Results and discussion. In a first series of experiments, the incorporation of ^{14}C from glycine into IMP was investigated. In these experiments, each tube contained: sodium bicarbonate: 200 µmoles, sodium formiate: 120 µmoles, glycine 2 ^{14}C: 120 µmoles (spec. act.: 2000 cpm/µmole). Furthermore all acidic fractions containing IMP, co-chromatographied with unlabelled IMP. In order to ascertain that radioactivity was located in the purine ring, each labelled spot was eluted from the plate and hydrolyzed. Then the hydrolysate was run under identical conditions and all radioactivity was found at the same position as unlabelled hypoxanthine. According to our results, cell suspensions of *R. spheroides* (1.8 mg dry wt/ml) incubated in aerobiosis and dark were able to synthesize labelled IMP using glycine 2 ^{14}C as substrate. 2% of the radioactivity was incorporated within 4 h (data not shown) instead of only 1% in Schulman's experiments[11] working with animal tissues.

In order to study the biosynthesis of the purine ring through δALA transamination to DOVA, similar experiments were performed using δALA 5 ^{14}C and δALA 4 ^{14}C as substrates. The table shows the comparative incorporation of ^{14}C from δALA 5 ^{14}C and δALA 4 ^{14}C into IMP fractions and porphyrins.

When δALA 5 ^{14}C was the substrate, anaerobic and light-grown bacteria incorporated more radioactivity into porphyrins (10%) than cells grown aerobically in the dark (1%). However, the ^{14}C incorporation into IMP was low in both conditions, in spite of the fact that cells grown aerobically and in the dark could favour this incorporation due to their low rate of porphyrin biosynthesis. Also with 4 ^{14}C, δALA incorporation of radioactivity into IMP is low in both conditions of growth.

These results are not in agreement with a direct incorporation of δALA into the purine ring of nucleotides, through its conversion to DOVA. According to this pathway, at least more than 2% incorporation into IMP would be expected, instead of 0.1–0.3% obtained when using δALA 5 ^{14}C as substrate, an intermediate closer to the end product than glycine.

Our results suggest a nonspecific incorporation of ^{14}C from δALA into IMP, after δALA degradation; without a direct transamination. In addition, our data support the results of Neuberger and Turner[7] and Lohr and Friedman[12] that the transamination works in the reverse sense from DOVA to δALA.

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Electrophoretic variation in esterases in 3 wild-type and respective mutant strains of *Aspergillus flavus*[1]

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Summary. Wild-type strains and auxotrophic mutants of *Aspergillus flavus*, differing regarding aflatoxin production, were tested for esterases isozymes. Esterases variation was found in all strains used, and a possible correlation between the pattern of esterase bands and aflatoxin production is suggested.

The present work was designed to obtain preliminary information about the extent and the nature of electrophoretic variation of esterases isozymes within 3 wild-type isolates of the filamentous fungus *Aspergillus flavus*. Mutants derived from such strains were also tested for esterases isozymes in an attempt to study the influence of such mutants in relation to esterase production.

Material and methods. 3 wild-type strains (A5a, A6e and B2d) were used. The strain A5a did not produce aflatoxin, either in culture medium or mycelia. A6e has a high production of aflatoxin in mycelia (400 ppm of Bl-aflatoxin and 300 ppm of Gl-aflatoxin) but a low production in culture medium. B2d produces a high amount of aflatoxin both in culture medium (40 ppm of Bl and 30 ppm of Gl) and in mycelia (400 ppm of Bl and 300 ppm of Gl). Mutants strains A5a arg, A5a w, A6e arg, A6e pur, A6e y and B2d lys were also used. Mutant alleles were designated as follows: w and y, white and yellow conidia respectively; arg, lys and pur, requirement respectively for arginine, lysine and purines. The isolation of mutants and the aflatoxin determinations were reported elsewhere[2]. Stock cultures were mantained on complete medium slants[3]. Cultures were transferred to liquid minimal medium and, after 7 days incubation at 28 °C, they were used for electrophoretic analysis both in mycelia and culture medium. The electrophoretic technique was as follows: mycelia were homogeneized in 0.05 ml of distilled water at 4 °C. The homogenate and the

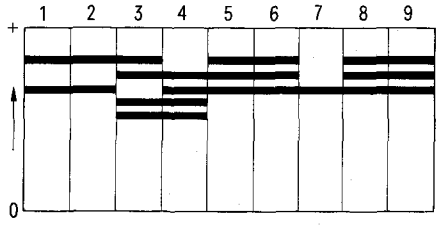

Fig. 1. Schematic representation of esterases zymograms from mycelia of wild-type strains: 2(A5a), 3(B2d), 8(A6e) and of mutants strains: 1(A6e y), 4(A5a arg), 5(A6e arg), 6(A5a w), 7(B2d lys) and 9(A6e pur).

