A Genetic Study of an Extracellular Elastin-hydrolysing Protease in the Ringworm Fungus *Arthroderma benhamiae*

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**SUMMARY**

Analysis of crosses between granular and downy self-sterile strains of *Arthroderma benhamiae* (= *Trichophyton mentagrophytes* var. *granulosum*) displaying high and low extracellular elastin-hydrolysing capabilities indicates that the locus for mating-type segregates independently of a colonial morphology locus and a locus determining elastin-hydrolysing capability. The colonial morphology locus consists of two alleles which govern the production of granular or downy phenotypes, and is closely linked to a locus consisting of two alleles which determine high or low extracellular elastolytic activity, showing a recombination frequency of approximately 5%.

**INTRODUCTION**

With the discovery by Ajello & Cheng (1967) of *Arthroderma benhamiae*, the cleistothecial phase of *Trichophyton mentagrophytes* (Robin, 1853) Blanchard, 1896, var. *granulosum* Neveu-Lemaire, 1911, and characterization of its two-allele incompatibility locus, it became feasible to investigate genetic affinities of the taxa of the *T. mentagrophytes* complex (Ajello, Bostick & Cheng, 1968; Padhye & Carmichael, 1969), and to begin a pursuit by meiotic analyses of the genetic and biochemical bases for pathogenicity in this fungus.

Recently, inheritance of granular (wild-type) and downy (a natural variant) colonial forms of *Arthroderma benhamiae* was shown to be governed by two alleles at a single locus, either non-linked, or distal to, the mating-type locus (Maniotis & Cheung, 1973).

Recent studies dealing with enzymes possibly involved in pathogenicity of this fungus have tended to demonstrate correlations between high or low protease activities and types of clinical lesions produced, or mating-types of the strains involved.

Genetic studies aimed at defining loci governing protease activities in natural variants would be of obvious value in this study area. This work reports the results of genetic crosses of downy and granular strains of *Arthroderma benhamiae* displaying high and low activities of an extracellular protease system which hydrolyses particulate elastin.

**METHODS**

*Fungal isolates and media.* Type cultures of *Arthroderma benhamiae* Ajello & Cheng, 1967, NCDCX797, mating-type *A*, and X798, mating-type *a*, were generously provided by Dr Libero Ajello, Center for Disease Control, Atlanta, Georgia, U.S.A. Strain X797 was used in this report, however, is a natural, downy variant which arose spontaneously in the granular parent culture, X797. It was shown that its phenotype was determined by a single allele, *cmd* (colonial morphology, downy), allelic to granular colony morphology, *cmg*, or wild-
type, and unlinked or distal to, the incompatibility locus (Maniotis & Cheung, 1973). Strains P46, P80, C55, C61 (ATCC 22778), 78-1 (ATCC 22780), 78-2, 78-6 (ATCC 22781), and 78-10 are monoascospore progeny produced from crossing X797D with X798. Strains of the 5546 series, 5546-52 (ATCC 22779), -59, -60, -63, and -77 were monoascospore progeny from a cross of P46 × C55. BM4 is a biochemical mutant derived by ultraviolet treatment of microconidia of strain X798.

Cultures were maintained on Sabouraud Dextrose Agar: 4% (w/v) dextrose, 176 (w/v) Difco Neopeptone, and 1.5% (w/v) Difco Bacto Agar. Culture stocks were maintained on Difco Bacto Potato Dextrose Agar at 5°C. Non-keratinous agar fruiting medium, prepared according to the recipe (Medium D) of Weitzman & Silva-Hutner (1967) was used for mating-type determination and production of cleistothecia.

Growth conditions. All inoculated plates were cultured in an incubator at 30°C in the dark.

Isolation of progeny. Ascospores were obtained from mature cleistothecia 21 days after inoculation of compatible strains on the fruiting medium. Cleistothecia were washed in a series of saline—Tween-80 solutions and violently agitated to remove adherent microconidia. The washed cleistothecia were then crushed in saline solution by violent agitation in glass beads to break the peridial walls. The saline suspension of ascospores obtained was filtered through Pyrex wool to remove peridial and hyphal debris, and appropriate dilutions of the suspension were made with saline employing a haemocytometer to obtain 250 to 300 monoascospore germlings per plate after the suspension was spread on to Sabouraud Dextrose Agar. Germlings were removed to individual plates after 18 h of incubation. Specific details of the procedures followed are listed elsewhere (Maniotis & Cheung, 1973).

