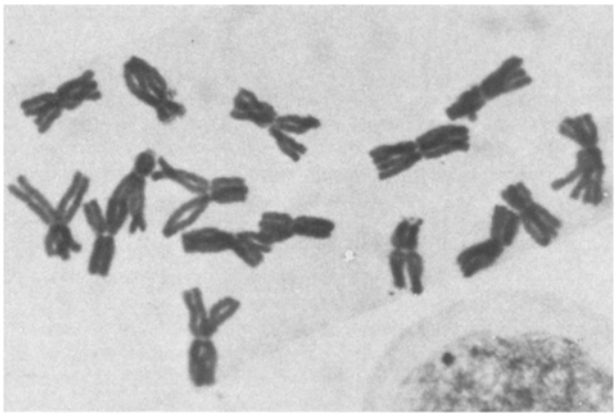

Endopolyploid root tip cell of *A. cepa* arrested in metaphase after a 2-h-treatment with 0.1% acetaldehyde, showing the typical diplochromosomes.

mitotic effect (arresting of the cells at metaphase) was evident. After the 2-h-treatment, no normal anaphases were observed. The highest dose employed (0.2%), resulted in a complete inhibition of mitosis (table). A 2-h-treatment with 0.1% acetaldehyde resulted in the highest frequency of endoreduplicated cells in mitosis (table and fig.), although, even so, the percentage of cells showing diplochromosomes was extremely low. A lengthening of the treatment time did not yield an increase in the frequency of endoreduplicated cells in mitosis but proved to be very toxic. The short treatment time rules out the possibility that such a treatment with acetaldehyde should have induced the endoreduplication of these cells. Instead, it seems that this chemical is able to stimulate spontaneous endopolyploid cells previously occurring in the meristem to divide.

In order to compare the short-term effect of acetaldehyde with that reported for excess thymidine[10], the roots were placed in colchicine for 2 h after a treatment with excess thymidine for 10 min. No endopolyploid mitosis was observed (table) indicating that a posttreatment of at least 3–5 h seems to be necessary for thymidine-stimulated cells to enter mitosis[10]. Nevertheless, when the treatment with thymidine was followed by a 2-h-posttreatment with 0.05% acetaldehyde, that per se did not induce endopolyploid mitosis, 2 cells showing diplochromosomes were observed (table). The extremely low number of endopolyploid cells in mitosis found by us is not surprising, if we consider that these are only a subset of the differentiated cells or the cells undergoing a process of differentiation, and therefore, their frequency in root tips cannot be expected to be high.

Since acetaldehyde has been shown to be a strong antimitotic agent in *A. cepa* root tip cells[9], it is rather surprising that a substance of this high toxicity could be able to stimulate mature cells to enter mitosis. Both auxins[5] and Kinetin[6] have been reported to act as triggers for mitosis in mature endomitotic plant cells. It has been also proposed that rhizobial infection might be analogous to the cytokinin-auxin stimulation, causing endomitotic cortical cells already existing in the root to undergo division and initiate nodules[7,11]. On this basis, the simplest explanation for the induction of endopolyploid mitosis by acetaldehyde appears to be that this chemical is able to induce a hormonal imbalance in the root. Nevertheless, a direct action at the cellular level to stimulate mitosis of mature cells cannot be excluded.

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Penicillium auxotrophic mutants can be detected by using xanthene dyes

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Summary. Auxotrophic mutants of *Penicillium* spp. have been directly isolated after mutagenic treatment from agar plates containing Xanthene dyes. They grow as characteristic small colored colonies. Some strains were tested and they showed a differential response depending on the Xanthene dye used.
Key words. Penicillium; Xanthene dyes; auxotrophs, selection of.

The dye Phloxin B (tetrabromotetrachlorofluorescein, Magdala Red) has been used to facilitate the detection of yeast mutants[1–4]. A common response of all of these mutants (respiratory-deficient[1], auxotrophic[2,3] and nitrogen-utilization mutants[4]) was the production of intensely stained small colonies that could be visually selected. In a previously described attempt to use this method with a filamentous fungus *(Aspergillus nidulans)*, auxotrophs and prototrophs were not clearly distinguished, but Magdala Red induced paramorphic colony growth of *A. nidulans* and *Penicillium* spp[5]. Of various Magdala Reds investigated by Scott[6], only Phloxin B induced paramorphic colonies in *Neurospora crassa*. Prompted by these results, we have examined the effects of Phloxin B, Eosin B and Eosin Y with *Penicillium* spp., and we have found that the dyes could indeed be used for the visual selection of auxotrophic mutants of these fungi. Xanthene dyes are used to color food and in hair treatment preparations. However, opinions differ as to whether these dyes are mutagenic[7]. We have also addressed this question here, and we have not detected mutagenicity or any effect on viability under our conditions.

