Sporogenesis in the Bryophyte *Anthocerosis*: Features shown by Fluorescence Microscopy

FLUORESCENCE microscopy has been found useful in bacterial identification, clinical diagnosis, fluorescent antibody techniques, and general cytological studies[1]. Recently, Shellhorn, Hull and Martin[2] have extended the use of fluorescence microscopy to the detection of fresh and fossil pollen grains by using autofluorescence of exines.

In an investigation of sporogenesis in *Anthoceros hawaiensis* Bartlett and related genera, fluorescence microscopy has proved useful in the detection of incipient deposition of the exosporium in the tetrad immediately following meiosis. In preparing the sporogenous tissue for examination, a median bisection of the living sporophyte was placed in distilled water on a glass slide and covered with a No. 1 cover glass. No fluorochrome was added.

The material was illuminated by an incident ultra-violet light from an Osram '*HB*' 200-W mercury lamp with current supplied from a 'Universal' light source, model 250. Observations were made through a Leitz 'Ortho-Lux' microscope. Filters used during the illumination by ultra-violet light were *BG* 38, *BG* 12 and an ocular blue-absorbing filter.

In Fig. 1, two sporocytes are shown as they appear under incandescent illumination. One sporocyte (top) has just formed tetrads, while the one below contains the four chloroplasts which are prominent in the pre-meiotic condition. The same field (Fig. 1 reversed) is shown in Fig. 2, but under ultra-violet illumination. In Fig. 2, the sporocyte containing the tetrad (top) shows the brightly autofluorescing exosporia of the individual spores; this layer shows a greenish-yellow autofluorescent. The exosporium of developing spores and mature spores shows this same greenish-yellow autofluorescence even after the spore is released from the ruptured sporocyte wall. The sporocyte wall itself is not autofluorescent. In the pre-meiotic sporocyte subjected to fluorescence, only the four chloroplasts can be seen because of the red autofluorescence of chlorophyll.

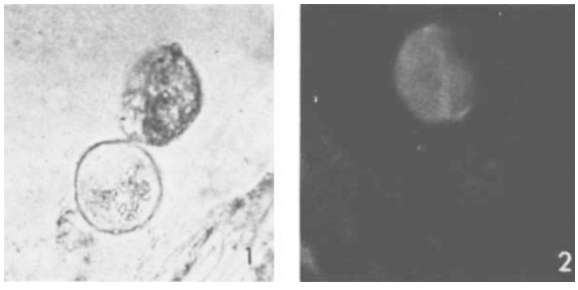

Fig. 1. Two living sporocytes under incandescent illumination. The post-meiotic sporocyte (top) contains a tetrad and the pre-meiotic sporocyte (below) contains four prominent chloroplasts. (× *c.* 770)

Fig. 2. Same field (reversed) as Fig. 1, but under ultra-violet illumination. The strong autofluorescence shows the exosporia of the tetrad in the post-meiotic sporocyte (top); whereas only the four chloroplasts of the pre-meiotic sporocyte (below) show autofluorescence of their chlorophyll. (× *c.* 770)

The phenomenon of autofluorescence in the exosporia of *Anthoceros* suggests that fluorescence microscopy may well be useful in investigations of sporogenesis among other bryophytes. Similar observations of other species of the same genus and representatives of other orders of bryophytes were made. Autofluorescence within the exosporium was found in the following species: *Reboulia hemisphaerica*, *Fossombronia salina Lindb*, *Anthoceros laevis* L., and *Anthoceros punctatus* L. With this technique, distinct developmental changes can easily be observed between the pre-meiotic and post-meiotic conditions. Indeed, fluorescence microscopy may also prove useful in studying exine development in angiospermous species. Exines of *Yucca treculeana* also exhibit bright yellow-green autofluorescence. The similarity of autofluorescence in bryophyte exosporia and pollen exines suggests a homologous chemical composition.

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MICROBIOLOGY

Transformation by Hybrid DNA in *Bacillus subtilis*

DOTY *et al.*[1] have reported that when an aqueous solution of DNA is heat-denatured and then annealed by slow cooling, the complementary strands separated by the thermal treatment recombine specifically. By this method, Marmur and Lane[2] made hybrid DNA molecules in which one of the complementary strands derived from the DNA of a streptomycin-resistant strain of *D. pneumoniae*, and the other strand from a wild-type strain. The hybrid molecules thus formed were said to be functional in transforming *D. pneumoniae* to streptomycin resistance.

In certain transformation experiments Herriott[3] used a molecular hybrid of the DNA extracted separately from two strains of *Hemophilus influenzae*, one streptomycin-resistant and the other catomycin-resistant. He found the number of double transformants to be significantly higher (fifty-fold) than with the control—a mixture of the two DNA materials denatured and renatured separately. This result may suggest that both strands of the hybrid molecule are effective in expressing the information into the recipient cell. Different results were obtained by Marmur *et al.*[4] and by Kent *et al.*[5] in *D. pneumoniae*.

Recently, Bresler *et al.*[6] obtained results for *B. subtilis* similar to those of Herriott. Using *his*⁻ and *try*⁻ strains as DNA donors, they observed that the hybrid DNA can transform a double auxotrophic strain to a prototrophic one. Moreover, when DNA synthesis by recipient cells is excluded, the hybrid molecules are still able to stimulate the ability of double auxotrophic cells to synthesize histidine and tryptophan. Therefore they assume that both strands of the double helix can serve as templates for messenger RNA synthesis.

We report here some experiments on transformation by hybrid DNA in *B. subtilis* in the system used by Bresler *et al.* The recipient strain was *B. subtilis SB* 25 ($his_2^-try_2^-$)—a double auxotroph for two linked markers[7,8]. In our experimental conditions, a 'cotransfer index' of 0·5 ± 0·03 was calculated. The two donor DNAs were isolated by Marmur's method[9]—one from a *B. subtilis* strain auxotrophic for histidine only (*SB* 25 his_2^-) and the second from a strain requiring tryptophan (*SB* 25 try_2^-). Hybrid DNA was formed by the procedure of Schildkraut *et al.*[10]. (For more details see the legend to Table 1.) In transformation assays the methods of Spizizen[11] and Nester *et al.*[8] were both used successfully. The most reliable results were obtained by a slight modification of the latter (20 μg/ml. of L-histidine in CHT 2 and CHT 10 media).

Table 1 shows that the number of double transformants obtained with the hybrid DNA is significantly higher than with the control mixture. A ratio his^+try^+/his^+ of 1×10^{-2} may be calculated. This value is close to that found by Bresler *et al.* in the same system.