Mating-type tests. To determine mating-types of strains, and to study the pattern of inheritance of mating-types, each strain was inoculated on to three agar fruiting medium plates, and after 2 days of growth, two of these plates received conidial suspensions of the tester strains (A and a), while the third plate served as a control. The plates were examined 7 and 14 days after receiving conidia to determine the mating reaction of the strain as evidenced by the appearance of mature cleistothecia on one of the plates.

Detection of elastin-hydrolysing activity. Detection of elastin hydrolysis was effected using plates of elastin medium prepared by adding 0.6 g of elastin powder (Worthington Biochemical Corporation, Freehold, New Jersey, U.S.A.) and 1.5 g of Difco Bacto Agar to each 100 ml of Difco Bacto Czapek Dox Broth. The pH of the medium before autoclaving was 7.3. The plates were inoculated, incubated at 30°C, and examined for zones of clearing in the elastin medium around and within growing colonies after 5, 7, and 10 days. A colony that displayed a zone of elastin hydrolysis (as evidenced by the degradation of elastin particles) greater than the diameter of the colony was considered to have high elastin-hydrolytic activity (Fig. 1). In contrast, a colony that displayed a clear area less than the diameter of the colony was considered to have low elastin-hydrolytic activity.

RESULTS

Ninety-six monoascospore progeny from a cross between two parents, one with a high and one with a low elastin-hydrolysing capacity, were scrutinized after 7 days of incubation to ascertain the relationship between radial growth and elastin-hydrolysing capability. A very definite relationship was apparent: 49 strains with high elastin-hydrolysing capabilities (range of zones of elastin clearance: 17.5 to 32.0 mm, µ (arithmetic mean) = 23.7 mm) had large colonial diameters (range of diameters: 20 to 28 mm, µ = 24.5 mm), while 47 strains with low elastin-hydrolysing capabilities (range of zones of elastin clearance: 0 to 18.5 mm,
Fig. 1. Extracellular elastin hydrolysis in four representative cultures of *Arthroderma benhamiae* derived from the cross of *cmd prt*<sup>+</sup> x *cmg prt*<sup>-</sup>. The two cultures on the left display high elastolytic activity, as evidenced by the degradation of particulate elastin in the medium. Cultures on the right display low elastolytic activity. × 0.6.

μ = 9·7 mm) showed small colony diameters (range: 13 to 24 mm, μ = 18·4 mm). Only six high elastin-hydrolysing cultures had growth rates in the range of the low elastin-hydrolysing cultures.

Monoascospore progeny obtained from three crosses (Table 1, crosses A), each between a strain showing high elastolytic activity and a strain displaying low elastolytic activity, can be categorized into two groups; one group with high, and one group with low elastolytic activity, and these in a ratio of 151:140, respectively. Two crosses each between strains displaying low elastolytic activity yielded progeny all of which had low elastolytic activity (Table 1, crosses C). These results strongly support an hypothesis that high or low elastolytic activity in these strains is mediated by a single locus consisting of two alleles, designated
Table 1. Linkage of protease and colonial morphology loci in Arthroderma benhamiae

<table>
<thead>
<tr>
<th>Crosses</th>
<th>prt&lt;sup&gt;+&lt;/sup&gt;</th>
<th>prt&lt;sup&gt;−&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cmd</td>
<td>cmg</td>
</tr>
<tr>
<td>A. cmd prt&lt;sup&gt;+&lt;/sup&gt; x cmg prt&lt;sup&gt;−&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. 5546-52 x -59</td>
<td>30</td>
<td>2</td>
</tr>
<tr>
<td>2. 555 x p46</td>
<td>52</td>
<td>1</td>
</tr>
<tr>
<td>3. 5546-60 x BM4</td>
<td>58</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>140</td>
<td>11</td>
</tr>
<tr>
<td>B. cmd prt&lt;sup&gt;+&lt;/sup&gt; x cmd prt&lt;sup&gt;+&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. 5546-60 x -52</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>2. 5546-63 x -77</td>
<td>64</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>124</td>
<td></td>
</tr>
<tr>
<td>C. cmg prt&lt;sup&gt;−&lt;/sup&gt; x cmg prt&lt;sup&gt;−&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. 78-1 x 78-2</td>
<td>0</td>
<td>0</td>
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<td>2. 78-6 x 78-10</td>
<td>0</td>
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*cmd, cmg = downy and granular colonial morphological phenotypes respectively.