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medium were subjected to horizontal starch-gel electrophoresis. The gels were prepared as described by Bush et al.[4]. Optimum resolution for esterases bands was obtained using borate 0.3 M electrolyte buffer, pH 8.2, and a gel buffer pH 8.7 containing 0.076 M tris (hydroxymethyl) aminomethane and 0.005 M citric acid. Each gel was prepared using 46.5 g of hydrolyzed starch (Sigma Chemical Company) and 300 ml of the gel buffer. The homogenate was absorbed into a small piece of Whatman No. 3 filter paper about 4×6 mm in size which was inserted in an incision made 3 cm from the cathode of the gels. Horizontal starch gel electrophoresis and enzyme assays were carried out using methods and solutions similar to those described by Toledo F° and Magalhães[5]. To prevent heat denaturation and loss of the enzyme activity, the runs were performed at 4 °C.

Each gel was incubated at 37 °C in a staining solution containing naphtyl acetate as substrate, Fast Garnet GBC salt as the dye Coupler and phosphate buffer in the mounts[5]. The stained gels were fixed in a solution of methanol, acetic acid and distilled water in the proportion of 5:1:5.

Also, in one strain (A 5a w), the pattern of electrophoretic isozymes of cultures from 1 to 7 days old was carried out.

Results and discussion. The esterase patterns of the wild-type and mutant strains are presented on figures 1 and 2. They differ both in mycelia and culture medium. In relation to the mutants, large variations were found in mycelia (figure 1) and only a slight variation on culture medium (figure 2).

The esterase pattern of A 5a w strain from 1 to 7 days development has shown that esterases are not produced during the first day and 3 bands are obtained in the 7th day (figure 3).

Esterases variations were found in all 3 strains of *A. flavus* used. Although only 3 strains were used, the strain with no aflatoxin production presented only 2 bands and 3 and 4 bands were found in strains with aflatoxin production in mycelia and medium respectively. A larger number of strains should be tested in order to see if the pattern of esterases production is related to aflatoxin production. Also, in relation to esterases in culture medium, the strain with no aflatoxin production, presented no bands, the strain which contained aflatoxin just in the mycelia, presented one band and the strain which synthetizes aflatoxin both in mycelia and culture medium, presented 2 bands. Mutants derived from A 6e wild-type strain gave the same pattern of esterase bands in relation to the original strain exception of A 6e y. Other mutants differ in relation to the wild-type in bands patterns. Several reasons can be given to explain such differences, such as: a) different rates of growth; b) the influence of the auxotrophic or morphological markers on esterase production; c) other mutation induced during the mutagenic treatment for the obtention of the auxotrophs mutants, which also interfere with esterase production. More extensive investigations, should be carried out making use of crosses between such strains. It has been shown[6] that *A. flavus* is ammenable to genetic studies through the parasexual cycle. The use of strains with different patterns in esterase bands can be useful in a genetic study with *A. flavus*. Also, if a correlation between esterases and aflatoxin production does occur, this can be of help in the study and detection of aflatoxin producing strains.

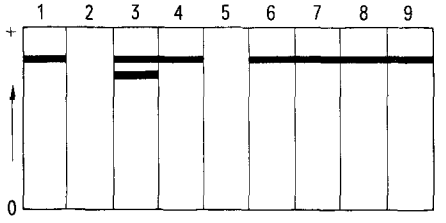

Fig. 2. Schematic representation of esterase zamograms from culture medium: 2, 3 and 8 are the culture medium of wild-type strains A5a, B2d and A6e, respectively. The 1, 4, 5, 6, 7 and 9 are the culture medium of mutants strains A6e y, A5a arg, A6e arg, A5a w, B2d lys and A6e pur, respectively.

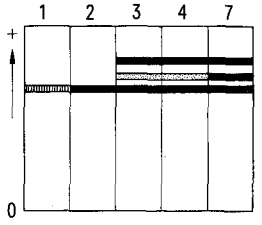

Fig. 3. Schematic representation of esterase zymograms from mycelia of A5a w-strain from 1 to 7 days development. Esterases are not produced during the first day and 3 bands are obtained in the 7th day.

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Activity tests of alcohol dehydrogenases in wheat, rye and triticale[1]

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Summary. The relative band staining intensities of ADH isoenzymes in wheat and triticale suggest alloploid genome interactions. Rye ADH is scarcely affected by anti-wheat-ADH. Despite the evolutionary divergence of their Adh genes, ADH monomers of wheat and rye form enzymatically active heterodimers in triticale.

A number of enzyme loci, found associated with homoeologous chromosomes in hexaploid wheat and diploid rye (*Secale cereale*), confirmed, at the biochemical level[3], the alloploid origin of wheat as well as of the wheat-rye alloploid triticale.

The data about the isoenzyme patterns of alcohol dehydrogenases of wheat, rye, and hexaploid triticale presented here, support the hypothesis of triplicate Adh genes in wheat and a 4th gene in rye[4]. However, minor differences in relative band staining intensities are revealed densitometrically, suggesting different activities of either the enzymes or the genes, which have hitherto been accepted to be equally expressed. Precipitation tests with antiserum against wheat ADHs should demonstrate