Fungal Genetics Reports

Volume 21 Article 4

Electrophoretic analysis of rhythmic morphological mutant strains

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Recommended Citation

Russell, P. J., and J.D. Hewitt (1974) "Electrophoretic analysis of rhythmic morphological mutant strains," *Fungal Genetics Reports*: Vol. 21, Article 4. https://doi.org/10.4148/1941-4765.1797

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Abstract

Electrophoretic analysis of rhythmic morphological mutants

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Electrophoretic analysis of rhythmic, morphological mutant strains.

Many morphological mutant strains of N. crassa have been shown to exhibit a hyphal branching rhythm on solid medium that is characteristically non-circadian. Four of these mutant strains, representing three functionally non-allelic loci, were the subject of this study; namely, Pk-1 (Russell and Srb 1972 Genetics 71: 233) and clock at the peak locus, and 22-214 (allelic with scumbo) and 24-310 at two other loci. After 72 hours of growth at 25 °C on Vogel's complete medium in petri plates, colony diameter (in mm.) were: Pk-1, 11-13; clock, 71-75, 22-214, 41-52; 24-310, 47-53. The number and type of growth bands at this time were: Pk-1, two to three, concentric; clock, three, concentric; 22-214, two to four, irregular and non-concentric; and 24-310, three, concentric. In addition to the mycelial phenotypes just described, all of the mutants are abnormal ascus mutants, all being zygote recessives with the exception of Pk-1, which is zygote dominant. All strains were obtained from A.M. Srb, except clock which was obtained from the FGSC.

Experimentally, we investigated the hypothesis that rhythmic hyphal growth might be characteristically the result of a particular change in the protein composition of the mycelium. Polyacrylamide gel electrophoresis was chosen as the means of detecting such a change. Only acidic proteins were compared in this study.

Crude extracts for electrophoretic analysis were prepared as follows: Strains were grown with shaking at 25°C in Vogel's minimal medium. The resulting mycelial growth was harvested on cheesecloth and ground with sea sand and 0. 1 M phosphate buffer, pH 7.0, in a pre-cooledmortar and pestle. The resulting slurry was centrifuged at 16,000 r.p.m. (35,000 x g) in a Sorvall RC-2, SS-34 rotor. The supernatant was retained and stored frozen until required.

The method employed for the separation of proteins on polyacrylamide disc gels was essentially that described by Barber et al. (1969 Dev. Biol. 20: 105). The 7 cm separating gel contained 7% acrylamide and 0.184% bisacrylamide, and the 0.5 cm stacking gel contained 2.5% acrylamide and 0.625% bisacrylamide. Both electrode chambers contained 0.025 M Tris, 0. 19M glycine buffer, pH 8.3. Approximately 700 µg of protein was applied to each gel, a current of 1 ma/gel was applied and electrophoresis carried out at 5°C until the tracking dye (bromphenol blue) was 0.5 cm from the bottom of the gels. The gels were removed from the tuber and stained with Coomassie Brilliant Blue (Sigma).

Figure 1 is a diagrammatic representation of the acidic proteins visualized following acrylamide gel disc electrophoresis of crude extracts prepared from wild type (74A) and the four mutant strains described. The Rf values for the protein bonds of these gels ore listed in Table 1. Gels from replicate experiments were not significantly different. Comparisons of the gels indicated that for each rhythmic mutant strain several protein band differences from the wild-type pattern were apparent. This might be expected since Barber, Srb and Steward (ibid.) demonstrated that morphologically different strains of N. crassa exhibited altered electrophoretic protein banding patterns from wild type.

Furthermore, these authors showed that a specific banding pattern was characteristic of a particular morphology, and slight variations in morphology were accompanied by minor changes in protein bonding pattern in acrylamide gels. Thus, since the morphologies of the rhythmic mutants examined in this study were distinct from that of wild type, their protein banding patterns were expected to be altered from that of wild type. Also, since the rhythmic mutants were themselves characterized by distinct morphological phenotypes, we hypotheiszed that their respective protein bands would differ. Indeed, each rhythmic mutant strain showed a number of protein band differences from wild type, these differences being unique for each strain.

In addition, we hypothesized that a common mechanism (oscillating system) might be responsible for rhythmic mycelial growth of Neurospora. If this were so, one might expect that rhythmically growing mutant strains might exhibit certain similarities in their protein compositions that ore nevertheless different from wild type. Examination of the gels diagrammed in Fig. 1 indicates that none of the protein differences from wild type were coincident for all four mutant strains. One band (Rf 52) present in wild type was absent from three of the strains, namely 22-214, 24-013 ond clock (although not unambiguously so in the latter), but the band was clearly present in the Pk-1 gel. Therefore, these results would tend to argue against the proposed hypothesis under there particular experimental conditions. Nevertheless, the results as a whole present further evidence for pleiotropic protein differences in morphological mutant strains of N. crassa

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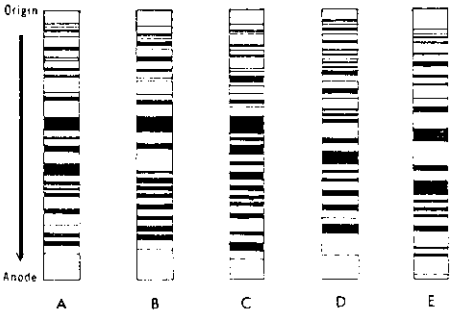

Figure 1.

Table 1. Rf values for the protein bands of the polyacrylamide gels depicted in Fig. 1.

74A	cl-11	Pk-1	22-214-A	24-310-A
5	6	6	5	8
6	10	8	6	9
8	11	10	8	10
10	13	14	10	12
14	16	17	13	17
19	19	18	16	20
20	25	23	18	25
23	28	25	20	29
27	29	27	21	33
34	35	30	23	37
36	37	33	25	46
44	44	36	29	60
52	56	39	32	66
56	68	43	35	71
64	70	52	40	74
70	74	55	42	77
73	75	61	44	82
75	81	65	52	90
82	86	71	59	92
89	91	74	66	
92	94	77	69	
55		82	74	
		90	78	
		95	85	
			88	

Rf values were measured from the origin to the cathodal side of each stained bond. The tracking dye position in each gel was given an Rf value of 100.