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Recommended Citation
Favraud, L., and J.L. Azevedo (1987) "Isolation and characterization of deteriorated sectors from duplicate strain A of A. nidulans.," Fungal Genetics Reports: Vol. 34, Article 4. https://doi.org/10.4148/1941-4765.1550

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Abstract
Isolation and characterization of deteriorated sectors from duplicate strain A of A. nidulans.

This regular paper is available in Fungal Genetics Reports: https://newprairiepress.org/fgr/vol34/iss1/4
The strain A of *Aspergillus nidulans* has a chromosome segment of linkage group I in duplicate - one in the normal position and the other translocated to linkage group II; the strain is unstable due to the excess of genetic material and produces two broad classes of sectors - improved and deteriorated (Azevedo and Roper 1970 Genet. Res. 16:79-93).

The aim of this work was to isolate deteriorated sectors spontaneously obtained from strain A, to analyze genetically the variants and see if their determinants of deterioration are located at random in the eight linkage groups.

The strain A was inoculated in the center of Petri dishes with Complete medium (Pontecorvo et al. 1953 Adv. in Genetics 5:141-238). Deteriorated variants were isolated and labelled as V53, V54, etc. Crosses were made between these variants and "Master" strains (MSE or MSF) carrying markers on all eight linkage groups (McCully and Forbes 1965 Genet. Res. 6:352-359). Mitotic haploidization was used for the assignment of determinants to their linkage groups. In some cases, further crosses were made to locate determinants more precisely and to test allelism among determinants on the same linkage group.

**Figure I. Location of the determinants of deterioration in the linkage groups of *Aspergillus nidulans.*
Nine variants and four derivatives of variants were genetically analysed. The determinants of deterioration acted as single genes; all of them were recessive in the diploid. They were found in all linkage groups, with the exception of groups V and VIII (Figure 1). In Figure 1, linkage maps are not drawn to scale and are approximate. The symbol \( \wedge \) designates a deletion. The symbol \( \wedge \) designates the determinant of deterioration while a square designates the genetic marker of the "Master" strain. The broken line represents the duplicate segment of linkage group I terminally attached to linkage group II. Variants allocated only to their linkage group are shown at the right. Centromeres are represented by open circles. The symbol /---\ indicates that the determinant of deterioration of the variant is linked to the genetic marker and may be located in any of the sides.

To perform the allelism tests, besides the variants isolated in this work, some deteriorated variants obtained by other authors were used. When the two determinants of deterioration are not alleles, we find normal and deteriorated colonies. If the determinants are alleles we find only deteriorated colonies. The determinants of deterioration were alleles in only two out of sixteen crosses performed. These results indicate that the determinants of deterioration must be located at random in the linkage groups, although there are spots in certain linkage groups in which an expressive number of determinants of deterioration is located. Supported by PIG/CNPq. ---^1Dept. de Genetica, I.B., UFRJ, Caixa Postal 68.011, Rio de Janeiro, Brazil; ^2Inst. de Genetica, ESALQ/USP, Caixa Postal 83, Sao Paula, Brazil

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Furukawa, K., K. Hasunuma and Y. Hamada

We have analyzed orthophosphate repressible enzymes produced in culture medium of wild type (74A) hyphae grown in low phosphate medium. Protein and enzyme relationships of alkaline phosphatase, 5'-nucleotidase, acid and alkaline nuclease, cyclic phosphodiesterase (cPDase), endonuclease and ribonuclease N1 were established using SDS-polyacrylamide gel electrophoresis and two dimensional gel electrophoresis (Furukawa, Hasunuma and Shinohara, submitted).

To analyze the regulation of these enzymes at the transcriptional level and to clone cDNAs for these genes, the development of an efficient method for the preparation of polyadenylated messenger ribonucleic acid from Neurospora mycelia was essential. In addition to the above nucleases, wild type hyphae grown in low phosphate medium contain numerous nuclease activities (K. Hasunuma, 1978, Molec. Gen. Genet. 160:259-265). To block these nuclease activities, the isolation method for mRNA using 6 M urea and 3 M LiCl (J.A.A. Chambers and V.E.A. Russo, 1986, Fungal Genet. Newsl. 33:25-26) is insufficient. For our method, we modified the phenol based procedure for mRNA isolation (W.R. Reinert, V.B. Patel and N.H. Giles, 1981, Molec. Cell. Biol. 1:829-835). The procedure results in a good yield of RNA (1.1 mg/g fresh wt.) compared with the other procedure (0.5-1 mg RNA/g fresh wt. with 6 M urea and 3 M LiCl). The procedure is also suitable for mini-preparations and for radioisotopic labeling. The procedure is as follows:

A conidial suspension (10^6 cells/ml; 10 ml) was inoculated into 1 l of low phosphate (1/20 Pi) Fries minimal medium in a Sakaguchi flask (2 l). The culture was shaken at 25° C for 24 hr. Fifteen minutes before harvesting hyphae, cycloheximide (final concentration, 0.5 mg/ml) was added. The hyphal pads were collected by filtration and stored frozen at -80° C.

Frozen hyphae (10 g) were powdered with a pestle in a mortar containing liquid nitrogen. Five volumes of 0.1 M Tris (pH 9.0)/1 mM EDTA/1% SDS were added and the same volume of 80% phenol equilibrated with the above buffer was added. The mixture was well ground in the mortar and further homogenized using a Polytron homogenizer (Kinematica type PT10/35) at full speed to 4 to 5 set (method II). In the original procedure we did not use a Polytron homogenizer (method I). (The Polytron homogenizer could also be used for mini-preparation of RNA and samples labeled with a radioisotope). A high-pH extraction buffer (pH 9.0) was used for efficient solubilization of RNA (C. Auffrang and F. Rougeon, 1980, Eur. J. Biochem. 107:303-314) and to avoid degradation of RNA by nucleases since most of the nucleases show pH optima of around 6.