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Note

Detection of 5-amino-4-imidazole-N-succinocarboxamide ribotide and hypoxanthine accumulation. A simple method for identification of some purine auxotrophs

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In micro-organisms, adenine auxotrophs can be divided into two different classes on the basis of growth on hypoxanthine-supplemented medium. It is known that mutants that cannot grow on this medium map in genes coding for adenylosuccinate lyase or adenylosuccinate synthethase[1], and it is impossible to distinguish between those specific adenine mutants on the basis of growth only. Assays of enzyme activity are tedious, as adenylosuccinate synthetase can be assayed only in strains free from adenylosuccinase[1]. Therefore, the study of accumulation of metabolites from the adenine biosynthetic pathway is often used for mutant classification[1,2]. It is known[1,2] that accumulation of 5-amino-4-imidazole-N-succinocarboxamide ribotide (SAICAR) is observed in adenylosuccinase-free strains, whereas hypoxanthine is accumulated in strains without adenylosuccinate synthetase activity. So far, however, no rapid and reliable method suitable for screening tests has been devised.

MATERIALS AND METHODS

Chromagram sheets 131181 (silica gel 6060, with fluorescent indicator) were purchased from Eastman-Kodak (Rochester, N.Y., U.S.A.). Hypoxanthine was obtained from Biomed (Warsaw, Poland) and Dowex 50W-X8 resin (200–400 mesh) from Serva (Heidelberg, G.F.R.).

The experiments were carried out on strains of *Aspergillus nidulans*: the *ad*A strain [lacking adenylosuccinate lyase (EC 4.3.2.2)] was from the Warsaw University collection, and the *ad*B strain [lacking adenylosuccinase synthetase (EC 6.3.4.4)] was obtained from the Fungal Genetics Stock Centre. (Humboldt State University Foundation, Arcata, Calif., U.S.A.).

The photographs were taken on Fotopan CD film under 254-nm illumination.

Sample preparation

Of the two methods used for sample preparation, the first was as described by Foley *et al.*[1]. In the second, mycelium was ground in a mortar with glass powder in a small volume of water, and the mixture was subsequently centrifuged at 20,000 g for 10 min. The supernatant liquid was deproteinised by adding 0.2 ml of 1.5 *N* perchloric acid per ml and the precipitated protein was removed by centrifugation.

The supernatant liquid was de-salted on a short Dowex (H^+) column, 2 *N* ammonia being used to elute the metabolites. Ammonia was evaporated from the eluate, and the residue was dissolved in a small volume of water for application to the chromatosheet.

Chromatography

The chromatogram was developed in 25% ammonia–isopropanol (3:7, v/v). After drying, the chromatogram was illuminated with 254-nm radiation; UV-absorbing compounds were easily detected in this way and were photographed.

RESULTS AND DISCUSSION

The experiments were performed on *ad*A and *ad*B mutants of *A. nidulans*. As shown in Fig. 1, accumulation of SAICAR was observed in the *ad*A mutant, whereas hypoxanthine was accumulated by the *ad*B mutant (SAICAR was below the limit of detection), which agrees with the expected results.

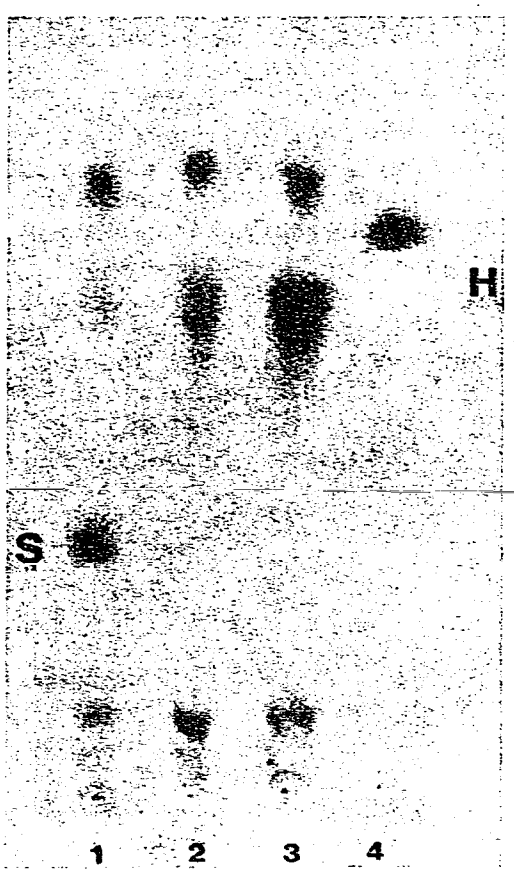

Fig. 1. One-dimensional chromatogram of metabolites of the adénine biosynthetic pathway. It should be noted that the hypoxanthine standard migrates in a different way when applied alone (ref. 4) than when co-chromatographed with an *ad*B strain extract (ref. 3); this is probably due to a salt effect. S = SAICAR; H = hypoxanthine; 1 = *ad*A strain; 2 = *ad*B strain; 3 = *ad*B strain + hypoxanthine; 4 = hypoxanthine.

The method described here allows rapid processing of many samples in a relatively short time. In comparison, the cross-feeding method of Nelson[3] is often difficult because of problems associated with sterility. The paper-chromatographic method of Bernstein[4], involving use of special colour reactions, permits distinction between several imidazole compounds, but is very time-consuming.

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