Table 1. Number of auxotrophic mutants produced by UV light in three *Penicillium* species, recovered from MEA plates containing 10 µg/ml of Phloxin B

Species	Number of total colonies		Number of stained colonies		Number of non-stained colonies	
	Normal	Auxotrophs	Normal	Auxotrophs	Normal	Auxotrophs
P. chrysogenum	5985	30	385	30	5600	0
P. expansum	5802	6	27	3	5775	3
P. urticae	5688	81	162	40	5526	41

Table 2. Number of auxotrophic mutants induced by UV light in three *Penicillium* species, recovered from MEA plates containing 10 µg/ml of Phloxin B, Eosin Y, Eosin B, and without a dye

Species	Total number of colonies	Total number of auxotrophs with			
		No dye	Phloxin B	Eosin Y	Eosin B
P. chrysogenum	3621	17	17	7	5
P. expansum	3836	4	2	2	–*
P. urticae	4317	60	30	56	52

* *P. expansum* does not stain with Eosin B.

Material and methods. Phloxin B (CI 45410) was obtained from Sigma, and Eosin B (CI 45400) and Eosin Y (CI 45380) were from Merck. Malt extract, mycological peptone and purified agar were from Oxoid; other chemicals were from Merck. Malt extract agar (MEA) was as described by Pitt[8], and minimal medium agar (MMA) was as previously described by Pontecorvo et al.[9]. The dyes were added to the media at a final concentration of 10 µg/ml if not otherwise specified, the pH was adjusted to 5.7, and the media were sterilized by autoclaving at 115 °C for 30 min (this treatment did not affect the properties of the stains). Conidia of the strains used in this work (*Penicillium chrysogenum, Penicillium expansum,* and *Penicillium urticae* wild types), were treated with UV light or diethylsulfate (DES) at a dose producing a survival rate of 1%. After mutagenesis, a series of MEA plates with and without each one of the dyes were inoculated and then incubated for 4 to 7 days at 28 °C in the dark. Auxotrophic mutants were isolated and tested for stability by replica plating in MEA and MMA media five times, and identified by auxanograms[10]. Colored and unstained colonies were studied separately for each treatment.

Results and discussion. In preliminary experiments, colonies of previously isolated auxotrophic strains of *P. chrysogenum* stained intensely pink when grown on MEA with 10 µg/ml of Phloxin B, whereas wild-type strains produced non-pigmented and bigger colonies. Similar results were obtained with *P. expansum* and *P. urticae,* and with Eosin B and Eosin Y; exceptions were the *P. expansum* mutants that did not stain with Eosin B. It thus appeared feasible to use dye plates for the detection of auxotrophic strains.

Subsequently, mutagenesis of conidia with UV light or DES was carried out, and colonies grown on MEA medium with 10 µg/ml of Phloxin B were replicated in MEA and MMA media. Auxotrophs were recovered and tested for stability, and further characterized by auxanograms. Samples of stained and unstained colonies were treated separately for each case. As can be seen in table 1, all of the *P. chrysogenum* mutants were stained with Phloxin B, and no auxotrophs remained within non-stained colonies. For *P. expansum* and *P. urticae,* half of the auxotrophs stained with Phloxin B. For a further characterization similar experiments were carried out but with three Xanthene dyes: Phloxin B, Eosin Y and Eosin B, and results are presented in table 2. All of the *P. chrysogenum* mutants were stained with Phloxin B, only half with Eosin Y and one fourth with Eosin B. On the other hand, most of the *P. urticae* mutants were colored by the two Eosins, and only half of them with Phloxin B. For *P. expansum,* Eosin B did not stain any colonies whereas about half of the auxotrophs were stained with Eosin Y or Phloxin B. It is thus clear that there is some species specificity in susceptibility towards different dyes. Similar results were obtained with diethyl sulfate (DES) as the mutagenic agent, but the number of recovered auxotrophic mutants was smaller.

In all cases the number of stained colonies was higher than the number of auxotrophic mutants, the ratios varying between 2 and 6.8% for *P. chrysogenum,* 19 and 50% for *P. urticae* and 10% for *P. expansum.* These data agree with results obtained by other authors with yeast mutants[2,4]. The explanation of this fact is as yet unknown, but we think it may involve a process related to the uptake of metabolites from the medium into the cell: auxotrophs might color, as do some other mutants, because of permeability differences between strains.

The published data concerning the toxicity of Xanthene dyes are inconclusive. A mutagenic effect was proposed by Kuroda[11] and Kada et al.[12], whereas Nagai[13] supports a counteracting effect of Eosins at low concentrations on the production of some mutants, pointing out, however, that Phloxin B and Rose Bengal could be mutagenic at high concentrations. More recently Haveland-Smith et al.[7] did not find any conclusive evidence for the toxicity of Xanthene dyes. We have, however, observed that these dyes do not produce a decrease in the viability of the strains and that they are not mutagenic under the conditions used. The concentrations applied were low enough to detect these effects. A decrease in viability was only found when higher quantities (like 1 mg/ml) were used.

Finally, we conclude that this technique may be of use for the isolation of auxotrophic mutants of filamentous fungi, at least for *Penicillium* spp., as an enrichment method based on direct visualization of the colored colonies.

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