prt<sup>+</sup> and prt<sup>−</sup>, respectively, referring to the high or low proteolytic (elastolytic) activities of the strains.

Strains displaying the prt<sup>+</sup> and cmd characteristics and strains displaying the alternate allelic characters, prt<sup>−</sup> and cmg, were selected and crossed on the fruiting medium. When these two characters were scored in monoascospore progeny (Fig. 1) resulting from the crosses, the data indicated a recombination frequency of approx. 0.05 between the loci (Table 1, crosses A). Reciprocal crosses could not be performed because all recombinant types produced from crosses A had the same mating type.

Three-point crosses, involving elastolytic activity, colonial morphology, and mating type, were carried out (Table 2). The results indicated that the proteolytic and the colonial morphology loci were strongly linked, and that segregation of the mating-type locus was independent of these two loci.

**DISCUSSION**

Data from the crosses described in this work clearly establish linkage relationships of three loci which are of practical importance in studies of Arthroderma benhamiae. The mating-type locus, of significance in genetic control of sexuality and recombination, segregates independently of the closely linked colonial morphology and protease loci. The colonial morphology locus consists of two naturally occurring alleles, cmg and cmd, which determine the expression of two colonial phenotypes, granular and downy, respectively. These phenotypes traditionally have been correlated with different clinical forms of Trichophyton mentagrophytes infection. Georg (1954, 1960), for example, demonstrated that the downy form of T. mentagrophytes is most commonly isolated from humans with chronic, low-grade infections, while the granular form is most commonly isolated from animals and humans with suppurating lesions. The nature of the protease system described in this paper is unknown.
It may be an example of two alternate forms of a structural gene for elastase, or it may represent two forms of a protease with elastin-hydrolysing activities, or the high-activity phenotype may be the result of a defective regulatory locus for a protease, or other possibilities may pertain. Nevertheless, high and low extracellular elastin-hydrolytic activities have been shown to be controlled by alternate forms of a single gene.

Rippon and his co-workers reported the presence of elastase in a number of dermatophytes usually producing more inflammatory type lesions (Rippon, 1967; Rippon & Varadi, 1968). Rippon & Garber (1969) and Rippon (1971) showed that in Arthroderma benhamiae strain x797 (mating type A) had no elastase activity and produced scaling and erythematous lesions, while strain x798 (a) had elastase activity, and produced extensive, weeping, crusted lesions in experimental guinea-pig infections. These workers concluded that the type of lesion produced on animals differed according to the amount of proteolytic activity displayed by the infecting fungus, and that mating-type was related to severity of infection and proteolytic enzyme production. In a related fungus, Nannizzia fulva, Rippon (1967, 1971), Rippon & Varadi (1968) and Rippon & Garber (1969) found that a strong association existed between mating-type and elastase activity: generally, positive mating-types were elastolytic, indicating to these workers that the locus governing elastase production was either strongly linked to, or identical to, the mating-type locus.

The present study of the pattern of inheritance of elastin-hydrolysing capability in Arthroderma benhamiae demonstrates unequivocally that the presence of high or low elastolytic activity is not associated with specific mating-types. Instead, the locus governing elastin-hydrolytic activity is in a different linkage group, closely linked to the colonial morphology locus. In this connexion it is of interest that Georg (1954) showed that downy forms of Trichophyton mentagrophytes produced less severe lesions in guinea pigs than the granular forms. This might be indicative that either the colonial morphology locus or the prt locus is closely correlated with the severity of lesions produced on animals, or, if there is another locus correlated with pathogenicity in this organism, one would expect it to be in the same linkage group as the colonial morphology and the prt loci.

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REFERENCES


