A new type of interaction between spore colour mutants of *Aspergillus niger*

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Summary. A new white-spored mutant of the imperfect fungus *A. niger* is described. Behind the heterokaryotic heads formed with non-allelic spore colour mutants, it shows as a result of crossfeeding a uniform change of colour of the conidial heads in a band up to 4–5 mm deep. This mutation with pleiotropic effects on colour and crossfeeding occurs relatively frequently in *A. niger,* although it has not been reported previously.

The only interaction reported so far between different mutant colonies of *A. niger* derived from the Glasgow strains and grown at 35 °C on complete medium[2] has been the formation of heterokaryotic heads on the line where the hyphae of the colonies meet and show anastomosis. When formed by non-allelic spore colour mutants, e.g. fawn (*a*) and olive (*o*)[2], these heads are easily recognized, as the colour of the conidia is not cell-determined in this species[3]. The colour of each heterokaryotic head depends on the proportions of the parental nuclei in the vesicle and therefore ranges from black to a colour just darker than that of either parental strain.

A completely different reaction to the colour mutants fawn and olive has now been noticed in a non-allelic white-spored mutant, *w7*. This mutant was obtained following nitrosoguanidine treatment[4] (25% viability) of conidia from a wild-type colour strain. Behind the isolated heterokaryotic heads formed at the contact with fawn- or olive-spored colonies, the white-spored colonies showed a uniformly coloured margin up to 4–5 mm deep. This reaction was first observed in a master plate of olive- and white-spored haploids (figure 1) obtained on *p*-fluorophenylalanine-supplemented medium from a diploid which had been synthesized from an olive strain and the *w7* mutant. In addition to the parental strains and olive-spored segregants and recombinants, the plate contained 12 white-spored colonies, 11 of which were in contact with the olive strains. 10 of the 11 showed black-spored margin(s) along the adjoining olive colonies, as did the parental strain *w7*. The other had an olive-spored margin; when used later in a diploid with an olive-spored strain it was shown to contain *o* as well as *w7*. Similar tests did not detect the *o* marker in the white-spored colonies showing crossfeeding to black. The width of the bands and their uniform colour led to the hypothesis that they did not result from heterokaryosis but from crossfeeding. This was confirmed by 2 experiments. First, conidia from the olive or black bands gave only white-spored colonies, which when grown in contact with olive strains showed bands of the same colour as that from which the conidia had been plated. Conidia from the heterokaryotic heads at the junction of the colonies, on the other hand, gave the expected mixture of white and olive parental colonies. Secondly, the *w7 o* recombinant was grown in contact with a fawn, an olive and a wild-type spore colour strain (figure 2). Although black heterokaryotic heads were found as expected between *w7 o* and the fawn strain, the band crossfed by the fawn strain was olive-spored and indistinguishable from that formed along the olive colony. The wild-type strain did not crossfeed *w7 o,* nor *w7* as was

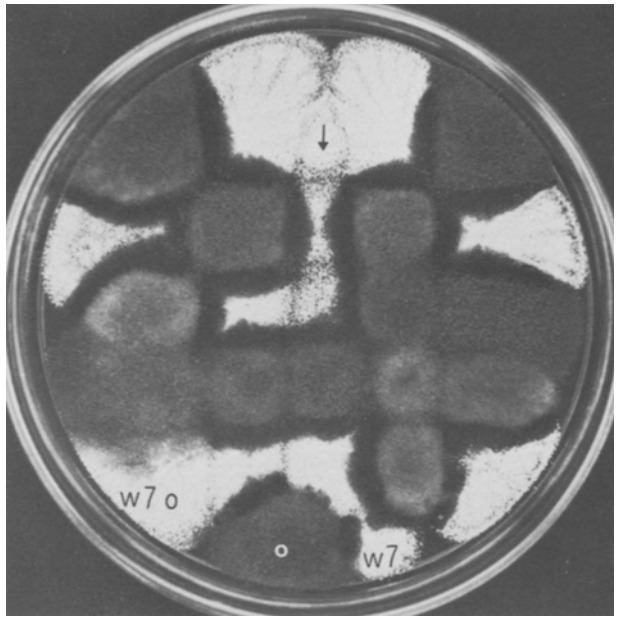

Fig. 1. Black- and olive-spored margins of white-spored colonies in contact with olive-spored strains. o and w7: respectively olive- and white-spored parents; w7 o: recombinant crossfed to olive. The centre of the only white-spored colony not in contact with olive-spored strains is shown by an arrow. The internal diameter of the petri dish is 100 mm.

