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Altered glycosaminoglycan of the pk mutant

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Abstract

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Mutants of the peak (pk) locus of N. crassa are aberrant in both hyphal and ascus morphology (Murray and Srb 1962 Can. J. Botany 40: 337). Recessive, dominant and temperature-sensitive alleles have all been isolated at the pk locus (Srb and Basl 1969 Genet. Res. 13:303) and unlinked modifiers of dominance effects of pk are also known (Russell and Srb 1972 Genetics 71:233). However, no consistent molecular alteration related directly to the morphology of the pk mutants has been shown. Reissig and Glasgow (1971 J. Bacteriol. 106:882) reported the isolation of a growth regulating glycosaminoglycan from the medium of N. crassa. They detected no difference in the isolated glycosaminoglycan from wild-type or cot-1 grown either at the permissive or the restrictive temperature. In view of the reported growth regulating capabilities of this substance, the glycosaminoglycan from pk mutants was examined by ion-exchange chromatography to determine whether differences existed between it and that isolated from wild-type.

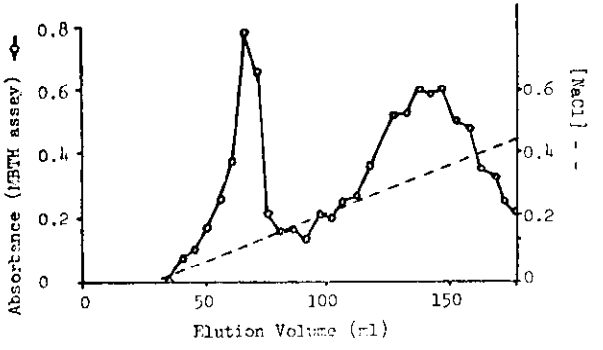

Figure 1

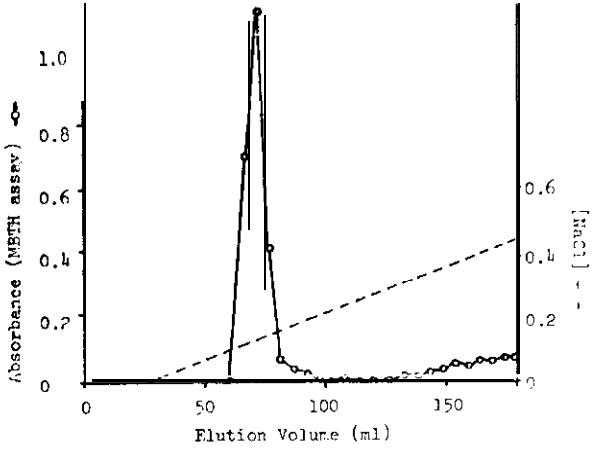

Figure 2

All stocks were maintained on Vogel's minimal N medium. Cultures were grown on a fresh slant of Vogel's N for five days, a heavy inoculum was transferred to 30ml liquid Vogel's N in a 125ml Erlenmeyer flask and incubated on a rotary shaker at 25° C for 48 hr. The medium was decanted and replaced with 50ml fresh medium, the culture was sheared in a Waring semi-micro blender cup, and used to inoculate 1000ml fresh medium in a 2.8 liter Fernbach. The culture was again incubated on a rotary shaker at 25° C for 48 hr. The contents of the Fernbach were filtered through several layers of cheesecloth and the medium collected for precipitation. Two volumes cold (6° C) 95% ethyl alcohol were added to the medium and it was allowed to precipitate overnight at 6° C. The resultant precipitate was collected via centrifugation, resuspended in 1 M NaCl (40ml/1000ml medium precipitated), and washed once with an equal volume of chloroform:isoamyl alcohol (24:1). A 5ml aliquot of the saline solution was applied to a 1.6 X 100 cm column of Sephadex G-50 (fine) equilibrated at pH 8 with 5mM TES (N-tris(hydroxymethyl) methyl-2-aminoethane sulfonic acid). The column was eluted with the same buffer at a flow rate of 0.8ml/min. The first 150ml from the Sephadex column was washed directly onto a 1.6 X 17 cm column of coarse mesh carboxymethylcellulose equilibrated with the same buffer. The column was then eluted with a 200ml 0-0.5 M NaCl linear gradient followed by a 50ml wash with 1 M NaCl. The linear gradient and the high salt wash were collected as 5ml fractions. Conductivity and hexosamine content of each fraction were assayed. The hexosamine content was assayed via the MBTH (3-methyl-2-benzothiazolinone hydrazone) assay (Tsuji, Kinoshita and Hoshino 1969 Chem. Pharm. Bull. 17:1505).

Application of the above technique shows that both St. Lawrence 74A and 77a have the same elution pattern (Figure 1). The two peaks and their relative positions with respect to the gradient are consistent. Each peak of hexosamine-containing material, when separated and rechromatographed, shows the same elution pattern as the original sample, indicating a reversible relationship between the material in the two peaks of wild-type. Five alleles of the pk locus were tested and all demonstrate the elution pattern shown in Figure 2. The alleles tested include three dominant alleles (Pk1, Pk3, and Pk4) and two recessive alleles (pk2 and pk5). The biochemical phenotype cosegregates with the morphological phenotype when Pk1 is crossed to wild-type.

This analysis is now being extended to non-allelic mutants which are characterized by altered hyphal and ascus morphology. Each locus tested to date demonstrates its own elution pattern. These preliminary results indicate that a single biosynthetic pathway might be affected by several of these loci. - - - Department of Genetics, Development and Physiology, Cornell University, Ithaca, New York 14853.