the leaf material. The effect of humic acids was considered particularly worthy of trial since KÜSTER[5] had reported that certain Actinomycetales may utilize these for growth. Humic acid samples were obtained from 3 separate sources, representing terrestrial, marginal, and aquatic environments.

The terrestrial source was an acid blanket bog peat collected from Red Tarn, Langdale, Westmorland. Following air-drying at 90 °C for 24 h it was first extracted with acetone in a Soxhlet for 3 h and redried at 50 °C overnight. The humic acids were then extracted[6] from the peat (117 g) with 0.2 *N* NaOH (2.2 l), by shaking occasionally, standing overnight at room temperature, and filtering off the insoluble residue on a No. 4 sintered glass funnel. The filtrate was acidified to pH 1.0 with c.HCl, the resulting precipitate was centrifuged off, washed twice with water and left overnight at − 20 °C to freeze. After thawing, the now granular particles were filtered off from the remaining liquors, washed, and air-dried at 50 °C giving a 14% yield of humic acids (16.3 g).

The marginal source consisted of compacted decomposing tree leaves (mainly oak and alder) collected from



a–d, Actinoplanaceae sporangia (unidentified form) observed following damp incubation of oak leaves. a, b, Mature sporangia; c, the onset of dehiscence showing spores beginning to emerge through wide pores; d, a later stage in dehiscence with tangled files of spores emerging; e, mature sporangium of Actinoplanaceae isolate S25 from a vertical section of a colony on humic acids agar; f, motile spores from sporangia in c; g, mature 'spore dome' colony on chitin agar; h, i, mature 'spore dome' colonies on chitin-yeast extract agar showing how on this medium the central sporing area tends to enlarge and become crustose at the expense of the peripheral mycelium which is a narrow zone only in h and non-existent in i,; j, motile spores obtained from such 'spore dome' isolates; k, germination of same. All photographs are of living material, except g, which is stained with cotton-blue. g-i; × 100. Remainder; × 1000.

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Ash Landing on the shore of Windermere, giving a 3% yield of humic acids by the same method.

The aquatic source was sand-filtered water from Windermere. This was passed down a column of De-Acidite K (a highly basic macroporous anion-exchange resin) for 4 weeks after which the absorbed anions were eluted with 10% NaCl[7]. The humic acid fraction was precipitated with c.HCl and treated as described above. The final yield was 54 µg humic acids/l of treated water, representing 2% of the total dissolved organic material.

In the experiments a final concentration of 0.5% humic acids was used, 2.5 g of the solid material being dissolved in approximately 5 ml of 0.2*N* NaOH and the solution made up to 500 ml with distilled water. The pH was then adjusted to 7.0–7.3. Addition of 2% agar (Oxoid Ionagar No. 2) was made and following autoclaving the medium was poured into sterile polystyrene Petri dishes. Together with plain agar (pH adjusted) controls these poured dishes were inoculated with test Actinoplanaceae. The latter comprised separate strains, 2 of each, of *Amorphosporangium* (isolates P8, S2), *Ampullariella* (isolates P2, S1), and of the unidentified form described above (isolates S23, S25). Previously these had formed sporangia only very rarely and unpredictably following manipulations involving the addition of free water (*Amorphosporangium* and *Ampullariella*) or had not formed them at all (unidentified form). With the Red Tarn sample of humic acids growth was sparse but of a slightly greater density than that on the plain agar control dishes. This indicated only limited utilization of the added material. However, sporangium formation occurred on the surface of the agar with 5 of the 6 isolates, the exception being 1 *Amorphosporangium* isolate (S2). In each case this fertile material matched that previously observed on other substrata very closely. Sporangia from the unidentified form were particularly striking (Figure e). Using the Ash Landing sample of humic acids results were less impressive, only 2 (P2 and S25) of the 6 isolates producing sporangia. Finally the humic acids sample from Windermere water was uniformly ineffective when used for the 6 isolates. This material was not toxic and supported abundant sporangium production in a stock *Actinoplanes* isolate which fruits readily on most standard Actinomycete media. Thus there is the suggestion of differences in the biological effects of humic acids from different sources.

Résumé. La culture sur un substrat à l'acide humique incorporé dans de la gelose a provoqué la formation de sporanges dans 5 colonies d'Actinoplanaceae (Actinomycetales) isolées, dont 2, soumises à quelques autres traitements, étaient restées stériles. Des acides humiques de provenances différentes (terrestre, marginale et aquatique) ont montré divers degrés d'efficacité.

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E- und I-Netzhäute von Urodelen

Granit[1] hat auf Grund des Elektroretinogramms bei den Netzhäuten verschiedener Tiere einen E-Typ und einen I-Typ unterschieden. Zur Zeit der Aufstellung dieses Schemas (1947) stand noch nicht endgültig fest, ob als Kriterium eine bestimmte Struktur der Netzhaut oder die Zugehörigkeit der Tierart zu einer bestimmten Tierklasse ausschlaggebend ist. Alle bis dahin untersuchten Netzhäute von Fischen, Amphibien und Sauropsiden gehörten nämlich dem I-Typ an, während sämtliche zunächst untersuchten Säuger E-Netzhäute aufwiesen. Als erste Ausnahme der letztgenannten Regel erwies sich das europäische Ziesel (*Citellus citellus* L.), dessen reine Zapfennetzhaut ein ERG vom I-Typ erzeugt[2]. Auch die später auf andere Sciuriden ausgedehnten Untersuchungen[3–5] sprachen eindeutig für eine Korrelation von Netzhautstruktur und ERG-Typ im Sinne von Granit[1]. In der vorliegenden Mitteilung wird nun beschrieben, dass Angehörige derselben Tierfamilie Netzhäute vom E-Typ und vom I-Typ aufweisen können.

Versuchstiere waren einerseits 11 Teichmolche (*Triturus vulgaris*) und andererseits 3 Feuersalamander (*Salamandra salamandra*). Die Bulbi wurden in Urethannarkose enukleiert, das ERG wurde mit Ag-AgCl-Elektroden abgeleitet und nach DC-Verstärkung (J. F. Tönnies, Freiburg Br.) mittels Direktschreiber (Cardiopan) oder KSO (Tektronix 502A mit Recordine N) registriert. Die Untersuchungstemperatur betrug bei beiden Species 22–25 °C. Als Reiz wurde «weisses» Licht verwendet (3200 °K), dessen Intensität 700 lux an Stelle des Auges betrug und dessen Dauer mittels eines elektronisch gesteuerten Magnetverschlusses begrenzt wurde (Multistim Disa). Die Tiere waren bei Versuchsbeginn durchwegs 2 h lang dunkeladaptiert.

Ergebnisse. Das ERG des Teichmolches weist einen deutlichen positiven off-Effect (d-Welle) auf, der mit wachsender Reizdauer an Höhe zunimmt; im Gegensatz dazu ist beim Feuersalamander bis zu einer Reizdauer von 1 sec kein deutlicher off-Effekt im ERG zu sehen; ausserdem beginnt das ERG des Teichmolches nach kurzer Latenz mit einer a-Welle, die beim Feuersalamander praktisch fehlt (Figur 1). Ausgeprägter off-Effekt und a-Welle sind charakteristische Merkmale einer I-Retina. Das zweite Charakteristikum ist die Fähigkeit, einem Flimmerlichtreiz bis zu weit höheren Frequenzen zu folgen, als dies die E-Retina vermag. Das ERG des Teichmolches folgt unter den gegebenen Reizbedingungen bis zu Frequenzen über 10/sec dem Flimmerlicht, beim Feuersalamander war auch bei sehr niedriger Reizfrequenz ein

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