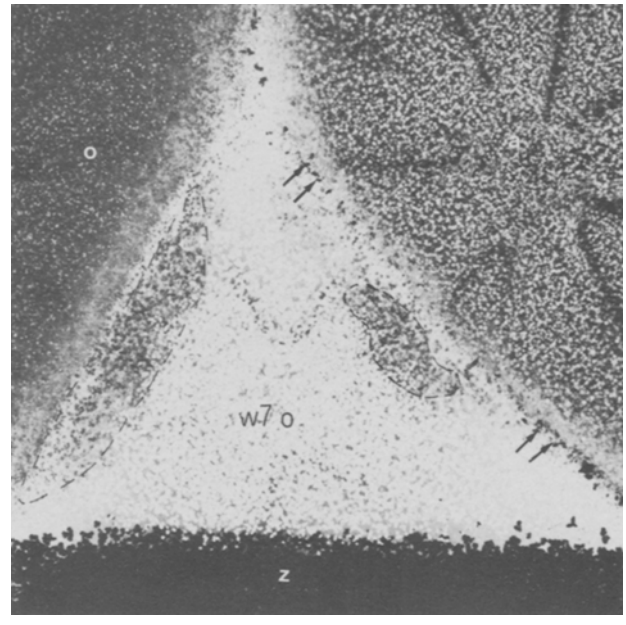

Fig. 2. Recombinant w7 o grown with an olive- (o), fawn- (a) and wild type-spored (z) strains. The line of dark heterokaryotic heads, some of which are shown by arrows, is present only between the fawn- and white-spored colonies. The only difference between the two bands crossfed to olive (their margin is indicated by black lines) is that the band crossfed by the olive colony is almost 3 times as long as that crossfed by the fawn colony. ×2.2. At this magnification, the uniform colour of all the heads in each mutant colony and in the crossfed bands is spoiled by the fact that all the conidial heads are not at the same level; for this reason, the heads located between the top layer and the medium appear as black spots even in the centre of the white-spored colony.

discovered in another experiment, but fawn strains crossfed them exactly as did olive strains, that is to olive and black respectively. One can therefore conclude that the bands are not due to heterokaryosis. Their presence depends on the block(s) responsible for the mutant phenotype of the crossfeeding strain, but their colour depends on the genotype of the crossfed strain. So far it has neither been possible to dissociate the ability of being crossfed from the light spore colour with which it was isolated, nor has it been possible to link crossfeeding to any growth requirement.

When it was realized that crossfed colour mutants might have been missed or discarded as unstable in previous mutagenic treatments, mutagenesis was repeated and isolation of spore colour mutants was done before contact had occurred between colonies. Among 18 phenotypically different spore colour mutants isolated under these conditions 2 showed crossfeeding, 1 of them in a gene different from *w7*. Both mutants showed the same basic crossfeeding reactions as *w7*. Details will be reported elsewhere.

It may be of interest to note that all *A. niger* spore colour mutants obtained so far, whether showing crossfeeding or not, show *p*-diphenol oxidase activity, while in *A. nidulans*[4] this enzyme has been identified as the crossfeeding material of yellow-spored mutants by wild-type green-spored strains. Another difference in the crossfeeding of the 2 species is that in *A. nidulans* crossfeeding is limited to the spores at the junction of the colonies[4].

The mechanism of the reaction is being investigated and it is hoped that the availability of crossfed mutants will help the study of spore pigmentation, pigment metabolism and perhaps conidiation.

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Photostimulation of ATP production, in cell-free extracts of insect integument

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Summary. Cell free extracts of *Pieris brassicae* integument or its 220,000 × g sonicated supernatant exhibit a light-dependent ATP production system (LAPS). ADP and Pi seem to be the substrates of the reaction. LAPS cannot be located in intact mitochondria.

One of the standard white-light experimental rhythms is 8 L:16 D (8 h white light followed by 16 h of dark). Under these conditions *Pieris* larval development lasts 23 days and 100% of pupae diapause[1-8]. In earlier experiments under the same conditions of quantic energy and rhythm we replaced, the white light with red light at the wavelength of the absorption maximum of pterobilin, the integumentary pigment. Larval development lasts 16 days without any diapausing pupae. Animals and their progeny are normal[9-11]. Red light has a particulary positive effect on the development of *Pieris*, since it prevents diapause and accelerates larval development[11].

These two spectacular changes in metabolism led us to assume that light, especially red light, can transfer its energy into some molecules such as ATP, which the integument can use directly.

In a previous paper we confirmed 1) that in vivo, the ATP content of the integuments increases together with the illumination time and 2) that in vitro, the ATP concentration in acellular integument-extracts also rises as a function of the illumination time[12].

This light-dependent ATP production system (LAPS) which is present in cell-free extracts of *Pieris* integuments is still far from being understood at the molecular level. In this paper we show that the LAPS can be split into small subunits which maintain their capacity for light-dependent ATP production, and that the substrates for such ATP production are probably phosphate and ADP.

Materials and methods. Animals. The 5th instar larvae of *Pieris brassicae* were chosen under equivalent physiological conditions[13] and grown under red or white lights as previously described[11]. Temperature was 19 ± 1 °C. Photoperiod was 8 L:16 D.

Preparation of acellular integument extracts. Animals were selected 30 min before the onset of the light period. Acellular extracts were prepared in the darkness at 4 °C. Integuments were homogenized (0.25 M sucrose, 2.5 mM $CaCl_2$ and 50 mM tris pH 7.2). Homogenates were centrifuged for 30 min at 60,000 × g. Certain supernatants were sonicated at 40 V for 40 sec and again centrifuged either at 100,000 × g for 60 min or at 220,000 × g for 30 min. The resulting supernatants were used for the experiments.

Acellular extract illumination. Extracts obtained in the dark were incubated in a large dish, so that a maximum surface

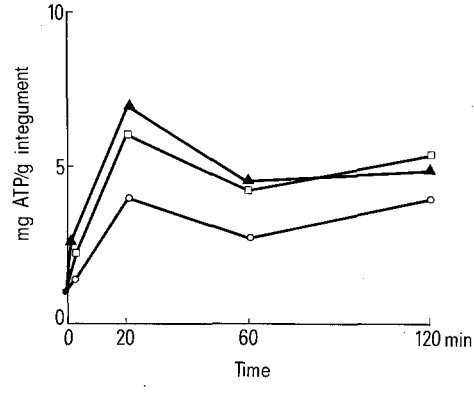

Fig. 1. LAPS activity in 60,000 × g supernatant after sonication (▲). For comparison, ATP concentrations were measured under the same conditions in non-sonicated 1000 × g supernatants (○) and in non-sonicated 60,000 × g supernatants (□).